Application of P isotope techniques to low-P acid tropical soils

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presented by
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General Conclusions

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List of Abbreviations

ANOVA  analysis of variance

$A_{d}$  dithionite-citrate-bicarbonate extractable Al

$A_{ox}$  oxalate extractable Al

Cas0  soil from cassava monocrop, without fertilizer application, or:

CasNK  • with 100 kg N and 100 kg K ha$^{-1}$ and year$^{-1}$

CasNP  • with 100 kg N and 100 kg P ha$^{-1}$ and year$^{-1}$

CasNPK  • with 100 kg N, 100 kg P and 100 kg P ha$^{-1}$ and year$^{-1}$

$C_{Chl}$  microbially bound carbon released by chloroform

CIAT  International Center for Tropical Agriculture, Centro Internacional de Agricultura Tropical

$C_{p}$  $P_{i}$ concentration in the soil solution (mg P L$^{-1}$), in soil:water ratio 1:10

CR  soil under continuous rice cropping

$E_{i}$  quantity of P isotopically exchangeable in one minute (mg kg soil$^{-1}$)

$E_{t}$  quantity of isotopically exchangeable P in time t (mg kg soil$^{-1}$)

$F_{e}$  dithionite-citrate-bicarbonate extractable Fe

$F_{ox}$  oxalate extractable Fe

GL  soil under grass-legume pasture

ICA  Instituto Colombiano Agropecuario

$L_{obs}$  $L$ value, describing the isotopically exchangeable P determined with the specific activity of a plant, without seed P correction

$L_{ah}$  $L$ value with seed P correction according to Truong and Pichot (1976)

$L_{ah,25}$  $L$ value with seed P correction according to Truong and Pichot (1976) with the assumption of 25 % seed P taken up in the respective cut

n  parameter obtained from isotopic exchange data, usually calculated using linear regression between log $r/R$ and log(t) for $t \leq 100$ minutes

$N_{Chl}$  microbially bound nitrogen released by chloroform

$P_{Chl}$  microbially bound phosphorus released by chloroform

$P_{i}$  inorganic soil phosphorus

$P_{o}$  organic soil phosphorus
<table>
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<tr>
<td>$P_{res}$</td>
<td>residual P fraction of the sequential P fractionation</td>
</tr>
<tr>
<td>$P_{sum}$</td>
<td>sum of all P fractions of the sequential P fractionation</td>
</tr>
<tr>
<td>$P_t$</td>
<td>total soil phosphorus</td>
</tr>
<tr>
<td>RGM</td>
<td>soil under rice green manure rotation</td>
</tr>
<tr>
<td>$r/R$</td>
<td>radioactivity remaining in the soil solution at the corresponding time (t) in relation to the radioactivity added at time zero</td>
</tr>
<tr>
<td>$r_\infty/R$</td>
<td>radioactivity remaining in the soil solution at infinite time of isotopic exchange in relation to the radioactivity added at time zero</td>
</tr>
<tr>
<td>SA</td>
<td>specific activity ($^{33}P/^{31}P$ or $^{32}P/^{31}P$)</td>
</tr>
<tr>
<td>SAV</td>
<td>soil under native savanna</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>$\Sigma P_t$</td>
<td>sum of inorganic P fractions of the sequential fractionation</td>
</tr>
</tbody>
</table>
Phosphorus (P) is the main limiting nutrient for crops and forage plants in acid tropical soils. Besides low total and available P contents, these soils are often highly P fixing due to their high aluminum and iron oxide concentrations. The correct determination of plant available P and profound knowledge about P dynamics are prerequisites for the evaluation of different soil/plant systems. The aim of this work was to improve this knowledge by using P-isotope techniques. While these techniques have successfully been used, mainly in temperate soils, the application to tropical, low-P soils has been shown to be rather difficult.

The isotopic exchange method was applied in soil/solution suspension in a batch experiment (E value determination) to quantify plant available P to soils of different P status from two sites in Colombia. E values, calculated from the P concentration ($C_p$) and the decrease of radioactivity in the soil solution ($r_t/R$), were determined during 4 to 5 weeks according to the method proposed by Fardeau (1996). Parameters ($r_t/R$, $n$, $r_{w}/R$) used to describe $r_t/R$ were derived from the first 100 minutes of isotopic exchange either as proposed by Fardeau (1996) or from a non-linear fitting procedure and used to extrapolate $r_t/R$ until 12 weeks. The results showed that the extrapolation of $r_t/R$ calculated as proposed by Fardeau (1996) leads to values which were very close to the measured values, but which were not in all cases identical. Especially in the P-poorest native savanna (SAV) soil the extrapolated $r_t/R$ were clearly and significantly lower than the measured values. Errors in $n$ and $r_{w}/R$, which in turn is dependent on $P_i$ and on the P concentration in the soil solution ($C_p$), might explain the differences. On the other hand, the measured $r_t/R$ values might have been influenced by microbial activity and therefore not strictly have followed the prediction of the model of isotopic exchange. For most soils $C_p$ was higher than the detection limit but for some soils $C_p$ was lower than the quantification limit (i.e. $< 4 \mu g \, l^{-1}$), showing that the determination of $C_p$ remains one of the major limitations of the methodology.

L values were determined in the same soils to quantify plant available P calculated from the specific activity of the test plant Agrostis capillaris grown on $^{33}$P-labelled soil. As in the
soils with the lowest P availability, the seed-P diluted the specific activity of the plant and therefore hindered the precise determination of the L value, too, it can be concluded that the isotopic exchange method, i.e. E and L values, can only be used with limitation in such low P acid soils. Due to these constraints, the comparison of L values of different plants (beans, maize, rice, the forage legume *Arachis pintoi*, and the forage grass *Brachiaria decumbens*) with the purpose to identify special P uptake mechanisms which would be manifested in increased L values, resulted in unsatisfying results. For *Brachiaria decumbens*, however, the resulting L value strongly indicated P uptake from normally unavailable P-pools. The application of carrier-P with the label to overcome the problem of limited plant growth was shown to modify the system, making it impossible to measure P exchange.

By the sequential P extraction of labelled soil samples derived from different land-use systems with varying P fertilizer inputs it was found that already 14 days after labelling, a significant amount (20 %) of the label was found in organic P forms in the non- or weakly fertilized soils whereas this percentage was lower in the soils with more P input. Most of the label, however, was recovered in inorganic P fractions in all soils, showing that they contained most of the available P.
Zusammenfassung


Wegen diesen Einschränkungen waren die Resultate des Vergleichs von L-Werten verschiedener Pflanzen (Bohnen, Mais, Reis, die Futterleguminose *Arachis pintoi* und das Futtergras *Brachiaria decumbens*) mit der Absicht, spezielle P-Aufnahmemechanismen aufzuzeigen, die sich in erhöhten L-Werten manifestieren würden, wenig zufriedenstellend. Für *Brachiaria decumbens* wies der erhöhte L-Wert aber klar auf P-Aufnahme von normalerweise nicht verfügbarem P hin. Die Anwendung von Carrier-P mit der Markierung, um das Problem des limitierten Pflanzenwachstum zu überwinden, veränderte das System so sehr, dass es unmöglich wurde, den P-Isotopenaustausch zu messen.

Durch die sequentielle chemische P-Fraktionierung von $^{33}$P markierten Bodenproben von verschiedenen Landnutzungssystemen mit unterschiedlicher P-Düngung wurde gezeigt, dass schon 14 Tage nach der Bodenmarkierung ein bedeutender Teil der Markierung (bis zu 20 %) in organische P-Formen eingebaut war und dass dieser Teil größer war in den Landnutzungssystemen mit geringerer P-Düngung. Der grösste Teil der Markierung wurde hingegen in allen Böden in anorganischen P-Formen gefunden, was zeigte, dass diese den grössten Teil des verfügbaren P enthalten.
General Introduction
Importance of phosphorus (P) in plant nutrition

Phosphorus, as orthophosphate, is an essential component of all living cells. It is present in a number of important plant cell compounds, such as sugar-phosphate, phospholipids in plant membranes, and nucleotides required for the accumulation and release of energy for cellular metabolism as well as in control processes and in the genetic information (Kirkby and Le Bot, 1994). Phosphorus is absorbed by plant roots from the soil solution mainly as orthophosphate ions (principally dihydrogen phosphate, $\text{H}_2\text{PO}_4^-$ and to a lesser extent $\text{HPO}_4^{2-}$).

The P-uptake is controlled by plant demand and is an active and energy consuming process by $\text{H}^+/\text{anion}$ cotransporter against a concentration gradient as the P concentration in root cells can be 100 to 1000 times greater than that in the soil solution (Johnston, 2000). The P requirement for optimal growth is in the range of 0.3 to 0.5 % of the plant dry weight during the vegetative stages of growth. Plants suffering from P deficiency exhibit retarded growth, and often a reddish coloration occurs because of enhanced anthocyanin formation (Marschner, 1986). Because of the functions of phosphorus in the growth and metabolism of plants, deficiency leads to a general reduction of most metabolic processes, including cell division and expansion, respiration and photosynthesis.

The soil P cycle

Soil P is derived from the weathering and breakdown of primary P minerals, mostly apatite. There are wide variations in the P content of soil parent materials and these are further increased by the geochemical and biological processes of pedogenesis, which change the total amount of P and alter the portion in soil that is available for plant uptake (Walker and Syers, 1976). During weathering and soil development, the P of apatite is liberated and subsequently acquired by plants and other organisms and recycled, incorporated into the organic matter and re-deposited as insoluble or slowly soluble mineral forms, such as Ca-, Fe-, and Al-phosphates and the occluded P of hydrous oxides. Figure 1 shows the different pathways of P in an agricultural soil system, including fertilizer inputs and P removal with harvest.
The Phosphorus Cycle

Fig. 1 P dynamics in an agricultural system, source: Potash & Phosphate Institute, (PPI), Georgia, USA

Soil P supply and reasons for P deficiency

In strongly weathered soils, total P contents tend to be low because of leaching and erosion. In the tropics and subtropics, in Oxisols, Ultisols and some Alfisols (if they have acid, loamy, or clayey topsoils), strong weathering is also correlated with an increase in the amount of sesquioxides. These exhibit high P sorption properties (Ryden et al., 1977; Parfitt, 1978), with a decrease in primary calcium minerals and with acidification (Stevenson, 1986). About 36 % of the tropical soils are highly weathered (defined as containing < 10 % weatherable minerals in the sand and silt fraction) and have low nutrient reserves while 23 % show a high P sorption capacity (Sanchez and Logan, 1992). High P sorption can also be related to high allophane contents in Andosols (Parfitt, 1980). This strong P sorption also reduces the efficiency of fertilizer P applications. Additionally, in
many tropical regions, soil P fertility is declining as a result of greater export of P, through removal of harvested products and erosion, than input of P (Stoorvogel et al., 1993; Smaling et al., 1997). Therefore, soil P infertility is recognised as a widespread and probably the major limitation to plant growth in the tropics (Fairhust et al., 1999).

**Phosphorus availability in soils**

Plant P uptake at the root surface rapidly depletes the $P_i$ in the adjacent soil solution, creating a diffusion gradient in the rhizosphere. The rate of uptake is then controlled by the effective diffusion rate to the root. On the other hand, the $P_i$ in the soil solution is in dynamic equilibrium with labile $P_i$ on the soil solid phase and organically bound P may be mineralised and also contribute to the replenishment of the soil solution (Randall, 1995). Therefore, the plant available P can be defined as the P that can get from the soil solid phase in to the soil solution within a time relevant for plant growth.

The availability of $P_i$ can be characterised according to Beckett and White (1964) by three factors: (i) the intensity factor, which is the concentration of orthophosphate ions in the soil solution, (ii) the quantity factor, which is the amount of P that can be released from the soil solid phase into the soil solution, and (iii) the buffer capacity, which describes the ability of a soil to maintain the intensity factor constant when the quantity varies.

**Strategies to alleviate P deficiency**

The most obvious strategy to alleviate P deficiency is to add large quantities of phosphate fertilizers to P deficient soils, either as soluble P fertilizer or as rock phosphate (Roche et al., 1980; von Uexküll and Mutert, 1995). However, amelioration of P deficiency with cost-intensive fertilizers is not a viable option for many resource-poor farmers. Moreover, as P is a non-renewable resource with relatively low concentrations in the biosphere, the use of fertilizer P inputs in any agricultural system must be carefully rationalised (Cathcart, 1980). Since 1960, the yearly P fertilizer consumption in Latin America has increased roughly ten times (IFA, 2001). The world's P reserves, defined as deposits which are or could be profitably mined under prevailing costs (Cathcart, 1980), market prices and technology, are
rather limited and will last for about 80 years (Benchekroun, 1995; Johnston, 2000). The resources that are at present not economically exploitable (e.g. because of low rock P content or difficult access to the deposit), but which could potentially become so, are much larger. It is however indispensable also to develop strategies for innovative local-scale farming systems based on the use of renewable and economically available P sources, such as animal manure, recaptured urban residual P, waste recycled P, or green manure P.

In recent years, much research has focused on biological aspects of soil fertility management through manipulation of factors such as mycorrhizal symbiosis, root distribution and function, and residue management, with the aim of increasing P use efficiency and P recycling. Crop and forage genotypes that can acquire and utilise scarce soil and fertilizer P resources more efficiently could improve and stabilise agricultural production (Rao et al., 1999a). However, such approaches do not result in a net addition of P to the soil and can not overcome P deficiency on the long term. As the quantity of P in the system is not increased, there is even the risk of soil P mining when P is exported in crop products and residues are removed. The beneficial effect of germplasm with a high P uptake efficiency may be to render recalcitrant P available to the system (Oberson et al., 1999). Therefore, a sustainable management of agroecosystems might consist in a combination of strategies of optimal fertilizer application and use, internal P recycling in the system and improvement of germplasm to enhance P acquisition efficiency.

To follow up the processes of P cycling in the system or for the evaluation of the adaptation of germplasm to low P conditions, adequate tools for research are needed. The application of radioactive P isotopes can deliver information, which is not available with other techniques.

**P isotope techniques**

Tracer studies with P involve the use of one or both of the radioactive isotopes $^{32}$P and $^{33}$P. Both isotopes emit β-radiation that can be detected and counted on a liquid scintillation counter. The half-life of $^{32}$P is with 14.3 days shorter than that of $^{33}$P with 24.4 days. Therefore, $^{33}$P allows conducting longer experiments. Additionally it emits lower energy β-
radiation than $^{32}\text{P}$ and hence poses less external radiation hazard. A reason to use $^{32}\text{P}$ may be its lower costs than for $^{33}\text{P}$.

P isotopes have been used to study P availability, soil P cycling and plant P uptake either from soil, plant residues, and organic or inorganic fertilizers. One possibility is to follow the fate of labelled compounds as plant residues (Friesen and Blair, 1988; McLaughlin et al., 1988; Daroub et al., 2000), P compounds as iron and aluminium phosphates (Armstrong et al., 1993) or mineral P fertilizers (Morel and Fardeau, 1989a). Daroub et al. (2000) combined the incorporation of labelled plant residues with the sequential P fractionation according to Hedley et al. (1982) to follow the P dynamics in the different soil P fractions. Another application of P isotopes is based on the kinetics of disappearance of radioactive phosphate ions from the solution of a soil-solution system at steady-state (i.e. at a constant $^{31}\text{P}$ concentration in the solution) resulting from the exchange with $^{31}\text{P}$ on the soil solid phase (Fardeau, 1996). This thesis is mainly focused on this second application using the principle of isotopic exchange to study soil P availability. This concept was used to determine E values or L values, both expressing a measure of P availability, either determined in a batch experiment in soil suspension (E) or with a plant grown on labelled soil (L). The E value concept was applied to estimate soil organic P mineralisation (López-Hernández et al., 1998; Oehl et al., 2001a) and can also give information about the availability of residual fertilizer (Morel and Fardeau, 1989b).

Studies comparing E and L values suggest that the isotopically exchangeable P is the main source of P for a large number of annual plant species (Frossard et al., 1994; Morel and Plenchette, 1994). Conversely, plants with a higher P uptake efficiency and specific adaptation to low P conditions might take up P which is not isotopically exchangeable. As a consequence, when grown on a labelled soil, such plants would take up comparatively more $^{31}\text{P}$ and with this have a higher L value than a plant using only isotopically exchangeable P. This approach was used by Braum and Helmke (1995) who found higher L values for lupine than soybean.
The overall aim of this thesis is to test P isotopic exchange methods, which are well established for well P supplied temperate soils, in low P acid soils. The application of these techniques to such soils was shown to involve considerable methodological problems (Amer et al. 1969, Wolf et al. 1986, Salcedo et al., 1991). It is therefore evaluated whether the methodology is applicable for the purpose of determination of soil P or residual fertilizer P availability or for the determination of differences in L values between plant species or varieties. Additionally, the concept of isotopic exchange is linked to the chemical sequential P fractionation method by fractionation of labelled soil samples.

The thesis is divided into four parts:

The first chapter briefly presents a review on already existing studies of P availability on low P tropical soils using radioactive P isotopes. It is discussed whether the often stated conclusion that the isotopically exchangeable P overestimates P availability on such soils is justified by the existing data.

In the second chapter, the application of the isotopic exchange method, either in soil/solution suspension in a batch experiment (E value determination) or with Agrostis capillaris grown as test plant on labelled soils (L value determination) was tested on soils from two sites in Colombia. From both sites, soils were used which had received different amounts of P fertilization during several years of long-term field studies. The aim of this part of the thesis is to evaluate the precision of the two methods on low P acid soils and to get information about their pitfalls and limitations.

In the third chapter, L values of different crops and forage plants are compared, in order to investigate the plants abilities to take up P from poorly available forms. In this thesis, the experiment was run twice, either with or without the use of $^{31}$P carrier with the application of the radioactive P label. By this it was checked whether the use of carrier P can overcome the problems of low plant growth, and with this high seed P influence on L values.
In the fourth chapter, Oxisols from different land-use systems with different amounts of residual fertilizer P were labelled and afterwards extracted according to the sequential P fractionation method described by Tiessen and Moir (1993). By this, the assignment of chemical P fractions to pools of different availability, as well as the effect of land-use systems and related P fertilizer inputs on size of P fractions and their isotopic exchangeability were investigated.
CHAPTER I

Introduction to P isotopic exchange methods and review of studies in low P acid soils
Introduction

Techniques using the principle of isotopic exchange allow assessing the total of exchangeable soil P. This approach is currently used for the determination of the E value in batch experiments using soil suspensions, introducing the label and measuring the decrease of radioactivity over time at condition of constant P concentration in the soil solution (Fardeau, 1996) (Figure 1.1). The isotopically exchangeable P is then calculated using the specific activity (i.e. the ratio $^{32}$PO$_4$:$^{31}$PO$_4$ or $^{33}$PO$_4$:$^{31}$PO$_4$) in the soil solution. A correspondent procedure is the growth of a plant on labelled soil in order to measure the specific activity of the plant P, which also allows the calculation of the isotopically exchangeable soil P (Larsen, 1952) (Figure 1.1). While these techniques are well established for temperate soils (Frossard et al., 1994), the application to very low P soils of the tropics was less successful (see literature compilation in Table 1.1). It is however often difficult to compare older studies with newer results as the applied methodologies are often not identical.

This chapter will give an introduction to the development of the isotopic exchange techniques and then focus on the studies that had been done using P isotope techniques on low P acid soils. It will be discussed whether the conclusion that the measured isotopically exchangeable P, on such soils, generally overestimates P availability for plants is justified by the results of these studies.

The origin and development of E and L values

Techniques using $^{32}$P and $^{33}$P isotopes for the determination of plant available P, either from soils or from fertilizers, have been used since the 1950ies (Wiklander, 1950; Russell et al., 1954; Talibudeen, 1958). In this time, the idea was developed to measure the quantity of labile soil P by isotopic exchange with radioactive P isotopes. The measured amount of exchangeable P determined in a batch experiment was called E value. The isotopically exchangeable P determined using plant P uptake from a labelled soil was called L value (labile P or after Larsen as one of the first scientists using this approach).
Table 1.1 List of publications dealing with P isotopic exchange measurements on low P acid soils

<table>
<thead>
<tr>
<th>authors</th>
<th>soil, culture and origin</th>
<th>time of exchange</th>
<th>method used</th>
<th>filtration</th>
<th>remarks, results and interpretation</th>
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<tr>
<td>Ipinmidun, 1973</td>
<td>slightly acid loamy fine sands or sandy loams from Nigeria, with different amounts of residual fertilizer</td>
<td>24 hours or 7 days</td>
<td>determined with Rhodes grass (Chloris gayana)</td>
<td>none</td>
<td>very low P concentration in solution, lack of correlation between E values and P uptake by Rhodes grass, poor correlation between E and L values</td>
</tr>
<tr>
<td>Dalal and Hallsworth, 1977</td>
<td>20 different soils, covering wide range of soil types, no further indications about cropping or origin</td>
<td>7 days</td>
<td>determined with wheat (Triticum aestivum cv. Gabo)</td>
<td>n.i.</td>
<td>for some soils discrepancy between E and L values was interpreted as error in E value determination</td>
</tr>
<tr>
<td>Le Mare, 1981</td>
<td>Entisol, Alfisol and Ultisol, maize in rotation with cowpea, beans or sweet potato, Nigeria</td>
<td>24 hours</td>
<td>(CaCl₂)</td>
<td>n.i.</td>
<td>good correlation between E values and plant P uptake after 51 days, worse for younger plants</td>
</tr>
<tr>
<td>Le Mare, 1982</td>
<td>Oxisols, Ultisols and Inceptisols from Colombia and Brazil, pH 4.0 - 4.9, no indications about cropping</td>
<td>24 h</td>
<td></td>
<td>Whatman 1</td>
<td>P sorption study, E values determined without added P did not indicate methodological problems</td>
</tr>
<tr>
<td>Wolf et al., 1986</td>
<td>Ultisols, Alfisols, Mollisols, no indication about cropping, USA</td>
<td>24 hours</td>
<td></td>
<td>0.2 µm</td>
<td>differences in E values determined without or with carrier bad correlation of E values and resin extractable P, especially for the Ultisols</td>
</tr>
<tr>
<td>Tran et al., 1988</td>
<td>58 different soils with different P-buffere capacity, some of them acid low P (mainly Spodosols and Inceptisols) no indication about cropping, Canada</td>
<td>18 hours</td>
<td>1, 10, 40 and 100 minutes</td>
<td>0.2 µm</td>
<td>correlation between plant P uptake and E worse, but still significant, for highly P sorbing soils than for less P sorbing soils</td>
</tr>
<tr>
<td>Naidu et al., 1991</td>
<td>no information, pH between 3.9 and 4.9, highly weathered</td>
<td>16 hours</td>
<td>2 hours</td>
<td>0.45 µm</td>
<td>no correlation between plant P uptake and isotopically exchangeable P</td>
</tr>
<tr>
<td>Salcedo et al., 1991</td>
<td>Ultisols, Alfisol and Oxisol, maize (Alfisol) or sugarcane (all others), Brazil</td>
<td>overnight</td>
<td>1, 10, 40 and 100 minutes</td>
<td>0.22 µm</td>
<td>very low soil solution P concentration measured by concentration of the solution with resin for one soil (Oxisol) of totally six soils extremely high E value, for the other soils (mainly Ultisols) good correlation with plant P uptake suggesting to use P concentration in soil solution as indicator for available P</td>
</tr>
</tbody>
</table>

*indicates soil:solution (deionized water, or if indicated 0.01 M CaCl₂) ratio

* not indicated
I. Introduction to P isotopic exchange methods - review of studies in low P acid soils

Classically, the E value was measured at a single time fixed between 30 min and 3 weeks (Russell et al., 1954; Amer et al., 1969; Triboi and Gachon, 1988) after the addition of the radioactive P to the soil solution suspension. This approach was based on the assumption that exchangeable P of the soil solid phase was a homogeneous pool and that the added labelled P would get equilibrated with this pool in a certain time (Amer et al., 1969; Wolf et al., 1986). This view was changed by the concept of Fardeau and co-workers (Fardeau et al., 1985; Fardeau, 1993) who characterised the kinetic parameters of the exchange in a

Figure 1.1 Concept of E value and L value, the graph on the left shows an example of a decrease curve of the radioactivity introduced to the soil solution over time.

<table>
<thead>
<tr>
<th>E value:</th>
<th>L value:</th>
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<tbody>
<tr>
<td>R: $^{33}\text{P}$ label</td>
<td>plant: $^{33}\text{P}/^{31}\text{P}$</td>
</tr>
<tr>
<td>solution: $^{33}\text{P}/^{31}\text{P}$</td>
<td>soil: $^{33}\text{P}/^{31}\text{P}$</td>
</tr>
<tr>
<td>batch experiment with soil suspension</td>
<td></td>
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</tbody>
</table>

![Graph showing decrease curve of radioactivity introduced to soil solution over time.](image)
steady-state system (i.e. constant P concentration in the soil solution) instead of considering only a single point in time. With this approach they showed that soil solid phase inorganic P represents an infinity of pools in more or less rapid exchange with the P ions in solution (Fardeau et al., 1991; Fardeau, 1993). The exchange reactions are regarded as continuous processes, which ultimately result in the exchange of all inorganic P in the soil. The decrease of the radioactivity in the soil solution after the addition of the $^{33}$PO$_4$ or $^{32}$PO$_4$ ions could be described by the following equation for exchange times until 4 months (Fardeau et al., 1985):

$$\frac{r}{R} = R_\infty \left[ t + \left( \frac{r_i}{R} \right)^{\frac{1}{\alpha}} \right]^{-\alpha} + \frac{r}{R}$$ \hspace{1cm} (1.1)

where $n$ is the slope of the linear regression equation between $\ln(r/R)$ and $\ln(t)$ for $t \leq 100$ minutes and $r_\infty/R$, i.e. the fraction of radioactivity remaining in the soil solution after an infinite isotopic exchange time, is estimated as follows (Equation (1.2)):

$$\frac{r_\infty}{R} = 10 \times \frac{C_p}{P_i}$$ \hspace{1cm} (1.2)

where $P_i$ is the total inorganic P expressed in mg P kg$^{-1}$ soil and $C_p$ is the P concentration in the soil solution. The factor 10 results from the 1:10 soil:solution ratio.

Equation (1.1) was developed on the basis of theoretical considerations (Sheppard, 1962, Fardeau et al., 1991) and connected with two models either of stochastic or functional nature (Fardeau, 1996). In the stochastic analysis, equation (1.1) can be considered as the Laplace transform of a $\gamma$ density function. This probability function describes the distribution of the individual rates of exchange, $k_i$, between the ions present in the soil solution and exchangeable ions located on the solid phase. There is a continuum of $k_i$ distributed between 0 and $\infty$, these two values being excluded (Fardeau et al., 1991). Fardeau (1996) also describes the calculation of a mean exchange rate of P ions between soil solid phase and solution from isotopic exchange experimental parameters.

The functional model relates isotopically exchangeable P to compartments organised as a
I. Introduction to P isotopic exchange methods - review of studies in low P acid soils

![Diagram of multi-compartment mamellary model of soil exchangeable P (Fardeau, 1993)](image)

Figure 1.2 Schematic representation of the multi-compartment mamellary model of soil exchangeable P (Fardeau, 1993)

- Pool of free ions
- Exchangeable ions
  - E1min - E1day
  - E1day - E3months
  - E3months - E1year
  - E>1year

Soil solution

Solid phase of the soil

The mamellary system (Sheppard, 1962) with the soil solution as central compartment (Figure 1.2). The other functional compartments of this mamellary system were defined according to root functioning and plant needs in cropping cycles (Fardeau, 1993).

The principle of the L value determination is based on the same theoretical background as the determination of the E value. Instead of following the decrease of radioactivity in the soil solution after the application of the radioactive P label, the specific activity of a plant grown on a labelled soil is measured. The growing plant takes up P with the specific activity resulting from isotopic exchange of the introduced $^{33}$PO$_4$ with $^{31}$PO$_4$ on the soils solid phase. The methodology and calculations for the determination of L values have not changed since the first studies in the 1950ies (Larsen, 1952) and there are many studies on the determination of L values in temperate soils either with or without prior fertilizer application. However, there are only few studies about L values in low P acid soils (Larsen, 1952; Ipinmidun, 1973; Dalal and Hallsworth, 1977; Braum and Helmke, 1995; Hocking et
especially on such soils, the determination of P in the plant, because of its relatively high concentration, avoids the problem of measuring the low P concentration in the soil solution. However, the specific activity measured in the plant is diluted by the P reserve in the plant seed and L values are overestimated by this influence (Truong and Pichot, 1976; Brookes, 1982). This error can be alleviated by cutting the plants several times and excluding the result of the first cut (Frossard et al., 1994). However, not all plants can be cut repeatedly. On very P limited soils, plant growth can be very limited, what also hinders the possibility of repeated plant cutting for perennial plants. Additionally, from a radio-safety point of view, the use of $^{32}$P or $^{33}$P has to be restricted to reasonably low amounts of activity. For L value determinations, this means that they are limited to pot studies with relatively low quantities of soil per pot (< 5 kg soil) and rapidly growing plants (Frossard et al., 1999).

**E and L value studies in low P acid soils**

In contrast to numerous studies in temperate soils and in tropical soils with a low P fixing capacity (Fardeau, 1993; Frossard et al., 1994), the application of the isotopic exchange method to low P and strongly P sorbing soils was of limited success and mostly resulted in an overestimation of P availability (Amer et al., 1969; Wolf et al., 1986; Salcedo et al., 1991). However, the methodology for the E value determination differed in most studies from the isotopic exchange approach used in this study and/or the description of the method is not detailed enough to comprehend the presented results (Table 1.1).

A major problem on soils with low P status and high P sorption capacity is the determination of the P concentrations in the soil solution. This is due to several reasons: (i) concentrations may be below the detection limit of the ammonium molybdate and ascorbic acid colorimetric method (Salcedo et al., 1991) (ii) non-orthophosphate P-forms may be measured due to hydrolysis from inorganic condensed (P-O-P) and organic (P-O-C) bonds (Gerke, 1992) or due to acid dissolution of P associated with colloids (Sinaj et al., 1998; Heens, 1999), (iii) other ions might form complexes with molybdate instead of phosphate, especially silicate might be problematic in soil solutions (Fardeau and Jappé, 1988). Another assumed error in the determination of E values is the fixation of a part of the label,
which then would not take part in the exchange process. As the total quantity of $^{33}$P ions introduced as label in isotopic exchange experiments is however very small in relation to $C_p$ and since it is assumed that $^{33}$P and $^{31}$P have the same fate in the system (Frossard and Sinaj, 1997), the specific adsorption of $^{33}$P in a steady-state system seems unlikely. If the condition of steady-state is however not fulfilled, the specific sorption of a part of the label can not be excluded. This might especially be likely when soil solution suspensions are not equilibrated before adding the label.

In all mentioned E value studies in low P acid soils (Table 1.1), $C_p$ was determined in solutions filtered at $\geq 0.2$ μm what could have resulted in an overestimation according to the results of Sinaj et al. (1998) who found higher $C_p$ values in 0.2 than 0.02 μm filtrates. In one study, Salcedo et al. (1991) concentrated the filtrated soil solution by the use of anion exchange resins. In their case interference of colloids on the $C_p$ values is unlikely. Wolf et al. (1986) additionally omitted the equilibration before the addition of the label what might have influenced their results. Their work, comparing E values determined with or without concomitant addition of $^{31}$P carrier with the label, resulted in decreasing and sometimes even negative E values with increasing amount of carrier added. This result was explained by the fixation or precipitation as Al-phosphate of the added label and carrier. As this effect was higher on Ultisols than on Alfisols or Mollisols, the method was declared as specifically inappropriate for highly P sorbing soils with high Fe and Al (oxy)hydroxides. However, the determination of E values with the addition of carrier changes the system, which will not be any more in steady-state, and should therefore generally be avoided (Sheppard, 1962; Fardeau et al., 1996). To sum up, with the exception of the unlikely high E value found for one (!) Oxisol in the study of Salcedo et al. (1991), there is very little evidence for the failure of the isotopic exchange method on low P tropical soils. Therefore, in this thesis, a first aim was to apply the isotopic exchange method to low P tropical soils strictly according to the newest knowledge and with special attention to the possible pitfalls.
CHAPTER II

Application of isotope methods for assessing the plant available phosphorus in acid tropical soils
II. Application of isotope methods for assessing the plant available P in acid tropical soils

Abstract

Isotope methods can provide relevant information on orthophosphate (P) availability in well supplied temperate soils. The aim of this study was to evaluate two isotope methods in low-P acid tropical soils. The isotopically exchangeable P measured in soil water suspensions (E value) and in pot experiments (L value) was assessed in soils which had received different amounts of P fertilizers in two field experiments in Colombia. E values were determined during 4 to 5 weeks according to the method proposed by Fardeau (1996). Parameters ($r_t/R$, $n$, $r_w/R$) used to describe the decrease of radioactivity in the soil solution ($r_t/R$) were derived from the first 100 minutes of isotopic exchange either as proposed by Fardeau (1996) or from a non-linear fitting procedure and used to extrapolate $r_t/R$ until 12 weeks. E values were calculated either using measured or extrapolated $r_t/R$ values. *Agrostis capillaris* was grown on the same soils labelled with carrier free $^{33}$P ions to calculate L values. Results show that, in these low P acid soils, the extrapolation of $r_t/R$ should be calculated as proposed by Fardeau (1996) and not from a non-linear fitting procedure. However, errors in $n$ and $r_w/R$ might still hinder the correct calculation of $E_t$. For most soils the P concentrations in the soil solution were higher than the detection limit (i.e. > 1 µg l$^{-1}$) of the malachite green method but lower than its quantification limit (i.e. < 4 µg l$^{-1}$). In the soils with the lowest P availability, the seed P interference hindered the precise determination of the L value. E values were highly correlated, but not identical, with the L values measured for the same time of isotopic exchange.

Key words: Isotopic exchange, P availability, L value, E value, *Agrostis capillaris*, low P acid tropical soils
**Introduction**

Plants take up orthophosphate (P) by roots from the soil solution. Even in well-supplied soils, the concentration of P in the solution represents less than 1 % of the up to 50 kg P ha$^{-1}$ annually taken up by crops (Morel et al., 2000). Thus, the amount of plant available P in a given soil can be defined as the amount of P which can leave the solid phase of the soil and arrive in the soil solution, either through abiotic or biotic processes, within a time frame relevant to duration of plant growth.

Techniques to measure the isotopically exchangeable P, either in soil/water suspensions, yielding the E value, or in soil/plant systems, yielding the L value, have been used since more than 50 years to assess soil P availability to plants (Wiklander, 1950; Russell et al., 1954; Talibudeen, 1958). Classically, the E value was measured at a single time fixed between 30 min and 3 weeks (Amer et al., 1969; Russell et al., 1954; Triboi and Gachon, 1988) after the addition of the radioactive P to the soil solution suspension. This approach was based on the assumption that the exchangeable P of the soil solid phase was a homogeneous pool and that the added labelled P would get equilibrated with this pool within a certain time (Amer et al., 1969; Wolf et al., 1986). Fardeau and co-workers (Fardeau et al., 1985; Fardeau, 1996) developed the isotopic exchange kinetics technique for the case of soil/water suspensions at steady-state, i.e. at constant P concentration in the soil solution, to characterise the kinetic parameters of the exchange instead of considering only a single point in time. With this approach, they showed that soil inorganic P is distributed on the solid phase of the soil in an infinity of pools which are in more or less rapid exchange with P in the solution (Fardeau, 1996). In the isotopic exchange kinetics the exchangeable P ($E_t$) is calculated according to Equation (2.1) assuming that, at any given time after the addition of carrier free radioactive P, the specific activity ($^{33}\text{P}/^{31}\text{P}$) in solution is equal to the specific activity of the P which has been isotopically exchanged (Fardeau, 1996):

$$\frac{E_t}{R} = \frac{10^8 C_p}{r_t}$$

(2.1)

where $R$ is the introduced radioactivity expressed in MBq, $r_t$ is the radioactivity remaining
in solution after t minutes, and $C_p$ the phosphate concentration in the soil solution expressed in mg l$^{-1}$. The factor 10 results from the 1:10 soil to solution ratio used in the experiment.

For exchange times between 30 seconds and 3 months the decrease of radioactivity in the solution with time (t) of exchange is described by Equation (2.2) (Fardeau, 1996):

$$\frac{r_t}{R} = \frac{r_i}{R} \left[ t + \left( \frac{r_t}{R} \right)^{(1/n)} \right]^{n} + \frac{r_\infty}{R}$$  \hspace{1cm} (2.2)

where n is the slope of the linear regression equation between ln($r_t/R$) and ln(t) for t≤100 minutes and $r_\infty/R$, i.e. the fraction of radioactivity remaining in the soil solution after an infinite isotopic exchange time, is estimated as follows (Equation (2.3)):

$$\frac{r_\infty}{R} = 10 \times \frac{C_p}{P_i}$$  \hspace{1cm} (2.3)

where $P_i$ is the total inorganic P expressed in mg P kg$^{-1}$ soil. In some instances, especially for soils with $r_i/R$ values higher than 0.2, the theoretical Equation (2.2) has been simplified as (Fardeau et al., 1985):

$$\frac{r_t}{R} = \frac{r_i}{R} \times t^{-n}$$  \hspace{1cm} (2.4)

The application of the isotopic exchange method to soils presenting a low P availability and a high P sorption capacity often resulted in an overestimation of $E_t$ values (Amer et al., 1969; Wolf et al., 1986; Salcedo et al., 1991). This was accounted for by an overestimation of the concentration of P in the soil solution (Sinaj et al., 1998), and by the specific sorption of a fraction of the added radioactive P onto soil particles (Barrow, 1991). Assuming that the system remains in steady-state conditions, $E_t$-values can be extrapolated to times as long as three months by using data obtained during the first 100 minutes of exchange in Equation (2.2) (Fardeau et al., 1985). Frossard et al. (1996a) pointed out, however, that small variations in the parameter n of this equation could result in over- or
underestimation of \( E_t \) values extrapolated for times longer than 24 hours. The application of the simplified Equation (2.4) to describe isotopic exchange kinetics and calculate \( E \) values, especially in high \( P \) sorbing soils \((r_\text{f}/R<0.2)\), can also result in erroneous results (Fardeau et al., 1985; Frossard et al., 1994).

The amount of soil isotopically exchangeable \( P \) can also be measured by labelling soil available \( P \) with radioactive \( P \) in a pot experiment and measuring the specific activity in the plant \((L \text{ value})\) after a given time of plant growth (Larsen, 1952; Sibbesen, 1984). Determining \( P \) in the plant, because of its relatively high concentration, avoids the problem of measuring the low \( P \) concentration in the soil solution. A specific sorption of radioactive \( P \) onto soil particles (Amer et al., 1969; Barrow, 1991) and/or an important contribution of the seed to plant \( P \) nutrition (Truong & Pichot, 1976) can result in an overestimation of the \( L \) value. The acquisition by a plant of \( P \) forms that are very slowly or not exchangeable can also result in a \( L \) value higher than that obtained with a plant exclusively taking up \( P \) which is isotopically exchangeable during the time span of plant growth (Braum & Helmke, 1995; Hocking et al., 1997). The effect of the seed on the \( P \) nutrition of the plant and on the measurement of \( L \) can be alleviated by cutting the plants several times and not considering the \( L \) value of the first cut (Frossard et al., 1994) and by using plants with low \( P \) seed content, such as \textit{Agrostis capillaris} (Truong and Pichot, 1976). The plant effect on the \( L \) value can be avoided by using plants which have been shown to take up only isotopically exchangeable \( P \) within the frame of plant growth such as \textit{Lolium perenne}, \textit{Hordeum vulgare} or \textit{Agrostis capillaris} (Fardeau and Jappé, 1976; Frossard et al., 1994; Morel and Plenchette, 1994). Finally, the use of a carrier, i.e. the addition of stable \( P \) with the radioactive \( P \), when labelling the soil has been proposed to avoid the specific sorption of radioactive \( P \) onto soil particles (Truong and Pichot, 1976).

As the mechanism of isotopic exchange of phosphate ions is the same in the determination of \( E \) or of \( L \) values, assuming no influence of experimental conditions, the two measurements conducted after the same isotopic exchange time should be identical. Fardeau and Jappé (1976), Frossard et al. (1994) and Morel and Plenchette (1994) showed for a wide range of soil types and for various plants that the \( L \) value measured after a given growth duration was not statistically different from the \( E \) value measured or calculated after the same exchange time. Studies comparing \( E \) values obtained using the isotopic exchange
kinetics method and L values in low P acid soils are however lacking. If such techniques could be used in these soils with a high degree of precision, they could be used to identify plant species or cultivars able to take up P from slowly or not exchangeable P pools (such as organic P or precipitated P-Ca compounds) or to quantify the rate of soil organic P mineralisation as proposed by Oehl et al. (2001a).

The aim of this paper was to ascertain the usefulness of the isotope exchange kinetic technique and of the L value to assess soil P availability in low P acid soils, and to identify the pitfalls of these techniques. To reach this objective, the topsoil of tropical acid soils from Colombia having received different P fertilizer applications were sampled and P availability was assessed using the isotopic exchange kinetics method (Fardeau, 1996) and the L value determination (Larsen, 1952; Sibbesen, 1984).

Materials and methods

Soils

The soils included in the study were sampled in September 1997 in two field experiments located in Carimagua (ICA: Instituto Colombiano Agropecuario; CIAT: Centro Internacional de Agricultura Tropical, Meta, Colombia), 150 m above sea level, 4°30'N, 71°19'W) and in Santander de Quilichao (CIAT, Cauca, Colombia, 990 m above sea level, 3°06'N, 76°31'W).

In Carimagua, the 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). The mean annual temperature in Carimagua is 27° C and the average rainfall is 2200 mm. The soils are well drained Oxisols (tropheptic Haplustox, isohyperthermic) with a clay loam texture (38 % clay, 44 % silt, 18 % sand) developed on a Pleistocene clay (Sanz-Scovino et al., 1992). For our study, the following treatments were included:

SAV (Native Savanna): native grassland, annually burned in February, not grazed; no fertilizer application.

GL (Grass-Legume pasture): rice in 1993, with under sown pasture, since then grass-
legume pasture with *Brachiaria humidicola* CIAT 679, *Centrosema acutifolium* cv Vichada CIAT 5277, *Stylosanthes capitata* CIAT 10280, and *Arachis pintoi* CIAT 17434. The pasture was partly re-sown for renovation in June 1996 with legumes (the same *Arachis pintoi*, and *Centrosema acutifolium* and additionally *Stylosanthes guianensis* CIAT 11833). Grazing intensity was on average 2.7 steers ha\(^{-1}\) during 15 d followed by a 15 d ley regrowth phase.

CR (Continuous Rice): rice (*Oryza sativa* cv Oryzica Sabana 6 and cv Oryzica Sabana 10 since 1996) grown in monoculture; one crop per year followed by a weedy fallow incorporated with early land preparation at the beginning of the rainy season before sowing rice.

RGM (Rice Green Manure rotation): Rice followed by cowpea (*Vigna unguiculata*, var. ICA Menegua) in the same year. The legume was incorporated at the maximum standing biomass level in the late rainy season before sowing rice in the following rainy season. The experiment had a split-plot design with four replicates with treatment sub-plots of 0.36 ha size. At the beginning of the experiment all treatments except SAV were limed using 500 kg dolomitic lime ha\(^{-1}\) (23 % Ca and 10 % Mg). Fertilization of rice was 80 kg N ha year\(^{-1}\) (urea, divided among three applications), 60 kg P ha year\(^{-1}\) (as triple superphosphate), 99 kg K as KCl, 15 kg Mg and 20 kg S (as MgSO\(_4\)) and 10 kg Zn ha\(^{-1}\) at establishment and according to plant needs afterwards. With cowpea additionally 20 kg N and 40 kg P ha year\(^{-1}\) (as triple superphosphate) and 60 kg K, 10 kg Mg, 13 kg S and 10 kg Zn ha\(^{-1}\) were applied at establishment and in adequate rates replacing plant nutrient removal afterwards. The introduced pasture (GL) received additional fertilization only in 1996 (per ha: 20 kg P as triple superphosphate, 20 kg Ca and 10 kg Mg as dolomitic lime, 10 kg S as elemental S and 50 kg K as KCl). Total applied P fertilization between 1993 and 1997 before sampling was 80 kg ha\(^{-1}\) for GL, 240 kg ha\(^{-1}\) for CR and 300 kg ha\(^{-1}\) for RGM. Soils were tilled to a maximum of 15 cm depth in CR and RGM.

In Santander de Quilichao, the soils (0-20 cm) were taken from a field experiment which 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, 1988). Annual mean temperature at this site is 23.7° C and the annual average precipitation is 1799 mm. The soil has been classified either as Oxisol or Inceptisol with a clayey texture.
II. Application of isotope methods for assessing the plant available P in acid tropical soils

(60% clay, 20% silt, 20% sand) and has developed on fluvially translocated volcanic, partly weathered material (Reining, 1992). All treatments were limed with 500 kg dolomite (22% Ca and 11% Mg) ha⁻¹ every two to three years. Samples were taken in the following treatments: no fertilizer applied (Cas0); 100 kg N, 100 kg P, 100 kg K ha⁻¹ yr⁻¹ (CasNPK); 100 kg N, 100 kg K ha⁻¹ yr⁻¹ (CasNK); and 100 kg N, 100 kg P ha⁻¹ yr⁻¹ (CasNP).

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

Soil Analysis

Bray-II P was extracted using dilute acid fluoride (0.03 M NH₄F, 0.1 M HCl) at 1:7 soil solution ratio using 2 g soil and 40 sec shaking time. Total soil P was determined using concentrated H₂SO₄ and H₂O₂ (Thomas et al., 1967). Soil Pᵢ was sequentially extracted, using the modified sequential Hedley P fractionation procedure as described by Tiessen and Moir (1993), with HCO₃-saturated resin strips (BDH # 55164, 9 mm x 62 mm), 0.5 M NaHCO₃, 0.1 M NaOH and hot concentrated HCl. Then, residual P was determined after digestion with concentrated H₂SO₄ and H₂O₂ (Thomas et al., 1967). Total Pᵢ was determined as the sum of the inorganic P fractions, either without (∑Pᵢ) or with adding the amount of residual P (∑Pᵢ + Pᵢ,res, assuming that residual P mainly consists of Pᵢ (Tiessen & Moir, 1993)). Additionally to the sequential extraction, Pᵢ was estimated by extracting 2 g of soil with 50 ml 1 M H₂SO₄ during 17 hours (Pᵢ,H₂SO₄), because this method has been used in other isotopic exchange studies (Frossard et al., 1994) as an estimate of total Pᵢ.

Dithionite-citrate-bicarbonate extractable and oxalate extractable Fe and Al (Feᵦ, Feₒₓ, Alᵦ, Alₒₓ) were determined according to Mehra and Jackson (1960) and McKeague and Day, (1966). Soil chemical characteristics are summarised in Table 2.1.
Table 2.1 Selected properties of the soil samples taken at the Carimagua and Quilichao sites.

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>Total C</th>
<th>Total N</th>
<th>Fe_d</th>
<th>Fe_ox</th>
<th>Al_d</th>
<th>Al_ox</th>
<th>pH in water</th>
<th>Al-saturation</th>
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<td>26.7B</td>
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<td>7.8A</td>
<td>2.0B</td>
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<td>26.4</td>
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<td>7.7</td>
<td>2.0</td>
<td>4.9a</td>
<td>71.7b</td>
</tr>
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<td>7.6</td>
<td>2.0</td>
<td>4.3b</td>
<td>75.4b</td>
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<td>7.8</td>
<td>2.0</td>
<td>4.3b</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>***</td>
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<td>36.9aA</td>
<td>3.4ab</td>
<td>7.0aB</td>
<td>2.9aA</td>
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<td>33.3b</td>
<td>3.5a</td>
<td>6.3b</td>
<td>2.8ab</td>
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<tr>
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<td>2.5</td>
<td>32.9b</td>
<td>3.3ab</td>
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<td>2.3</td>
<td>32.4b</td>
<td>3.1b</td>
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<td>n.s.</td>
<td>**</td>
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<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*,**,*** Significant at the 0.05, 0.01 or 0.001 probability level, respectively. Means within columns of one site followed by the same or no lower case letter are not significantly different (P<0.05) according to Tukey's test. Different upper case letters for SAV and CasO in one column show significant difference between the two sites for these unfertilized treatments according to Student's t-Test (P<0.05).

**E value determination**

Fifteen grams (dry weight) of soil, with three replicates per sample, were equilibrated for 16 hours in 148.5 ml deionised water. Before equilibration two drops of toluene were added to each sample to prevent microbial growth. At t=0, 1.5 ml of a solution containing 5.8 MBq carrier-free $^{33}$PO$_4$ were added and mixed with a magnetic stirrer. Eight to nine subsamples were taken from the suspension after 1, 10, 100 min, and five to six times between 1 day and four (GL, CR, all Quilichao soils) or five weeks (SAV and RGM). The
suspensions sampled at 1, 10 and 100 minutes were immediately filtered through a 0.2 µm pore size 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 deionised water to remove minor P contamination found on these filters. The 0.025 µm filter was preferred as soil colloids carrying $^{33}$P and $^{31}$P can pass through 0.2 µm (Sinaj et al., 1998). However, at 1, 10 and 100 minutes, filtration through 0.025 µm is not possible, as, due to the high resistance of the filter, it would take several minutes to filter one subsample. This would render any precise $r_t$ determination impossible. The influence of the filter pore size on $r_t$ was tested additionally comparing solutions filtered at 0.2 and 0.02 µm after 120 minutes. At that time, the $r_t$ values were not affected by the filter pore size (results not shown). The radioactivity in solution ($r_t$) was determined with a liquid scintillation counter (Packard 2500 TR) using Packard Ultima Gold scintillation liquid in the soil: solution ratio 1:5. All values were corrected for radioactive decay back to the day of soil labelling.

After 100 minutes, and each sampling time afterwards, the P concentration ($C_p$) in the 0.025 µm-filtrates were determined using the method of Murphy and Riley (1962) with a Kontron spectrophotometer Uvikon-810 using a 4 cm cell and with the malachite green method (Ohno & Zibilske, 1991) with a Shimadzu UV-1601 spectrophotometer using a 1 cm cell. As the concentrations in some solutions were close to the detection limit, all solutions were additionally measured after a five-fold concentration, obtained by evaporation. The detection limit, i.e. the smallest concentration which 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 for the malachite green and the Murphy and Riley method with standard P solutions ranging from 0 to 500 µg P l$^{-1}$.

The isotopically exchangeable P ($E_t$) was calculated as described in the Equation (2.1) using

i) the experimental $r_t/R$ data measured during 4 or 5 weeks
ii) \( \frac{r}{R} \) values extrapolated up to 5 weeks after having statistically fitted Equation (2.2) to the experimental data obtained between 0 (\( \frac{r}{R}=1 \)) and 100 min with a non-linear procedure, giving modelled values for the \( \frac{r}{R} \), \( n \) and \( \frac{r_s}{R} \) parameters, and

iii) \( \frac{r}{R} \) values extrapolated to 5 weeks according to the Equation (2.2) after having calculated the parameters \( \frac{r}{R} \), \( n \) and \( \frac{r_s}{R} \) as proposed by Fardeau (1996), i.e. \( n \) is calculated as the slope of the log/log regression between \( \frac{r}{R} \) and \( t \) for times between 1 and 100 minutes, \( \frac{r}{R} \) is the interception of this regression when \( t=1 \), \( \frac{r_s}{R} \) is \( 10C_p/\text{total } P_i \).

The comparison of measured values (i) and extrapolated values (ii and iii) was made to evaluate the significance of the extrapolation of \( r_i \)-values based on experimental data up to 100 minutes of isotopic exchange for the conditions of these low P acid soils. The non-linear fitting procedure (ii) was applied to compare the best fit of the single equation parameters of Equation (2.2) with the parameters determined according to Fardeau (1996) (iii). By this, it was evaluated whether and with which accuracy an extrapolation was possible without use of the (possibly erroneous) information of \( \frac{r}{R} \) and \( n \) values determined according to (iii), and without the information of total \( P_i \) needed to express \( \frac{r_s}{R} \) according to Equation (2.3).

**L value determination**

Prior to 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) in a climate chamber at a temperature of 20° C and 80 % relative air humidity in portions of 4 kg. After this incubation, a P-free nutrient solution, providing N, Ca, K, Mg and S was added (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\(_2\)SO\(_4\), and MgCl\(_2\), respectively). Soil P was labelled with a quantity, \( R \), of carrier free \(^{33}\)PO\(_4\) of 7.4 MBq kg\(^{-1}\). The \(^{33}\)PO\(_4\) was added in a volume of 7 ml per kg soil, and portions of 1.5 kg soil were thoroughly mixed to ascertain an even distribution of the isotope. Portions of 600 g labelled soil were filled into pots, sown with 100 mg of common bentgrass (Agrostis capillaris) seeds, corresponding to about 800 seeds, and put into the
IL Application of isotope methods for assessing the plant available P in acid tropical soils

Agrostis capillaris was chosen because of the relatively low P content of its seeds, of its ability to grow after successive cuts (Truong & Pichot, 1976) and because it has been shown to take up only P that is isotopically exchangeable within the time span of plant growth (Frossard et al., 1994). The pots were covered with a polythene sheet to maintain an adequate humidity. After three days, the plants were exposed to a rhythm of 18 h of light (300 μmol s⁻¹ m⁻²) and 6 h darkness, at a night temperature of 18 °C and a day temperature of 22 °C. The study included five replicates per soil and treatment. The totally random distribution of the pots in the growth chamber was rotated every three days. The pots were weighed and watered daily to readjust humidity to 50 % of the soils’ water holding capacity. Nitrogen (100 mg kg⁻¹ in the form of NH₄NO₃ after the 1st cut and in the form of (NH₄)₂SO₄ after the 2nd cut), S and K (as K₂SO₄, S additionally with the (NH₄)₂SO₄ after the second cut) were applied after each cut at the rate of 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 plant dry matter was weighed after 48 h drying at 80 °C. About 200 mg of the plant material, cut in pieces < 2mm, was calcinated at 550 ° for 4 hours. Plant P content was determined after solubilisation of the ashes in 1 ml of 11.3 M HCl. Aliquots of the samples were diluted and measured using the method of Murphy and Riley (1962). The same method was used for the determination of the seed P content, measuring five seed samples of 100 mg. The plant ³³P content was measured by scintillation counting.

As the L value (mg P kg soil⁻¹) measured after the 1st cut can be strongly affected by the P derived from the seeds (Truong and Pichot, 1976; Frossard et al., 1994), L values were calculated only after the 2nd and 3rd harvest using Equation (2.5) (Sibbesen, 1984):

\[
L_{obs(n)} = \frac{R - \sum_{i=1}^{n-1} r_i}{P_n} + \sum_{i=1}^{n-1} P_i
\]

where R is the total introduced radioactivity (MBq kg soil⁻¹), \( r_n \) is the amount of ³³P (MBq kg soil⁻¹) and \( p_n \) the quantity of ³¹P (mg kg soil⁻¹) in the shoots of the common bentgrass measured at the n-th harvest, respectively. \( \Sigma r_i \) is the sum of radioactivity taken up by the aerial parts of the plants between the 1st and the n-1th cut.
As a part of the P present in the seed was probably still taken up by the common bentgrass after the first cut, the following correction was applied on the L value of the 2nd and 3rd cuts (Truong & Pichot, 1976):

\[ L_{\text{cor}(n)} = L_{\text{obs}(n)} \left( \frac{P_n}{P_n + aP_{\text{seeds}}} \right) \]  

(2.6)

where \( L_{\text{cor}(n)} \) is the corrected L value of the \( n \)th cut, \( L_{\text{obs}(n)} \) the L value calculated of the \( n \)th cut with Equation (2.5) and \( P_{\text{seeds}} \) the P content of 100 mg seeds sown per pot which amounted to 480 µg with a CV of 11.4 %. For the factor \( a \) the value 0.25 was chosen assuming that 25% of the seed P could have been taken up by the plant first in the 2nd cut and then in the 3rd cut.

**Statistical Analysis**

Soils within a given site were compared by ANOVA and Tukey's multiple range test for differences in kinetic exchange parameters, L values and plant parameters of the pot experiment. Student's t-test was used to compare the unfertilized soils (SAV and CasO) from both sites. Linear regression analysis was used to compare L and E values extrapolated for a comparable exchange time, i.e. for 8 and 12 weeks. Isotopic exchange kinetics data (\( r_t/R \)) was extrapolated using \( r_t/R \) measured between 0 and 100 minutes to fit Equation (2.2) using Table Curve (Table Curve 2D, Version 4, SPSS Inc.) non-linear fitting procedure. Measured and extrapolated \( \ln(r_t/R) \) values, as well as E values calculated either from measured \( r_t/R \) values or from \( r_t/R \) values extrapolated according to Fardeau (1996) were compared with paired Student's t-test (at \( P=0.05 \)). The detection limit and the limit of quantification of the two methods used for \( C_p \) determination were calculated according to Wilson (1961), Roos (1962), and Gabriels (1970) using a level of confidence of 95 % (limit of quantification) or 90 % (detection limit).
Results and discussion

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

The total P content was lower in the unfertilized soils sampled from Carimagua compared to the total P measured in the unfertilized soils sampled in Quilichao (Table 2.2), reflecting probably the higher clay content (Reining, 1992) and the lower degree of weathering of the Quilichao soil. The P fertilization was reflected in higher total P contents in the fertilized soils (RGM and CR in Carimagua and CasNP and CasNPK in Quilichao) on both sites in comparison to their non fertilized counterparts (SAV, Cas0, Cas NK) or to GL with had received very little P. Total P\textsubscript{i} 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 equality observed between SP\textsubscript{i} + P\textsubscript{res} and P\textsubscript{i} for CasNPK (Table 2.2) suggests that none of these measurements is perfect and that the results are additionally dependent on the soil type (Condron et al., 1990; O’Halloran, 1993). For the calculation of the E values as for correlation of total P\textsubscript{i} with other parameters, the sum of the P\textsubscript{i} fractions plus residual P (ΣP\textsubscript{i} + P\textsubscript{res}) was used.

Isotopic exchange parameters

Radioactivity remaining in the solution (r/R)

The decrease of radioactivity remaining in the solution (r/R) until 4 to 5 weeks in comparison to the r/R values extrapolated with the two described procedures is shown in the Figures 2.1 and 2.2. The measured r/R values decreased until 4 or 5 weeks for all fertilized soils (CR, RGM, CasNPK and CasNP) while r/R reached a plateau after 2 weeks of isotopic exchange in the non-fertilized SAV soil.

The r/R values extrapolated according to Fardeau (1996) with Equation (2.2) decreased until four or five weeks for all soils except SAV where the 3 last extrapolated r/R values were identical. The comparison of the extrapolated with the measured values showed significant differences, at least for one sampling time, in all soils, with exception of GL and CasNPK (Figures 2.1 and 2.2).
Table 2.2 Amount of P extracted with a selection of methods from the soils sampled at the Carimagua and Quilichao sites.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Bray II P</th>
<th>Resin P</th>
<th>P$_{1\text{H}_2\text{SO}_4}$</th>
<th>$\Sigma P_1 + P_{\text{res}}$</th>
<th>$\Sigma P_1$</th>
<th>$P_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carimagua:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>0.9 dB</td>
<td>0.3 cB</td>
<td>12 dB</td>
<td>105 bB</td>
<td>61 bB</td>
<td>175 cB</td>
</tr>
<tr>
<td>GL</td>
<td>2.0 c</td>
<td>0.5 c</td>
<td>30 c</td>
<td>115 b</td>
<td>72 b</td>
<td>213 c</td>
</tr>
<tr>
<td>CR</td>
<td>17.2 b</td>
<td>3.9 b</td>
<td>98 b</td>
<td>220 a</td>
<td>171 a</td>
<td>293 b</td>
</tr>
<tr>
<td>RGM</td>
<td>35.5 a</td>
<td>8.3 a</td>
<td>156 a</td>
<td>255 a</td>
<td>208 a</td>
<td>376 a</td>
</tr>
</tbody>
</table>

ANOVA: *** *** *** *** *** ***

Quilichao:

<table>
<thead>
<tr>
<th>Soil</th>
<th>Bray II P</th>
<th>Resin P</th>
<th>P$_{1\text{H}_2\text{SO}_4}$</th>
<th>$\Sigma P_1 + P_{\text{res}}$</th>
<th>$\Sigma P_1$</th>
<th>$P_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CasO</td>
<td>9.0 cA</td>
<td>3.0 cA</td>
<td>112 dA</td>
<td>415 cA</td>
<td>324 cA</td>
<td>429 cA</td>
</tr>
<tr>
<td>CasNPK</td>
<td>40.6 a</td>
<td>19.3 a</td>
<td>361 a</td>
<td>671 a</td>
<td>587 a</td>
<td>671 a</td>
</tr>
<tr>
<td>CasNK</td>
<td>14.0 b</td>
<td>4.5 c</td>
<td>180 c</td>
<td>410 c</td>
<td>336 c</td>
<td>486 c</td>
</tr>
<tr>
<td>CasNP</td>
<td>40.1 a</td>
<td>15.8 b</td>
<td>333 b</td>
<td>613 b</td>
<td>532 b</td>
<td>594 b</td>
</tr>
</tbody>
</table>

ANOVA: *** *** *** *** *** ***

*** Significant at the 0.001 probability level; 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 CasO) in one column followed by different upper case letters are significantly different according to Student’s t-test (P<0.05).

a Inorganic P extracted with 1 M H$_2$SO$_4$

b Inorganic P measured as the sum of the P$_1$ extracted with the modified Hedley sequential fractionation procedure with resin, NaHCO$_3$, NaOH, HCl plus the total residual P extracted with concentrated H$_2$SO$_4$ and H$_2$O$_2$.

c Inorganic P measured as the sum of the P$_1$ extracted with the modified Hedley sequential fractionation procedure without the residual P.

d Total P extracted with concentrated H$_2$SO$_4$ and H$_2$O$_2$. 

II. Application of isotope methods for assessing the plant available P in acid tropical soils

Figure 2.1 Decrease of radioactivity ($r_v/R$) in the soil solution of the soils sampled in Carimagua. Comparison between measured values, $\vee$; values modelled as proposed by Fardeau (1996), $\bullet$; and values derived from the non-linear fitting procedure, $\blacklozenge$. Values show means ± standard error for $\bullet$ and $\vee$. * or ** show significant differences, at the 0.05 or 0.01 probability level, respectively, between $\bullet$ and $\vee$. 

measured values
\bullet modelled according to Fardeau (1996)
\blacklozenge derived from non-linear fitting procedure

log($r_v/R$)

log(t) (minutes)
Figure 2.2 Decrease of radioactivity ($r/R$) in the soil solution of the soils sampled in Quilichao. Comparison between measured values, $\nabla$; values modelled as proposed by Fardeau (1996), •; and values derived from the non-linear fitting procedure, ♦. Values show means ± standard error for • and $\nabla$. * or ** show significant differences, at the 0.05 or 0.01 probability level, respectively, between • and $\nabla$. 
Figure 2.3 Extrapolation of the isotopic exchange until 5 weeks for SAV and CR, by fitting Equation (2.2) to the measured \( r/R \) values between 0 and 100 minutes. Measured values used for extrapolation are shown as rectangles, measured values for times > 100 minutes as circles. Dashed lines show the 95% confidence limits.
The curve fitted with the non-linear fit using data obtained between 0 and 100 minutes were generally lower than the measured values for the Carimagua soils, whereas they were higher than the measured values for CasNPK and similar for the other Quilichao soils. The confidence intervals for the fitted equation parameters (Table 2.3) were broad and show that there was no significant difference between modelled or measured $r_t/R$ values (Figure 2.3). The low accuracy of this extrapolation can be explained by the difficulty to determine the three parameters of Equation (2.2) by fitting it to only four experimental values assessed for $t<100$ minutes.

The comparison of the parameters characterising the kinetics of radioactivity decrease according to Equation (2.2) ($r_t/R$, $n$, and $r_\infty/R$) either calculated as proposed by Fardeau (1996) or estimated by a non-linear fitting procedure using the $r_t/R$ values measured between 0 and 100 minutes (Table 2.3) shows that $r_t/R$ estimates were identical with both determinations and that $n$ tended to be lower (Carimagua soils) or higher (Quilichao) when calculated according to Fardeau (1996) in comparison to the estimation from the non-linear fitting procedure. For the only soil (SAV), for which the experimental $r_t/R$ values reached a clear plateau, and therefore where the measured $r_{4\text{weeks}}/R$ can be assumed to correspond to $r_\infty/R$, this measured value was 5 times higher than the calculated $r_\infty/R$, according to Equation (2.3). This suggested underestimation of the calculated $r_\infty/R$ can be accounted for by one or a combination of the following reasons: i) the determined total $P_i$ is not totally isotopically exchangeable, ii) an underestimation of $C_P$, iii) the specific sorption of a portion of the added radioactivity, iv) an overestimation of total $P_i$. Soil microorganisms activity during such a long-term incubation might also have affected the measured $r_\infty/R$ through uptake and release of $^{33}P$ (Oberson et al., 2001).

Frossard et al. (1994) used the amount of $P_i$ extracted with 1 M H$_2$SO$_4$ as an estimation of total $P_i$ to calculate the parameter $r_\infty/R$ according to Equation (2.3). For the soils used in the present study the soil $P_i$ calculated as the sum of the $P_i$ pools obtained from a sequential extraction either with or without the residual $P$ was much higher ($P<0.0001$) than the amount of $P_i$ extracted by 1 M H$_2$SO$_4$ (Table 2.2). This comparison shows that the use of $P_i$ extracted by 1 M H$_2$SO$_4$ would result in a higher $r_\infty/R$. This means that, although the $P_i$ extracted with 1 M H$_2$SO$_4$ is very probably only a part of total $P_i$ the $r_\infty/R$ value calculated
Table 2.3 Estimation of the parameters used in the Equation (2.2): $\frac{r_t}{R} = \frac{r_1}{R} \left[ t + \left( \frac{R_1}{R} \right)^{\frac{1}{n}} \right] + \frac{r_0}{R}$ either using the approach proposed by Fardeau (1996) or a non-linear fitting procedure (fit of the model to the experimental data for $0 \leq t \leq 100$ was in all soils $r^2=0.99$) in the soil samples taken at the Carimagua and Quilichao sites.

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>Parameters calculated according to Fardeau (1996)</th>
<th>Parameters determined from a non-linear fitting of Equation (2.2) to the experimental data for $0 \leq t \leq 100$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_1/R$ $N$ $r_0/R$ $(=10C_\rho/(\Sigma P + P_{res}))$</td>
<td>$r_1/R$ $n$ $r_0/R$ $(/\pm$ 95% Confidential limit)</td>
</tr>
<tr>
<td>Carimagua:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>0.02c 0.51a $1 \times 10^{-4}$c</td>
<td>0.02 0.57 $2 \times 10^{-4}$ $(0.014/0.023)$ $(0.45/0.69)$ $(-1 \times 10^{-3}/1 \times 10^{-3})$</td>
</tr>
<tr>
<td>GL</td>
<td>0.03b 0.43b $3 \times 10^{-4}$b</td>
<td>0.03 0.45 $1 \times 10^{-4}$ $(0.027/0.028)$ $(0.42/0.49)$ $(-5 \times 10^{-4}/7 \times 10^{-4})$</td>
</tr>
<tr>
<td>CR</td>
<td>0.03b 0.41b $3 \times 10^{-4}$b</td>
<td>0.03 0.44 $1 \times 10^{-4}$ $(0.029/0.031)$ $(0.39/0.48)$ $(-8 \times 10^{-4}/1 \times 10^{-3})$</td>
</tr>
<tr>
<td>RGM</td>
<td>0.04a 0.41b $5 \times 10^{-4}$a</td>
<td>0.04 0.44 $2 \times 10^{-4}$ $(0.041/0.046)$ $(0.38/0.50)$ $(-1 \times 10^{-3}/2 \times 10^{-3})$</td>
</tr>
<tr>
<td>ANOVA</td>
<td>*** *** ***</td>
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</table>
continuation Table 2.3

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<tbody>
<tr>
<td></td>
<td>Quilichao:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cas0</td>
<td>0.02d</td>
<td>0.46a</td>
<td>5 x 10^{-5}c</td>
<td>0.02</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>CasNPK</td>
<td>0.05a</td>
<td>0.38c</td>
<td>4 x 10^{-4}a</td>
<td>0.05</td>
<td>0.29</td>
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<td></td>
</tr>
<tr>
<td>CasNK</td>
<td>0.03cd</td>
<td>0.43ab</td>
<td>1 x 10^{-4}b</td>
<td>0.03</td>
<td>0.42</td>
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<tr>
<td>CasNP</td>
<td>0.04bc</td>
<td>0.39bc</td>
<td>5 x 10^{-4}a</td>
<td>0.04</td>
<td>0.34</td>
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<td>***</td>
<td>***</td>
<td>***</td>
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</tbody>
</table>

*** Significant at the 0.001 probability level, means followed by the same letter are not significantly different (P<0.05) according to Tukey’s test
with this amount of $P_i$ according to Equation (2.3) would be closer to the measured $r_i/R$ for SAV.

The $r_i/R$ values, fitted or measured, were in all cases lower than 0.2, showing that the studied soils had a high to very high $P$ sorbing capacity (Fardeau, 1996). Although the soils from Quilichao had a higher total $P_i$ content than the soils from Carimagua, the $r_i/R$ values of the unfertilized soils from the two sites were not statistically different. This indicates a higher $P$ sorption capacity for the Quilichao soils, which could be due to higher concentrations in $Fe_d$ and in $Al_{ox}$ in the soils from Quilichao (Table 2.1). For each site a positive linear correlation (Carimagua: $r^2=0.52$, $P=0.008$, Quilichao: $r^2=0.72$, $P=0.001$) was found between total $P_i$ and $r_i/R$ confirming that $P$ fertilization with water soluble fertilizer results in an increase of the $r_i/R$ value. The relations between $n$ calculated according to Fardeau (1996) and total $P_i$ observed in each site were not significant. However for each soil the $n$ values were significantly higher in the absence of $P$ fertilization in comparison to values of the regularly fertilized soils (Table 2.3).

Concentration of $P$ in the soil solution ($C_p$)

With the Murphy and Riley method using the 4 cm cell, the limit of quantification (95% probability level) was determined as 4.2 $\mu g$ P l$^{-1}$ and with the malachite green method using the 1 cm cell as 3.6 $\mu g$ P l$^{-1}$. The detection limit (90% probability level) was 0.9 $\mu g$ P l$^{-1}$ or 1.3 $\mu g$ P l$^{-1}$ for the malachite green and the Murphy and Riley method, respectively. The concentration in the solution samples, which were evaporated from 10 to 2 ml and measured with the malachite green method did not differ significantly from the $C_p$ directly measured, with exception of RGM where the directly measured concentration was higher (Table 2.4, $P<0.05$). There were no significant differences between the concentrations measured with malachite green or Murphy and Riley, respectively. The malachite green method can however be used with smaller volumes in the 1 cm photometer cell. This is an important criterion, as it is difficult to gain large volumes of soil solution using the 0.025 $\mu m$ filters. Additionally, the coefficients of variation were lower for the malachite green than for the Murphy and Riley method (Table 2.4). Generally, the coefficients of variation
indicated a higher variability of the results than reported for other isotopic exchange studies (Morel et al., 1994; Sinaj et al., 1997). All $C_p$ values determined with both methods lay above the detection limit, but below the limit of quantification for SAV, GL, Cas0 and CasNK. The $C_p$ values determined with malachite green, using the 1/5 concentrated solutions, were used for the calculations of the $E$ values.

Although the non-fertilized soil from Quilichao had a higher total inorganic $P$ content, its $C_p$ was not statistically different (t-Test at $P<0.05$) from that measured in the non-fertilized Carimagua soil (SAV). This can be explained by the higher $P$ sorption capacity of the Quilichao soils, for example due to higher $Fe_{ox}$ and $Al_{ox}$ contents. $C_p$ values were positively linearly correlated with the total $P_i$ content of each soil (Carimagua: $r^2=0.82$, $P<0.001$, Quilichao: $r^2=0.94$, $P<0.001$).

$E_t$ values

The $E_t$ values calculated from the experimental data ($C_p$, $r/R$) after 4 to 5 weeks of isotopic exchange varied from 26 mg P kg$^{-1}$ in SAV to 121 mg P kg$^{-1}$ in RGM and from 71 mg P kg$^{-1}$ in Cas0 to 242 mg P kg$^{-1}$ in CasNPK. The $E_t$ values calculated from the experimental data might have been affected by errors in the determination of $C_p$ (Frossard et al., 1994), the specific sorption of a fraction of the added radioactivity (Amer et al., 1969; Wolf et al., 1986) or the influence of microbial processes (Oberson et al., 2001). As shown above, slight errors in $C_p$ determinations can not be ruled out in SAV, GL, Cas0 and CasNK. Small variations on $C_p$ will however have a large effect on $E_{5\text{weeks}}$. The possible specific sorption of a fraction of added $^{33}P$ can not be disproved by the presented results but seems unlikely. Amer et al. (1969) observed a decrease in radioactive $P$ concentration in the solution of a soil in which increasing rates of iron oxides had been applied prior to measuring isotopic exchangeable $P$. Such an artificial experimental set-up does however not correspond to the conditions of natural soils. Additionally, as the soil/iron mixtures were only shaken for 30 minutes before applying the isotope, the system was hardly in a steady-state condition. The total quantity of radioactivity introduced in our experiment corresponded to 39 kBq per ml soil solution i.e. to a mass of about $1.13 \times 10^{-5}$ $\mu g P$ ml$^{-1}$ soil solution (Amersham product specification, July 2000).
Table 2.4 Concentration of P in the soil solution (C_p) measured within the first two hours of isotopic exchange batch experiment and amount of P isotopically exchangeable within one minute in the soils sampled at the Carimagua and Quilichao sites.

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>E_l</th>
<th>Malachite green method</th>
<th>Murphy and Riley</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg kg(^{-1})</td>
<td>(1:5)(^a)</td>
<td>(1:1)(^b)</td>
</tr>
<tr>
<td>Carimagua:</td>
<td></td>
<td>mg l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>0.7c</td>
<td>0.002 (19.0)</td>
<td>0.001 (27.4)</td>
</tr>
<tr>
<td>GL</td>
<td>1.1c</td>
<td>0.003 (6.0)</td>
<td>0.003 (15.3)</td>
</tr>
<tr>
<td>CR</td>
<td>2.0b</td>
<td>0.006 (3.4)</td>
<td>0.006 (5.1)</td>
</tr>
<tr>
<td>RGM</td>
<td>3.2a</td>
<td>0.014 (3.1)</td>
<td>0.02 (7.8)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Quilichao:</td>
<td></td>
<td>mg l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Cas0</td>
<td>0.9c</td>
<td>0.002 (17.5)</td>
<td>0.002 (15.3)</td>
</tr>
<tr>
<td>CasNPK</td>
<td>5.9a</td>
<td>0.03 (3.5)</td>
<td>0.03 (4.1)</td>
</tr>
<tr>
<td>CasNK</td>
<td>1.0c</td>
<td>0.003 (13.2)</td>
<td>0.002 (13.2)</td>
</tr>
<tr>
<td>CasNP</td>
<td>4.4b</td>
<td>0.017 (4.2)</td>
<td>0.02 (5.1)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*** significant at the 0.001 probability level, means followed by the same or no letter (lower case in columns for soil comparison within one site, upper case in rows for comparison of C_p-values) are not significantly different (P<0.05) according to Tukey's test, coefficient of variation in parenthesis. There were no significant differences between the non fertilized soils of the two sites for all parameters.

\(a\) determined with malachite green method, using solutions concentrated by evaporation from 5:1 or

\(b\) without concentration step

\(c\) determined with the method of Murphy and Riley (1962) method using solutions concentrated by evaporation from 5:1
This is at least 50 times lower than the concentration of $^{31}$P present in the solution of poorest soils (SAV, CasO). As the addition of $^{33}$P has not perturbed the P steady-state equilibrium and since $^{33}$P and $^{31}$P have the same fate in the system (Fardeau, 1996), we consider the specific sorption of $^{33}$P onto soil particles as unlikely. Although toluene has been added at the beginning of the experiment, microbial activity might also have affected the $E_i$ calculation through the cycles of P ($^{33}$P and $^{31}$P) uptake and mineralisation. However, in soil water suspensions to which no sources of C and N are added, it can be assumed that these microbial processes reach on the long term an equilibrium and that after 4 to 5 weeks they do not have a major influence on the determination of the $E_i$ values (Oehl et al., 2001b).

In all soils except GL and CasNPK, the $E_i$ values calculated from the experimental data after 4 to 5 weeks of exchange were at least at one sampling date significantly different ($P<0.001$) from the extrapolated values using the parameters determined according to Fardeau (1996) in Equation (2.2) (Figures 2.4 and 2.5). In SAV, the extrapolated $E_{5\text{weeks}}$ value was twice as large as the $E_{5\text{weeks}}$ value derived from measured $r/R$ and $C_p$ values. Furthermore, the $E$ value extrapolated to 4 weeks according to Fardeau (1996) was higher 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, show that SAV contained much less available P than GL. Therefore, the extrapolation overestimates $E_{5\text{weeks}}$ for SAV. This demonstrates that the difference between measured and extrapolated values can also result from errors made in estimating $n$ and $r_{\infty}/R$ using Equation (2.2).

The $E$ values deduced from the non-linear regression of the $r/R$ measured between 0 and 100 minutes and extrapolated to 4 to 5 weeks resulted in values higher than the values calculated from measured values for the Carimagua soils (Figure 2.4) or almost equal (CasO, CasNK and CasNP) or lower (CasNPK) for the Quilichao soils (Figure 2.5), but are all not significantly different due to the broad confidence intervals, as shown previously for $r/R$. Consequently, due to the low precision, the extrapolation of experimental data for $t \leq 100$ minutes using this method can not be applied to calculate $E_i$-values.

The calculated $E_i$ values (Table 2.4) are in the range of values already published for similar soils (Salcedo et al., 1991). For the Carimagua soils, all $E_i$ values lay below the
value of 5 mg P kg\(^{-1}\) regarded as the limit under which P becomes limiting for plant growth (Tran et al., 1988). For the soils from Quilichao, the \(E_1\) of the P fertilized treatments (CasNP and CasNPK) reached values close to 5 mg P kg\(^{-1}\) whereas the treatments, which had not received any P fertilization, had \(E_1\) values close to 1 mg P kg\(^{-1}\). \(E_1\) values were for each site positively linearly correlated to the total P content, to the resin extractable P content, and to the Bray II P content (Table 2.5). The same linear correlation was found for \(E_{4weeks}\) or \(E_{5weeks}\), either measured or extrapolated according to Fardeau (1996), with P\(_i\), Bray II and resin P (Table 2.5). As \(E_{4weeks}\) or \(E_{5weeks}\) for a given soil do not differ much, the correlation for the measured E values could be calculated including both values (i.e. \(E_{5weeks}\) for SAV and RGM and \(E_{4weeks}\) for all other soils).

Table 2.5 Coefficients of determination \((r^2)\) of the linear regression between \(E_1\) values, E values derived from the experimental parameters \((E_{4 or 5weeks} measured)\), E values calculated as proposed by Fardeau (1996) \((E_{4weeks} calculated parameters)\), E values derived from the non-linear fitting procedure \((E_{4weeks} fitted parameters)\) and the amount of P extracted by various methods in the soils sampled at the Carimagua and Quilichao sites.

<table>
<thead>
<tr>
<th>site</th>
<th>Value</th>
<th>(\Sigma P_i + P_{res}^a)</th>
<th>Bray II P</th>
<th>Resin P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carimagua</td>
<td>(E_1)</td>
<td>0.97***</td>
<td>0.97***</td>
<td>0.97***</td>
</tr>
<tr>
<td></td>
<td>(E_{4 or 5weeks} measured)</td>
<td>0.91***</td>
<td>0.73***</td>
<td>0.71**</td>
</tr>
<tr>
<td></td>
<td>(E_{4weeks} calculated parameters)</td>
<td>0.87***</td>
<td>0.96***</td>
<td>0.96***</td>
</tr>
<tr>
<td></td>
<td>(E_{4weeks} fitted parameters)</td>
<td>0.88***</td>
<td>0.97***</td>
<td>0.97***</td>
</tr>
<tr>
<td>Quilichao</td>
<td>(E_1)</td>
<td>0.86***</td>
<td>0.81***</td>
<td>0.85***</td>
</tr>
<tr>
<td></td>
<td>(E_{4 or 5weeks} measured)</td>
<td>0.97***</td>
<td>0.99***</td>
<td>0.99***</td>
</tr>
<tr>
<td></td>
<td>(E_{4weeks} calculated parameters)</td>
<td>0.89***</td>
<td>0.94***</td>
<td>0.94***</td>
</tr>
<tr>
<td></td>
<td>(E_{4weeks} fitted parameters)</td>
<td>0.76**</td>
<td>0.63*</td>
<td>0.63*</td>
</tr>
</tbody>
</table>

***,** Significance at the 0.05, 0.01 or 0.001 probability level, respectively

\(^a\) determined as the sum of P\(_i\) fractions + residual P of the sequential P fractionation
Excepted for SAV, for which the $E_{4\text{weeks}}$ value extrapolated as proposed by Fardeau (1996) was overestimated, $E_{4\text{weeks}}$ values increased with increasing fertilization. Assuming that the increase in soil total P compared to the non-fertilized soils was solely due to fertilization, increase in the $E_{4\text{weeks}}$ values extrapolated as proposed by Fardeau (1996) in the fertilized soils accounted for 48 and 69% of the increase in soil total P in Quilichao and Carimagua. This result suggests that a significant fraction of the added P fertilizers remained plant available.
Figure 2.4 Changes in the concentration of isotopically exchangeable P (E value) with time in the soils sampled in Carimagua. Comparison between measured values, ▼; values modelled as proposed by Fardeau (1996), ●; and values derived from the non-linear fitting procedure, ◆. Values show means ± standard error for ● and ▼. * or ** show significant differences, at the 0.05 or 0.01 probability level, respectively, between ● and ▼.
Figure 2.5 Changes in the concentration of isotopically exchangeable P (E value) with time in the soils sampled in Quilichao. Comparison between measured values, $\nabla$; values modelled as proposed by Fardeau (1996), $\bullet$; and values derived from the non-linear fitting procedure, $\blacklozenge$. Values show means ± standard error for $\bullet$ and $\nabla$. * or ** show significant differences, at the 0.05 or 0.01 probability level, respectively, between $\bullet$ and $\nabla$. 
Shoot dry weight production and P uptake

Shoot biomass production, P concentrations and resulting total P export by *Agrostis capillaris* were lower in the Carimagua soils than in the soils from Quilichao, and were lower in the soils which had not been fertilized with P or which received very little P than in the soils which had received a regular P fertilization (Table 2.6). After the second cut, plant re-growth was very low on SAV (Table 2.6). Biomass production decreased from the second to the third cut on all soils with exception of GL. On this soil the biomass in the third cut was almost the double of the second cut. Plant P concentrations ranged between 0.3 mg g\(^{-1}\) (SAV, 2\(^{nd}\) cut) and 2.2 mg g\(^{-1}\) (CasNP, 3\(^{rd}\) cut). Phosphorus concentrations in temperate grasses below 2 mg P g\(^{-1}\) plant dry matter indicate P deficiency (Mays et al., 1980). Therefore, with exception of the fertilized soils from Quilichao (CasNPK and CasNP), with plant P concentrations above this limit in the second cut, the P supply was limiting growth of *Agrostis capillaris* on all soils. Total P export in shoots during the entire pot experiment ranged between 0.13 mg in SAV, reaching merely one quarter of the P applied in the seeds (0.48 mg), and 16.4 mg in CasNPK.

Total P uptake was linearly positively and highly significantly (P<0.001 for all correlations) correlated to resin P (Carimagua \(r^2=0.89\), Quilichao \(r^2=0.94\)), to Bray II P (Carimagua \(r^2=0.90\), Quilichao \(r^2=0.87\)), to total P (Carimagua \(r^2=0.99\), Quilichao \(r^2=0.94\)), to \(E_t\) (Carimagua \(r^2=0.93\), Quilichao \(r^2=0.93\)), to \(E_{4\text{weeks}}\) or \(E_{5\text{weeks}}\) derived from measured \(r/R\) after 4 or 5 weeks of isotopic exchange (Carimagua \(r^2=0.91\), Quilichao \(r^2=0.99\)) and to \(E_{4\text{weeks}}\) extrapolated according to Fardeau (1996) (Carimagua \(r^2=0.85\), Quilichao \(r^2=0.94\)).

**L-values**

In the 2\(^{nd}\) cut the uncorrected L value (\(L_{\text{obs}}\), Table 2.7) ranged between 42 mg P kg\(^{-1}\) in GL and 198 mg P kg\(^{-1}\) in Cas NPK. SAV had the highest \(L_{\text{obs}}\) values of the Carimagua soils in the 2\(^{nd}\) cut. Since other analyses showed that SAV had the lowest P availability, the \(L_{\text{obs}}\) value overestimated P availability in this soil. For the Quilichao soils, the ranking of the \(L_{\text{obs}}\) results was consistent with the other P analyses.
Table 2.6 Shoot dry weight, P concentration and total P export in shoots for three cuts of *Agrostis capillaris* in the soils sampled at the Carimagua and Quilichao sites

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>first cut (after 1 month)</th>
<th>second cut (after two months)</th>
<th>Third cut (after 3 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry weight</td>
<td>P concentration</td>
<td>P export</td>
</tr>
<tr>
<td></td>
<td>mg g pot&lt;sup&gt;1&lt;/sup&gt;</td>
<td>mg g plant dry matter&lt;sup&gt;1&lt;/sup&gt;</td>
<td>mg</td>
</tr>
<tr>
<td>Carimagua:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>0.17cB</td>
<td>0.4cB</td>
<td>0.07cB</td>
</tr>
<tr>
<td>GL</td>
<td>0.17c</td>
<td>0.6c</td>
<td>0.11c</td>
</tr>
<tr>
<td>CR</td>
<td>0.3b</td>
<td>1.1b</td>
<td>0.4b</td>
</tr>
<tr>
<td>RGM</td>
<td>0.6a</td>
<td>1.6a</td>
<td>1.0a</td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Quilichao:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CasO</td>
<td>1.0dA</td>
<td>1.0bA</td>
<td>1.0cA</td>
</tr>
<tr>
<td>CasNPK</td>
<td>2.4a</td>
<td>1.7a</td>
<td>4.2a</td>
</tr>
<tr>
<td>CasNK</td>
<td>1.2c</td>
<td>1.1b</td>
<td>1.3c</td>
</tr>
<tr>
<td>CasNP</td>
<td>2.1b</td>
<td>1.3b</td>
<td>2.7b</td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

**,** *** Significant at the 0.01 or 0.001 probability level, respectively, means within one column of one site followed by the same lower case letter are not significantly different according to Tukey's test \((P<0.05)\), means of the unfertilized soils (SAV and CasO) followed by different upper case letters are significantly different according to the two-samples Student's t-test \((P<0.05)\).  

<sup>a</sup> Total P export in shoots per pot  
<sup>b</sup> Sum of the three cuts
Table 2.7 L-values calculated with or without seed P correction at the second and third cut of *Agrostis capillaris* in the soils sampled at the Carimagua and Quilichao sites

<table>
<thead>
<tr>
<th>Soil and site</th>
<th>Second cut</th>
<th>Third cut</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L_{obs}^{a}$</td>
<td>$L_{th0.25}^{b}$</td>
</tr>
<tr>
<td>Carimagua:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>143cA</td>
<td>38aB</td>
</tr>
<tr>
<td>GL</td>
<td>42aA</td>
<td>31aB</td>
</tr>
<tr>
<td>CR</td>
<td>91b</td>
<td>87b</td>
</tr>
<tr>
<td>RGM</td>
<td>118c</td>
<td>114c</td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Quilichao:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CasO</td>
<td>95a</td>
<td>92a</td>
</tr>
<tr>
<td>CasNPK</td>
<td>198c</td>
<td>194c</td>
</tr>
<tr>
<td>CasNK</td>
<td>92a</td>
<td>89a</td>
</tr>
<tr>
<td>CasNP</td>
<td>149b</td>
<td>146b</td>
</tr>
<tr>
<td>ANOVA:</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*** Significant at the 0.001 probability level, means within columns of one site followed by the same lower case letter are not significantly different according to Tukey's test ($P<0.05$), different upper case letters for $L_{obs}$ and $L_{th0.25}$ for one soil and cut show significant difference according to Student's t-test.

a $L_{obs}$: L-value calculated without correction for seed P

b $L$ value corrected with the assumption of 25 % ($L_{th0.25}$) seed P uptake in the respective cut

The assumption of 25 % uptake of total seed P in the second cut resulted in a decrease of the L value ranging between 2 % (CasNPK) and 73 % (SAV). In the 3rd cut, the uncorrected L value ($L_{obs}$, Table 2.7) ranged between 35 mg P kg$^{-1}$ for GL and 225 mg P kg$^{-1}$ for...
CasNP. Significant decreases ($P<0.001$) were observed in the $L_{obs}$ values of SAV and GL between the 2nd and 3rd cut whereas these values should either be constant or increase (as in the other studied soils) since the radioactive P gets diluted in increasing amounts of P with time. The assumption of 25 % of seed P uptake in the third cut leads to a reduction of the $L$ value of less than 5 % for all soils, with the exception of SAV (89 %) and GL (16 %). This shows that the seed P correction, at least at the third cut, has little impact on L values and that L values calculated without correction should not be affected by errors > 5 % due to seed effect on all soils, except SAV and GL. Thus, on these soils (SAV and GL) an accurate L value determination with *Agrostis capillaris* is not possible.

$L_{th0.25}$ values observed in the 3rd cut logically increased with P fertilization. Assuming that the increase in soil total P compared to the non-fertilized soils was solely due to fertilization, the increase in the $L_{th0.25}$ values accounted for 50 and 73% of the increase in soil total P in Quilichao and Carimagua. This result is similar to that obtained with $E_{4weeks}$ values and suggests that a significant fraction of the added P fertilizers remains plant available.

For each site, the corrected L values are positively linearly correlated with plant parameters (plant dry matter and log P concentration) at the second and the third cuts (data not shown). $L_{obs}$ at the third cut was positively correlated for both sites to resin P (if not mentioned, $P<0.001$) (Carimagua: $r^2=0.93$, Quilichao: $r^2=0.88$), to Bray II P (Carimagua: $r^2=0.93$, Quilichao: $r^2=0.90$), to total P (Carimagua: $r^2=0.89$, Quilichao: $r^2=0.85$), to $E_1$ (Carimagua: $r^2=0.88$, Quilichao: $r^2=0.86$) and to $E_t$ derived from measured $r_t/R$ for 4 or 5 weeks (Carimagua: $r^2=0.65$, $P<0.01$, Quilichao: $r^2=0.82$).

**Comparison of E and L values**

Figure 2.6 shows the highly significant correlation between the E values extrapolated with Equation (2.2) with parameters calculated as proposed by Fardeau (1996) to 8 and 12 weeks of isotopic exchange and $L_{th0.25}$ measured after the same isotopic exchange time. The slope of the regression line of 0.91 was not statistically different from 1. The exclusion of SAV and GL, with the most uncertain L values, did not improve the determination of the regression (not shown on figure).
This set of data shows that, with the exception of the CasNK and CasNP in the 3rd cut, almost all soils (including the high P soil CasNPK) had a higher extrapolated E value than the $L_{0.25}$ value. The possible reasons for errors in E and L values in low P soils have been reviewed in the previous sections of this paper. The overestimation of the E value in the P rich CasNPK is difficult to explain, but most likely due to errors in the determination of the

![Graph showing comparison of E-values extrapolated according to Fardeau (1996) and seed P corrected L values measured with Agrostis capillaris for 8 or 12 weeks of isotopic exchange, indicated by the respective number to the soil name, in the soils sampled at the Carimagua and Quilichao sites. The solid line shows the calculated regression with 95% confidence interval shown by dashed lines.](image-url)
equation parameters for extrapolation. In CasNK and CasNP the L values increased strongly from the 2nd to the 3rd cut. The increase in isotopically exchangeable P as deduced by the isotopic exchange kinetics approach can not account for these large increases in L values. Such an increase could be related to the increased release of stable P to the soil solution and uptake by the plant, e.g. due to the mineralisation of organic P.

Conclusions

This study was carried out to ascertain the interest of isotope methods (the isotopic exchange kinetics approach and the L value measurement) in assessing the availability of soil P to plants in tropical acid soils. It was shown that the most reliable results for E values were obtained when the P concentration in solution was higher than the detection and the quantification limits of the malachite green method (i.e. for concentration higher than 3.6 µg P l⁻¹). The E values can be extrapolated according to the Equation (2.2) provided that the approach suggested by Fardeau (1996) for the estimation of the parameters is followed. An extrapolation of the short time kinetic using a non linear fitting procedure was shown to result in very low precision of the extrapolated values and can not be recommended.

The most reliable L values results were obtained in soils where the soil P availability was large enough to enable a higher P uptake from soil in comparison to the P uptake from seeds, at least in the third cut. E and L values were strongly linearly positively correlated when measured after the same exchange time. Comparison of E values extrapolated after 4 weeks according to the approach by Fardeau (1996) in non fertilized and fertilized soils as well as comparison of L values in non fertilized and fertilized soils, showed similarly that 48 to 73% of the added P fertilizer has remained plant available. In most of the cases however, the E values were higher than L values measured after the same exchange time, and in some cases the contrary was observed. In addition to the experimental problems encountered in measuring C_p or due to seed-P interference, incertitude in n or in r_o/R when calculated as proposed by Fardeau (1996) can be a source of error when extrapolating E values. In very low P soils, as in this study SAV, the term r_o/R has a high impact on the extrapolation of r/R using Equation (2.2) because the isotopic equilibrium is rapidly
II. Application of isotope methods for assessing the plant available P in acid tropical soils

attained. Therefore small errors in the determination of \( r_{\text{e}} / R \) can lead to miscalculation of \( E_t \).

These results show that the studied isotope techniques can be used to coarsely estimate the fraction of P which has been added with fertilizers and which remained available to plants. However, the theoretical adequacy between E values extrapolated as proposed by Fardeau (1996) and L values measured after the same exchange time is not precise enough in these soils. As a consequence, neither E values extrapolated from the isotope exchange kinetics experiment as proposed by Fardeau (1996) nor the L value measured in the presence of a plant using isotopically exchangeable P as its main source of P, can be used as a baseline either to detect small differences between plants in their ability to access slowly exchangeable P forms or to assess organic P mineralisation. Furthermore, it is not advised to use the isotope exchange kinetic technique when the concentration of P in the solution is below 4 \( \mu \text{g} \text{ l}^{-1} \) when using the malachite green colorimetric method.

**Acknowledgements**

I thank the field staff at CORPOICA-CIAT Carimagua and CIAT Quilichao research station for taking soil samples, and Mrs Roesch (ETH Zurich, Institute for Plant Science) for measuring the Al and Fe concentrations. This research was funded by ZIL (Swiss Centre for International Agriculture) and SDC (Swiss Development Co-operation).
CHAPTER III

L values of different crops and forage species on a low P Oxisol: Limits of comparison of L values and influence of P carrier application
Abstract

Through selection and plant breeding, germplasm adapted to low P soils is available, but it is not known whether these plants are able to access P forms which are not isotopically exchangeable and whether different species take up P from different soil P forms. The L value is derived from the isotopic composition of the P taken up by the plant and expresses the total amount of soil P which is potentially plant available. L values were determined on a Colombian Oxisol labelled with $^{33}$PO$_4$ for the forage grass *Brachiaria decumbens*, the forage legume *Arachis pintoi*, and low P adapted rice, maize and bean cultivars in order to investigate their capacities to acquire soil phosphorus from less available, not isotopically exchangeable forms. The experiments was carried out twice, once without and a second time with KH$_2$PO$_4$ carrier application with the label. The L values were compared to the L value of *Agrostis capillaris*, which was used as reference plant without special P uptake mechanisms, and to the E value of the same soil, determined in an isotopic exchange kinetic batch experiment.

A higher L value than E value for *Brachiaria decumbens* suggested P uptake from not isotopically exchangeable forms. For all other plants, the contribution of the seed P to plant P uptake did not allow the calculation of exact L values. Therefore, drawing conclusions about the access of different P forms by different plants was not possible. L values determined with or without carrier P differed widely and suggested that a carrier application is not recommendable on very P limited soils.

Introduction

Phosphorus is the main limiting nutrient for crop and forage production on infertile, acid tropical soils with a high phosphorus fixation capacity. Additionally, it is a limited and non-renewable resource. Often P limitation is not primarily due to low total soil P contents but rather to the restricted P availability. The ability to grow on low P soils differs widely between plant species and even among varieties. These differences have been attributed to several strategies for optimizing P uptake or P use efficiency (Rao et al., 1999a). The
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strategies for P uptake improvement include root system morphology, root hair density, symbiosis with mycorrhizal fungi and modification of the rhizosphere by root exudates or phosphatases to access P forms of low availability. It was also shown that plants differ in their abilities to extract nutrients from low ambient concentrations, i.e., their threshold levels (Itoh, 1987).

A study of Rao et al. (1999b) suggested that *Arachis pintoi* accesses sparingly soluble P sources by high response to fertilization with Al-phosphate, Ca-phosphate or organic phosphate (phytic acid). *Brachiaria* species are reported to be well adapted to low P acid soils with the variety *Brachiaria decumbens* CIAT 606 being planted on over 40 million ha of low P acid soils in Latin America (Rao et al., 1999b). Mainly, the low-P adaptations of *Brachiaria* species are attributed to the extensive fine root system, mycorrhizal association and low internal P requirement (Rao et al., 1997a). Another adaptation consists in the enhanced secretion of phytase under low P conditions as shown for *Brachiaria decumbens* by Li et al. (1997). Infertility of acid tropical soils is caused by multiple stress factors, among others Al-toxicity. Additionally to the adaptation to low P, *Brachiaria decumbens* tolerates high Al-concentrations in the soil solution, shown to result from the intracellular complexation of Al-ions with organic acids (Wenzl et al., 2001). The adaptation of maize cultivars to low-P conditions may be related to several traits, as higher root dry matter and length, higher exudation of citric and malic acids, and enhanced acid phosphatase activity (Gaume et al., 2001). Plant breeders could also improve low-P tolerance for naturally very low-P susceptible crops as beans (Lynch and Beebe, 1995). Plants with special P uptake mechanisms may contribute to more efficient soil P use or could increase the recovery of applied fertilizer if they take up P which is normally not available to other plants. This might reduce the need of high P fertilizer inputs, although on the long-term the use of germplasm with special P uptake mechanisms would lead to soil P mining. A strategy to contribute to agricultural sustainability would be to minimize the P fertilizer requirement needed to produce an economic return through the use of more efficient crop and forage germplasm (Rao et al., 1999c).

Differences between plants in the accessed P forms can be detected by growing plants on soils labelled with radioactive P isotopes and comparison of their isotopic composition. The L value named after Larsen (1952) is derived from the isotopic composition of the P taken
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up by the plant and expresses the total amount of soil P which is potentially plant available. This amount can be compared to the E value of the same soil, which is based on the isotopic composition, measured or extrapolated, in the solution of a labelled soil suspension after a defined time of isotopic exchange. It was shown by Frossard et al. (1994) that, for a large range of soils, L values determined with Agrostis capillaris and E values calculated for the same time of isotopic exchange are not significantly different, and that therefore Agrostis capillaris takes up isotopically exchangeable P. In contrast, significant differences between E values and L values on the same soil for the same time of isotopic exchange are explained by plant P uptake from non-exchangeable P pools. Different L values of different plants on the same soil indicate that these plants do access P pools with different isotopic exchangeability. Several studies compared L values in order to identify plants with special P uptake mechanisms (Russell et al., 1958; Smith, 1981; Braum and Helmke, 1995; Hocking et al., 1997). A higher L value was found for lupine than for soybean by Braum and Helmke (1995) or L values in the order white lupine > pigeon pea > canola, sunflower and wheat > narrow leaf lupine and soybean (Hocking et al., 1997). Higher L values were attributed to special P uptake mechanisms, especially exudation of organic acids, as citric acid in the case of lupine or piscidic, malonic or oxalic acid in the case of pigeon pea.

Smith (1981), on the other hand, found the L values of four tropical pasture legumes and of buffel grass grown on two alkaline clay soils to be equal although the plants differed considerably in the rate of P removal and total P uptake. Seed P is an important factor affecting the isotopic composition of the P taken up by the plant and with this L values, especially under P-limitation and with little plant P uptake. Brookes (1982) suggested to add carrier-P, i.e. a simultaneous $^{31}$P addition with the radioactive label, to increase plant growth and P uptake, with the assumption that L values are independent of the amount of carrier added to the soil (Larsen, 1952; Russell et al., 1957). Besides the effect of enhancing plant growth, the application of carrier with the $^{33}$P label is recommended to avoid the fixation of the label (Amer et al., 1955, Ipinmidun, 1973, Barrow, 1991). Another approach to correct for the influence of seed-P was used by Truong and Pichot (1976), who investigated the utilization of seed P depending on soil P supply for two grasses in sand culture using labelled P solutions of different P concentrations. From their results they established a correction equation for L values considering the relation
between plant P uptake and P seed content.

For our study, crop (a maize, bean and rice variety) and forage germplasm (Arachis pintoi and Brachiaria decumbens) adapted to low P soils were selected to compare their ability for P uptake from sparingly available P pools by comparing their L values. A soil was chosen with very low available P (determined with Bray II) in order to guarantee P limited growth conditions. The L value of Agrostis capillaris was determined as reference assuming that this plant does not access any non isotopically exchangeable P. E values were determined in a batch experiment without carrier application to determine the isotopically exchangeable soil P.

L values were determined in two experiments, one without and one with a P carrier application of 10 mg P kg⁻¹ soil. This amount was chosen as, with the application of the same amount to a similar soil, Brachiaria species and Arachis pintoi increased biomass production and P uptake but remained P limited (Rao et al., 1997a; Rao et al., 1997b).

Materials and Methods

Soil

The soil for this study was sampled in September 1998 at Carimagua (Llanos Orientales, Colombia) ICA-CIAT (Instituto Colombiano Agropecuario- Centro Internacional de Agricultura Tropical) research station. It is a well-drained Oxisol (tropeptic Haplustox, isohyperthermic). Surface soil (0-15 cm) was sampled in a long-term field experiment which was established in 1993 with the objective to test the effect of different farming systems on plant productivity and soil fertility (Friesen et al., 1997). The soil chosen for this study was cultivated as improved grass legume pasture starting with rice in 1993, with under sown pasture. Since then a grass-legume pasture was maintained with Brachiaria humidicola CIAT 679, Centrosema acutifolium cv Vichada CIAT 5277, Stylosanthes capitata CIAT 10280, and Arachis pintoi CIAT 17434. The pasture was partly resown for renovation in June 1996 with legumes (the same Arachis pintoi and Centrosema acutifolium and additionally Stylosanthes guianensis CIAT 11833). At the beginning of the experiment the soil was limed using 500 kg dolomitic lime ha⁻¹. Fertilization of rice was 80
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kg N (urea, divided among three rates), 60 kg P (triple superphosphate), 99 kg K as KCl, 15 kg Mg, 20 kg S (as MgSO₄) and 10 kg Zn ha⁻¹. At renovation in 1996 the pasture received additional fertilization of 20 kg P ha⁻¹. The soil samples were air-dried and sieved at 5 mm before use.

Bray-II P was extracted using dilute acid fluoride (0.03 M NH₄F, 0.1 M HCl) at a 1:7 soil:solution ratio and 40 sec shaking time. Total soil P was determined by digestion with a mixture of 2 parts hot concentrated (15.6 M) HNO₃ and 1 part concentrated (13.7 M) HClO₄, using 5 ml per 0.5 g soil. Total P was determined as the sum of the inorganic fractions of the modified sequential Hedley P fractionation procedure as described by Tiessen and Moir (1993), thus including P sequentially extracted with resin strips charged with HCO₃, 0.5 M NaHC₃, 0.1 M NaOH, and hot concentrated HCl (Buehler et al., 2001a). Soil properties are shown in Table 3.1.

Table 3.1 Soil (0-15 cm) properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>total P, mg kg⁻¹</td>
<td>242</td>
</tr>
<tr>
<td>total P, mg kg⁻¹</td>
<td>86.4</td>
</tr>
<tr>
<td>resin P, mg kg⁻¹</td>
<td>1.5</td>
</tr>
<tr>
<td>Bray-II P, mg P kg⁻¹</td>
<td>3.1</td>
</tr>
<tr>
<td>pH (in H₂O)</td>
<td>4.3</td>
</tr>
<tr>
<td>total C, g kg⁻¹</td>
<td>23.7</td>
</tr>
<tr>
<td>total N, g kg⁻¹</td>
<td>1.6</td>
</tr>
<tr>
<td>aluminum-saturation, %</td>
<td>68</td>
</tr>
<tr>
<td>bulk density, g cm⁻³</td>
<td>1.3</td>
</tr>
</tbody>
</table>

**Determination of isotopically exchangeable P**

E values were determined according to the method of Fardeau (1996), as described in (Buehler et al., 2001b). The procedure is based on the measurement of the specific activity (³¹PO₄/³²PO₄) of phosphate ions in the soil solution after an addition of carrier free ³²PO₄ in
a soil-solution system at steady-state (Fardeau et al., 1991; Frossard et al., 1992). The isotopically exchangeable P (Et) was calculated assuming that, at any given exchange time, the specific activity of phosphate in solution is equal to the specific activity of the phosphate which has been exchanged on the solid phase (Fardeau, 1993):

\[
\frac{r_t}{10^*C_p} = \frac{R}{E_t}
\]

or:

\[
E_t = R \times \frac{10^*C_p}{r_t}
\]

and \( r_t/R \) is extrapolated as:

\[
\frac{r_t}{R} = \frac{r_t}{R} \left[ t + \frac{r_t}{R} \left( \frac{1}{n} \right) \right]^{-n} + \frac{r_\infty}{R}
\]

where \( R \) is the introduced radioactivity in MBq ml\(^{-1}\) and \( r_t \) is the radioactivity remaining in the solution after \( t \) minutes. The other parameters can be determined experimentally (Fardeau, 1993): \( n \) is a parameter calculated as the slope of the linear regression between ln(\( r_t/R \)) and ln(\( t \)) for \( t \leq 100 \) minutes of the measured values, \( r_t/R \) is the interception of the regression when \( t = 1 \), and \( r_\infty/R \) is calculated as:

\[
\frac{r_\infty}{R} = 10^* \frac{C_p}{P_t}
\]

where \( P_t \) is total inorganic P and the ratio \( r_\infty/R \) represents the radioactivity remaining in the soil solution at infinite time. The results, taken over from the analysis in chapter II, of the isotopic exchange are given in Table 3.2.
Table 3.2 Parameters of isotopic exchange of the used soil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>r_i/R^a</td>
<td>0.03</td>
</tr>
<tr>
<td>c_p</td>
<td>0.003 mg l^{-1}</td>
</tr>
<tr>
<td>n</td>
<td>0.43</td>
</tr>
<tr>
<td>E_1</td>
<td>1.1 mg kg^{-1}</td>
</tr>
<tr>
<td>E_{8weeks}</td>
<td>64 mg kg^{-1}</td>
</tr>
</tbody>
</table>

^a ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1 minute of isotopic exchange

^b P concentration in the soil solution measured at soil:water ratio 1:10

^c parameter of isotopic exchange describing the decrease of radioactivity in the soil solution

^d quantity of P exchangeable within 1 minute or within 8 weeks (calculated with Equation 3.3)

L value determination

The experimental conditions of the two pot experiments carried out to determine L values are summarized in Table 3.3 The cultivars used were Brachiaria decumbens (CIAT 606), Arachis pintoi (CIAT 18744) and rice (Oryza sativa var Savanna-6) in experiment one. Additionally beans (Phaseolus vulgaris AFR 475) and maize (Zea mays NST 90201(s) co-422-2-3-1-7-2-1) were used in the second experiment. This inbred line was derived from a triple hybrid developed by the Thai Department of Agriculture and selected as tolerant to low-P conditions. It was shown to exudate more organic acids and to have higher activity of acid phosphatase than non low P tolerant cultivars (Gaume et al., 2001). All these cultivars were chosen following the recommendation of CIAT plant breeders as being selected for acid soils especially low in P availability. In both cases common bentgrass (Agrostis capillaris) was grown as control plant without adaptation to low P conditions (Frossard et al., 1994).

Before labelling, the soil was incubated for two weeks at 50 % of water holding capacity
### Table 3.3 Experimental conditions in pot experiment 1 and 2 for determination of L values

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>plant species, quantities of soil and plants per pot</td>
<td><em>Arachis pintoi</em>, 2 kg soil, 2 plants</td>
<td><em>Arachis pintoi</em>, 0.9 kg soil, 1 plant</td>
</tr>
<tr>
<td></td>
<td><em>Brachiaria decumbens</em>, 2 kg soil, 2 plants</td>
<td><em>Brachiaria decumbens</em>, 0.9 kg soil, 2 plants</td>
</tr>
<tr>
<td></td>
<td>Rice, 2 kg soil, 2 plants</td>
<td>Rice, 0.9 kg soil, 2 plants</td>
</tr>
<tr>
<td></td>
<td><strong>Beans</strong>, 0.9 kg soil, 1 plant</td>
<td><strong>Beans</strong>, 0.9 kg soil, 1 plant</td>
</tr>
<tr>
<td></td>
<td><strong>Maize</strong>, 3.4 kg soil, 1 plant</td>
<td><strong>Maize</strong>, 3.4 kg soil, 1 plant</td>
</tr>
<tr>
<td></td>
<td><em>Agrostis capillaris</em>, 500 g, 100 mg seeds</td>
<td><em>Agrostis capillaris</em>, 400 g soil, 100 mg seeds</td>
</tr>
<tr>
<td>labelling</td>
<td>$^{32}\text{PO}_4$, 5.2 MBq kg$^{-1}$ soil</td>
<td>$^{33}\text{PO}_4$, 3.7 MBq kg$^{-1}$ soil</td>
</tr>
<tr>
<td>carrier</td>
<td>none</td>
<td>10.26 mg P as KH$_2$PO$_4$ kg$^{-1}$ soil, applied with labelling solution</td>
</tr>
<tr>
<td>replicates</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>location</td>
<td>greenhouse, CIAT, Colombia</td>
<td>Biotron, ETH, Switzerland</td>
</tr>
<tr>
<td>experimental conditions</td>
<td>maximum photosynthetic photon flux density during the day 1100 μmol s$^{-1}$ m$^{-2}$</td>
<td>16 h daylight, photon flux density ~ 300 μmol s$^{-1}$ m$^{-2}$</td>
</tr>
<tr>
<td>temperature</td>
<td>38/20 °C (max/min d/n, over whole growth period)</td>
<td>24/20 °C (constant)</td>
</tr>
<tr>
<td>humidity</td>
<td>90/40 % (max/min)</td>
<td>65 % (constant)</td>
</tr>
<tr>
<td>duration of plant growth:</td>
<td>2 months</td>
<td>11 weeks</td>
</tr>
</tbody>
</table>
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(=500 mg water kg\(^{-1}\) soil) with N (urea, 41 mg N kg\(^{-1}\) soil), K (KCl, 51 mg K kg\(^{-1}\) soil), S (sulfur powder, 10 mg S kg\(^{-1}\) soil), dolomitic lime (153 mg kg\(^{-1}\), corresponding to 33.7 mg Ca and 15 mg Mg kg\(^{-1}\) soil in the first and 256 mg kg\(^{-1}\) corresponding to 56.3 mg Ca and 25 mg Mg kg\(^{-1}\) in the second experiment) and minor elements. The soil was labelled by adding the quantities of \(^{32}\)PO\(_4\) or \(^{33}\)PO\(_4\) ions indicated in Table 3.3 in 10 ml water to portions of 1.5 kg incubated soil and was thoroughly mixed to ascertain an even distribution of the isotope. *Agrostis* was grown from 100 mg of seeds (corresponding to about 800 seeds) in both experiments, which were sown directly into each pot. All other plants were pregerminated on filter paper before planting into the pot at numbers indicated in Table 3.3. The pots with beans and *Arachis pintoi* were inoculated with a suspension of the *Rhizobium* strains CIAT 899 and CIAT 3101, respectively. During the experiment soil humidity was controlled by weighing and kept at 50% of the water holding capacity.

After two months or eleven weeks, respectively, plant shoots were harvested and dry matter was weighed after 48 h drying at 80° C. About 200 mg of a homogenized sample of the whole shoot biomass in the first or half of the total shoot in the second experiment, cut in pieces < 2 mm, was calcinated at 550° for 4 hours. Plant P content (p) was determined after solubilization of the ashes in 1 - 5 ml of 1.3 M HCl. Aliquots of the samples were diluted and measured using the method of Murphy and Riley (1962). The same method was used for the determination of the seed P content, measuring ball milled samples of 100 mg (*Agrostis capillaris*), two seeds (*Arachis pintoi*, rice, beans and maize) or five seeds (*Brachiaria decumbens*), with five replicates each. The plant \(^{33}\)P (r) content was measured by scintillation counting of diluted (to avoid quench effect) samples using a liquid scintillation analyzer (Packard 2500 TR) and Packard Ultima Gold scintillation liquid. The measured radioactivity was decay corrected back to the day of soil labelling.

The L values, expressed as mg P kg\(^{-1}\) soil, were calculated with the P-concentrations and activities measured in the total shoot.

**Experiment 1:**

Without carrier (Larsen and Sutton, 1963):

\[
L = \frac{R \ast p}{r}
\]

(3.5)
Experiment 2

With carrier (Frossard et al., 1994):

\[ L = Q \left( \frac{R \cdot p}{Q \cdot r} \right) - 1 \]  

(3.6)

The source of P taken up by the plant in the experiment with carrier addition can be calculated as (Frossard et al., 1994):

\[ P_{\text{soil}} = p - P_{\text{carrier}} \]  

(3.7)

\[ P_{\text{carrier}} = \frac{Q \cdot r}{R} \]  

(3.8)

where \( R \) is the quantity of \(^{33}\text{P}O_4\) or \(^{32}\text{P}O_4\) used to label exchangeable soil P (MBq kg\(^{-1}\) soil) and \( Q \) the quantity of carrier added (mg P kg\(^{-1}\) soil), \( r \) is the quantity of \(^{33}\text{P}O_4\) or \(^{32}\text{P}O_4\) (MBq kg\(^{-1}\) soil) and \( p \) is the quantity of \(^{31}\text{P}O_4\) (mg kg\(^{-1}\) soil) in the plant shoots. \( P_{\text{carrier}} \) and \( P_{\text{soil}} \) are the total amount of P derived from the carrier solution or from soil respectively. However, the P content of the seed is a third P source and uptake from this source can not be distinguished from the P taken up from soil. Therefore, \( P_{\text{soil}} \) is actually the sum of the P taken up from soil and from the seed, and the specific activity of the P taken up from soil is diluted. This results in an overestimation of the \( L \) value in both experiments. To increase the accuracy of the \( L \) value, Truong and Pichot (1976) suggest the following correction:

\[ L_{\text{th}} = L \frac{P}{(P + P_{\text{seeds}})} \]  

(3.9)

where \( L_{\text{th}} \) is the corrected value, \( L \) the value calculated with Equation (3.5) or (3.6) and \( P_{\text{seeds}} \) the P content of the sown seeds per pot. Another possibility to correct for the seed P influence is to subtract the total seed P content from plant P uptake for the calculation of
the L value (Smith, 1981):

\[ L = \frac{R(p - P_{\text{seed}})}{r} \]  \hspace{1cm} (3.10)

This correction assumes that 100% of seed P was taken up by the plant and allocated to the shoot. Therefore, it corrects for the highest possible influence of seed P.

**Acid phosphatase activity determination**

Three bulk soil samples were taken at random after harvesting plants in all pots, air dried and roots were removed carefully by sieving soil at 2 mm. Acid phosphatase activity at pH 6.5 of soil samples derived from the planted pots and soil incubated without plants at the same conditions was measured using 1 g air-dried soil according to the method of Tabatabai (1982).

**Statistical Analysis**

The effect of plants in the pot experiment and the effect of the experimental conditions on parameters of isotopic exchange in the E value determination were tested by analysis of variance (ANOVA). If the F-test was significant \((P< 0.05)\), the means were compared using Tukey’s multiple range test.
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Results and discussion

$L$ values determined without carrier and correction for seed $P$ influence

The biomass production of all plants in the first experiment was very low and the total $P$ uptake was hardly higher than the $P$ content of the seeds (Table 3.4 and 3.5). The $P$ concentrations in the plant tissue were below 1 mg g$^{-1}$ for all plants except beans. This is lower than the critical $P$ concentrations indicated for *Brachiaria decumbens* (1 mg g$^{-1}$, Rao et al., 1997a), beans (2.5-4.5 mg g$^{-1}$, Bergmann, 1988), temperate grasses (> 2 mg g$^{-1}$, Mays et al., 1980) or rice (>1 mg g$^{-1}$, Nelson 1980). The correction for the contribution of seed $P$ applying Equation (3.9) caused a strong reduction of the uncorrected $L$ values (Table 3.4). It is, however, doubtful whether this correction, which was established for $L$ value determination with *Agrostis capillaris* and *Lolium perenne* as model plants on sand culture (Truong and Pichot, 1976), is also valid for other test plants and all soil types. The correction with Equation (3.10) was only applicable in the case of *Brachiaria decumbens* as for all other test plants the seed $P$ content was higher than the total $P$ uptake in the plant shoot. Consequently, the corrected $L$ values may rather show the order of magnitude of the seed $P$ influence than represent exact values. Most studies comparing $L$ values of different plants on low $P$ soils may have underestimated the problem of seed $P$ influence. As the $P$ reserves in the seed are in most cases relatively high in comparison to the $P$ taken up from soil, the $L$ value can not be calculated without correction for seed $P$ uptake. This was not always taken into consideration (Ipinmidun, 1973; Dalal and Hallsworth, 1977) and inconsistencies in results (e.g. higher $L$ values in first than in second cuts) were ascribed to other factors (Ipinmidun, 1973). Corrections for seed $P$ influence were applied by Hocking et al. (1997) who subtracted the whole seed $P$ content from total plant $P$ uptake before calculating specific activities (Equation 3.10), or by Braum and Helmke (1995) who subtracted total seed $P$ from the $L$ value. As total $P$ uptake in both studies mentioned was rarely higher than the $P$ stock in the plant seeds, there might have been considerable error in the $L$ value calculation.

The correction applied by Hocking et al. (1997) to subtract the total seed $P$ from plant $P$ uptake is affected by two errors. (i) Part of the mobilized seed $P$ is transferred to the roots.
As in most cases L values are calculated on shoot P basis, the dilution effect of seed P would be overestimated. (ii) The amount of P taken up from seed is variable, highly depending on soil P availability and hardly ever 100%. High amounts of available P inhibit the synthesize of phytase and therefore suppress seed P exploration. On the other hand also very low soil P supply can lead to a lower P export from the seed (Truong and Pichot, 1976)

Due to the uncertain influence of seed P, the interpretation of the L values remains limited. Additionally, the L value of Agrostis capillaris can not be used as reference on these low P soil as the ratio of plant P uptake to seed P was very low, too. However, in the case of Brachiaria decumbens with the smallest influence from seed P (Table 3.4 and 3.5), the corrected Lth-value remains much higher (131 mg kg⁻¹ with Equation (3.9) or 127 mg kg⁻¹ with Equation (3.10), respectively) than the extrapolated E8weeks-value of 64 mg kg⁻¹ determined for the same soil (Table 3.2).

As Equation (3.10), with the subtraction of total seed P from P export in the plant shoot, corrects for the highest theoretically possible influence of seed P, the L value of Brachiaria decumbens indicates that P additional to the isotopically exchangeable P was taken up (Frossard et al., 1994). It has however to be mentioned that the extrapolation of E values on such very low P soils is difficult and the precision of the calculated E8weeks is therefore limited (Buehler et al., 2001). On the other hand, the L value determined using Brachiaria decumbens is also higher than the total soil Pi extracted with the sequential P fractionation (Table 3.1). This fact reinforces the assumption that organic P or very recalcitrant inorganic P forms contributed to the P uptake of Brachiaria decumbens. The adaptation of Brachiaria species to low P soils is mainly attributed to soil exploration by an abundant fine root system and mycorrhizal association (Wenzl, 1999). In addition, it was shown in a pot experiment with different added P sources, that Brachiaria dictyoneura cv. Llanero can acquire P from less available inorganic (aluminum phosphate, as AlPO₄) and organic (phytic acid) forms (Rao and Kerridge, 1994). Acid phosphatase activity in roots of Brachiaria dictyoneura was increased with decreasing soil P supply (Rao et al., 1997b), and Brachiaria decumbens grown under low P condition in nutrient solution was shown to secret the highest amount of phytase in comparison to 15 other plant species (Li et al., 1997). In our study acid phosphatase activity measured in the pot soil samples, and in turn
### Table 3.4 Biomass production, P uptake and L values of the compared plants in experiment 1 and 2

<table>
<thead>
<tr>
<th>Plant</th>
<th>Shoot dry weight</th>
<th>P uptake</th>
<th>Shoot P concentration</th>
<th>L&lt;sup&gt;a&lt;/sup&gt;</th>
<th>L&lt;sub&gt;th&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot dry weight</td>
<td>P uptake</td>
<td>Shoot P concentration</td>
<td>L&lt;sup&gt;a&lt;/sup&gt;</td>
<td>L&lt;sub&gt;th&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>g per pot</td>
<td>mg per pot</td>
<td>µg g&lt;sup&gt;-1&lt;/sup&gt; dry weight</td>
<td>mg P kg&lt;sup&gt;-1&lt;/sup&gt; soil</td>
<td>mg P kg&lt;sup&gt;-1&lt;/sup&gt; soil</td>
</tr>
<tr>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 1</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>1.6a</td>
<td>2.4b</td>
<td>0.9a</td>
<td>2.1b</td>
<td>561ab</td>
</tr>
<tr>
<td><em>Brachiaria decumbens</em></td>
<td>0.3bc</td>
<td>1.9bc</td>
<td>0.22b</td>
<td>1.1b</td>
<td>729a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>0.6b</td>
<td>2.3b</td>
<td>0.25b</td>
<td>1.1b</td>
<td>417b</td>
</tr>
<tr>
<td>Maize</td>
<td>-</td>
<td>6.3a</td>
<td>-</td>
<td>3.9a</td>
<td>-</td>
</tr>
<tr>
<td>Beans</td>
<td>-</td>
<td>1.0c</td>
<td>-</td>
<td>1.5b</td>
<td>-</td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>0.2c</td>
<td>1.0c</td>
<td>0.14b</td>
<td>0.4 b</td>
<td>697ab</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
</tbody>
</table>

*<sup>a</sup>, **<sup>b</sup>, ***<sup>c</sup>** Significant at the 0.05 or 0.001 probability level, respectively. Values within columns followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

<sup>a</sup> L value without seed-P correction

<sup>b</sup> L value with the seed-P correction after Truong and Pichot (1976), Equation (9)

<sup>c</sup> second value: corrected with seed P correction according to Brookes (1982), Equation (10)

<sup>d</sup> not significant
Table 3.5 Average seed weight and seed P content of the used varieties

<table>
<thead>
<tr>
<th>plant</th>
<th>weight per seed</th>
<th>total P in sown seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>µg</td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>158</td>
<td>1200/2 seeds</td>
</tr>
<tr>
<td><em>Brachiaria decumbens</em></td>
<td>4.6</td>
<td>26.7/2 seeds</td>
</tr>
<tr>
<td>Rice</td>
<td>44</td>
<td>282/2 seeds</td>
</tr>
<tr>
<td>Maize</td>
<td>306</td>
<td>900/1 seed</td>
</tr>
<tr>
<td>Beans</td>
<td>174</td>
<td>540/1 seed</td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>0.125</td>
<td>480/100 mg seeds</td>
</tr>
</tbody>
</table>

the potential to mineralize available phosphomonoesters (Renz et al., 1999), was only significantly increased ($P<0.001$) for *Arachis pintoi* in the first experiment and was increased significantly ($P<0.001$) for all plants but *Agrostis capillaris* in comparison to the control soil without plant in the second experiment (Table 3.6). However, as the measurements were not restricted to rhizosphere soil, local effects in that zone would not have been detected.
Table 3.6 Phosphatase activity in soil samples derived from pots after plant harvest

<table>
<thead>
<tr>
<th>Plant</th>
<th>Phosphatase activity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg nitrophenol g(^{-1}) h(^{-1})</td>
<td></td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>426a</td>
<td>322a</td>
<td></td>
</tr>
<tr>
<td><em>Brachiaria decumbens</em></td>
<td>285b</td>
<td>342a</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td>295b</td>
<td>295ab</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>-</td>
<td>332a</td>
<td></td>
</tr>
<tr>
<td>Beans</td>
<td>-</td>
<td>286b</td>
<td></td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>236b</td>
<td>242c</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>219b</td>
<td>225c</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>***</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

*** Significant at the 0.001 probability level, values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey’s test

The influence of carrier application

As the correction for seed P influence was difficult, the L value determination without carrier application was unsatisfying for the tested plants, with exception of *Brachiaria decumbens*. To overcome the difficulties of small total P uptake and biomass production, the second experiment was carried out with the application of KH\(_2\)PO\(_4\) (10.3 mg P kg\(^{-1}\) soil) as a carrier with the labelling solution. The duration of plant growth was extended from two month to eleven weeks to get higher biomass production and smaller pots were used to get a higher soil exploration by the roots. The application of a P carrier resulted in much smaller L values (mean of all plants 2.7 mg P kg\(^{-1}\) soil) than without carrier (mean 148 mg kg\(^{-1}\) and there were no significant differences between plants. One possible explanation of the difference found between L values determined with or without carrier application is that an application of 10 mg P kg\(^{-1}\) to a soil with a very low P concentration in the solution (in this case approximately 3 µg l\(^{-1}\)) could have a high impact on the processes in this system.
Table 3.7 Amount of P derived from applied carrier and percentage of P derived from other sources in exp. 2 (calculated with Equations 3.7 + 3.8)

<table>
<thead>
<tr>
<th>Plant Shoot</th>
<th>P Derived from Carrier</th>
<th>P Derived from Other Sources (soil and seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg per pot</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><em>Arachis pintoi</em></td>
<td>2.1b</td>
<td>1.5b</td>
</tr>
<tr>
<td><em>Brachiaria decumbens</em></td>
<td>1.1b</td>
<td>1.0b</td>
</tr>
<tr>
<td>Rice</td>
<td>1.1b</td>
<td>0.9b</td>
</tr>
<tr>
<td>Maize</td>
<td>3.9a</td>
<td>2.6a</td>
</tr>
<tr>
<td>Beans</td>
<td>1.5b</td>
<td>1.0b</td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>0.4b</td>
<td>0.3c</td>
</tr>
<tr>
<td>(average)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA *** *** ***

*** Significant at the 0.001 probability level, values within columns followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

Instead of isotopic exchange, a net diffusion process may dominate and sizes of pools are changed (Fardeau et al., 1996). High influences of carrier application on E values, especially for highly P sorbing soils, were found by Dalal and Hallsworth (1977) and Wolf et al. (1986) and were explained by the influence of carrier P on the process of isotopic exchange as well as by the fact of $^{32}$PO$_4$ fixation. Additionally to the carrier application also the different experimental conditions, especially the smaller pot size, might have influenced the L-value (Andersen et al., 1961). However, a smaller soil volume and therefore higher root exploration and biomass production per kg soil should, if at all, lead to an increase of L values by higher root activity and P mobilization and not to a decrease (Andersen et al., 1961).

In our experiment, the nearly identical values of the specific activities of the plants and the applied carrier indicate that the carrier P was the main source for the P taken up by the plant. Separation of the P sources using Equation (3.7) and (3.8) shows that on average
81% of P taken up by the plant derived from the carrier (Table 3.7). Of the 19% of the total plant P uptake derived from another source a part is actually seed P. Therefore it can be assumed that almost no soil P was taken up and that the application of carrier is not valid for the determination of L values on low-P highly P sorbing soils.

**Conclusions**

Generally, due to the influence of seed P on the L value calculation, the determination of L values can only provide useful information if the plant P uptake is at least five times higher than the P reserve of the seeds. Therefore, in this study, only for *Brachiaria decumbens*, with a very small seed P content, it could be demonstrated that P from less available forms was taken up. The comparison of L values of different Brachiaria species or varieties (or similar plants with small seed P reserves) could therefore be useful to distinguish germplasm with adaptation to low P conditions. For other plants with large seeds, as e.g. legumes, the correction for the seed P influence is difficult if the plant can not be cut several times. The application of carrier P with the radioactive label to enhance plant P uptake does not solve the problem as it modifies the system.

**Acknowledgements**

I thank the field staff at CORPOICA-CIAT Carimagua research station for their help at taking soil samples and Gloria Marcela Rodriguez and Gonzalo Borrero for their help in the laboratory and in the greenhouse at CIAT. This research was funded by ZIL (Swiss Center for International Agriculture) and SDC (Swiss Development Cooperation).
CHAPTER IV

Sequential phosphorus extraction of a $^{33}$P-labelled Oxisol under contrasting agricultural systems
Abstract

Chemical sequential extraction procedures are widely used to divide soil phosphorus (P) into different inorganic and organic fractions, but the assignment of these fractions to pools of differing plant availability, especially for low P tropical soils, is still matter of discussion. To improve this assignment, the effect of land-use systems and related P fertilizer inputs on size of P fractions and their isotopic exchangeability was investigated. A Colombian Oxisol, sampled from a long-term field experiment with contrasting management treatments was labeled with carrier free $^{33}$P and sequentially extracted after incubation times of 4 hours, 1 and 2 weeks. Phosphorus concentrations (inorganic=P$_i$ and organic=P$_o$) and $^{33}$P recovery in fractions sequentially extracted with anion exchange resin (P$_i$), 0.5 M NaHCO$_3$ (Bic-P$_i$, Bic-P$_o$), 0.1 M NaOH (P$_i$, P$_o$), hot concentrated HCl (P$_i$, P$_o$) and residual P were measured for each incubation time. Resin-P$_i$, Bic-P$_i$, NaOH-P$_i$ and hot HCl-P$_i$ were increased with P fertilization, with the highest increase for NaOH-P$_i$. The recovery of $^{33}$P in the two treatments with annual P fertilizer inputs and large positive input-output P balances indicate that resin-P$_i$, Bic-P$_i$ and NaOH-P$_i$ represented most of the exchangeable P. In these treatments, label P transformed with increasing incubation time from the resin to the Bic-P$_i$ and NaOH-P$_i$ fractions. As the $^{31}$P content of these fractions remained constant, the transfer of $^{33}$P suggests P exchange among these fractions. The organic or more recalcitrant inorganic fractions contained almost no exchangeable P. In contrast, in soils with low or no P fertilization, more than 14% of the added $^{33}$P was recovered in NaOH-P$_o$ and HCl-P$_o$ fractions two weeks after labeling, showing that organic P processes are important to plant availability when soil P reserves are limited.
**Key words:** Oxisol, land-use system, sequential P fractionation, short term P dynamics, $^{33}$P labelling, metallic (oxy)hydroxides, soil microbial biomass
Introduction

Phosphorus (P) is an essential nutrient for plants and often the first limiting element in acid tropical soils. Profound understanding of the P dynamics in the soil/plant system and especially of the short- and long-term fate of P fertilizer in relation to different management practices is essential for the sustainable management of tropical agroecosystems (Friesen et al., 1997). Chemical sequential extraction procedures have been and still are widely used to divide extractable soil P into different inorganic and organic fractions (Chang and Jackson, 1957; Bowman and Cole, 1978; Hedley et al., 1982; Cross and Schlesinger, 1995). The underlying assumption in these approaches is that readily available soil P is removed first with mild extractants, while less available or plant-unavailable P can only be extracted with stronger acids and alkali. In the fractionation procedure developed by Hedley et al. (1982) and modified by Tiessen and Moir (1993), the P fractions (in order of extraction) are interpreted as follows. Resin-P\textsubscript{i} represents inorganic P (P\textsubscript{i}) either from the soil solution or weakly adsorbed on (oxy)hydroxides or carbonates (Mattingly, 1975). Sodium bicarbonate 0.5 M at pH 8.5 also extracts weakly adsorbed P\textsubscript{i} (Hedley et al., 1982) and easily hydrolysable organic P (P\textsubscript{o})-compounds like ribonucleic acids and glycerophosphate (Bowman and Cole, 1978). Sodium hydroxide 0.5 M extracts P\textsubscript{i} associated with amorphous and crystalline Al and Fe (oxy)hydroxides and clay minerals and P\textsubscript{o} associated with organic compounds (fulvic and humic acids). Hydrochloric acid 1 M extracts P\textsubscript{i} associated with apatite or octacalcium P (Frossard et al., 1995). Hot concentrated HCl extracts P\textsubscript{i} and P\textsubscript{o} from more stable pools. Organic P extracted by concentrated HCl may also come from particulate organic matter (Tiessen and Moir, 1993). Residual P that remains after extracting the soil with the already cited extractants represents very recalcitrant P\textsubscript{i} and P\textsubscript{o} forms.

Several studies have related these different P fractions in tropical soils to plant growth (Crews, 1996; Guo and Yost, 1998) or showed the influence of land-use and the fate of applied fertilizers (Iyamuremye et al., 1996; Linquist et al., 1997; Lilienfein et al., 1999; Oberson et al., 1999), and partly resulted in contrasting assignments of fractions to pools of different availability. By comparing the amounts of P extracted from the surface horizons
of Brazilian Oxisols that had been under different land-use systems for 9-20 years, either unfertilized or fertilized with mineral P fertilizer applications, Lilienfein et al. (1999) showed that most of the fertilizer was recovered in the Bic- and NaOH-P\textsubscript{i} fractions, irrespective of the land-use system (resin-P\textsubscript{i} was not measured). In a 4-year field study conducted on a Hawaiian Ultisol, Linquist et al. (1997) recovered, one year after fertilizer application, almost 40% of the applied triple super phosphate (TSP) fertilizer in the hot HCl and H\textsubscript{2}SO\textsubscript{4} fractions. Oberson et al. (1999) showed that in an Oxisol managed as a legume-grass pasture for 15 years, resin-P\textsubscript{i}, Bic- and NaOH-P\textsubscript{i} as well as NaOH-P\textsubscript{o} levels were maintained at a higher level over the whole year in comparison to the same soil with the same total P content but managed as a grass only pasture. Iyamuremye et al. (1996) found an increase in resin-P\textsubscript{i}, Bic-P\textsubscript{i} and -P\textsubscript{o} as well as NaOH-P\textsubscript{i} after addition of manure or alfalfa \textit{(Medicago sativa)} residues to acid low-P soils from Rwanda. In the study of Guo and Yost (1998), resin-P\textsubscript{i}, Bic- P\textsubscript{i} and NaOH-P\textsubscript{i} were most depleted by plant uptake on highly weathered soils. NaOH-P\textsubscript{i} was important in buffering available P supply while significant depletion of organic fractions could rarely be measured.

A possible method of gaining information about the availability of different P fractions is to label soil P, P fertilizers or plant residues before applying the sequential fractionation scheme (MacKenzie, 1962; Weir and Soper, 1962, Dunbar and Baker, 1965). Two studies followed the movement of labelled P from plant residues to soil P fractions applying a modified Hedley (Daroub et al., 2000) or the Chang and Jackson (1957) fractionation procedures (Friesen and Blair, 1988). They found that at six or eleven days, respectively, after plant residue addition between 20 and 50% of the label was extractable as P\textsubscript{i} with resin (Daroub et al., 2000) or with NH\textsubscript{4}Cl and NH\textsubscript{4}F (Friesen and Blair, 1988). For longer incubation periods up to 34 days, Daroub et al. (2000) showed a subsequent movement of the label from the resin-P\textsubscript{i} fraction to the NaOH-P\textsubscript{i} fraction. The results obtained in these studies suggest that, in tropical soils, the amounts of P in the different pools measured by sequential P extraction procedures and the fluxes of P between pools are controlled both by physico chemical factors (sorption/desorption) and by biological reactions (immobilization/mineralization). However, the importance of these different reactions for different land-use systems, such as monocropping, pasture or intercropping, remain largely unknown.
The objective of this study was to assess the effect of different land-use systems (native savanna, rice (*Oryza sativa*) monocropping, rice green manure rotation, grass legume pasture) on some physico chemical and biological reactions involved in P cycling in a Colombian Oxisol.

**Materials and methods**

**Soils**

Soils included in the study were sampled during the rainy season in September 1997 from a field experiment (Friesen et al., 1997) located at CORPOICA-CIAT ( CORPORacion Colombiana de Investigacion Agropecuario; Centro Internacional de Agricultura Tropical) research station, Carimagua, Meta, Colombia (4°30'N, 71°19'W). Mean annual temperature is 27° C and average rainfall is 2200 mm. The soils are well drained Oxisols (tropeptic Haplustox, isohyperthermic).

The surface soil layer (0-20 cm) was sampled in the long-term “Culticore” field experiment, which was established in 1993 with the objective to test the effect of different farming systems on plant productivity and soil fertility (Friesen et al., 1997). The experiment had a split-plot design with four replicates with treatment sub-plots of 0.36 ha size. Soil samples used for this study were taken at random in two replicates of each treatment and the replicates were mixed for laboratory analysis. For our study, the following treatments were included:

**SAV** (Native savanna): native grassland annually burned in February, not grazed; no fertilizer application.

**GL** (Grass-legume pasture): rice (*Oryza sativa* cv Oryzica Sabana 6) in 1993, with undersown pasture, since 1993 grass-legume pasture with *Brachiaria humidicola* CIAT 679, *Centrosema acutifolium* cv Vichada CIAT 5277, *Stylosanthes capitata* CIAT 10280, and *Arachis pintoi* CIAT 17434. The pasture was partly resown for renovation in June 1996 with legumes (the same *Arachis pintoi*, and *Centrosema acutifolium* and additionally *Stylosanthes guianensis* CIAT 11833). Grazing intensity was on average 2.7 steers ha⁻¹ during 15 d followed by a 15 d ley regrowth phase.
CR (Continuous rice): rice (*Oryza sativa* cv Oryzica Sabana 6, cv Oryzica Sabana 10 since 1996) grown in monoculture; one crop per year followed by a weedy fallow incorporated with early land preparation at the beginning of the rainy season before sowing rice.

RGM (Rice green manure rotation): Rice followed by cowpea (*Vigna unguiculata*, var. ICA Menegua) in the same year. The legume was incorporated at the maximum standing biomass level in the late rainy season before sowing rice in the following rainy season.

At the beginning of the experiment all treatments except SAV received 500 kg dolomitic lime ha$^{-1}$. Annual fertilization of rice consisted of 80 kg N ha$^{-1}$ (urea, divided among three applications), 60 kg P ha$^{-1}$ (triple superphosphate). In addition 99 kg K as KCl, 15 kg Mg and 20 kg S (as MgSO$_4$) and 10 kg Zn ha$^{-1}$ were applied at establishment and at recommended rates thereafter. With cowpea an additional 20 kg N and 40 kg P ha year$^{-1}$ and 60 kg K, 10 kg Mg, 13 kg S and 10 kg Zn ha$^{-1}$ were applied at establishment and at recommended rates thereafter. The introduced pasture (GL) received additional fertilization only in 1996 (per ha: 20 kg P, 20 kg Ca (lime), 10 kg Mg (lime), 10 kg S (elemental) and 50 kg K (KCl)). Phosphorus input-output balances were estimated by subtracting the P removed from the system by grain and/or with animal live weight gains from the P applied in mineral fertilizers. Phosphorus exports in grain were calculated by multiplying weighed rice grain yields with measured P content in the grain. Phosphate exported in the animals was assumed to be 8 g per kg of live weight gain. Live weight gains in GL were on average 68 kg ha$^{-1}$ yr$^{-1}$ (Oberson et al., 2001). Cultivated soils were tilled to a maximum of 15 cm depth.

Topsoil samples (0-20 cm) were air-dried and sieved to pass a 2 mm sieve before chemical analysis in the analytical service laboratory of CIAT or shipped to Switzerland where they were stored in an air-dried condition until use for the fractionation experiment in 2000.

**Soil Characterization**

Bray-II P (0.03 M NH$_4$F, 0.1 M HCl) was extracted using 2 g subsamples of soil at a 1:7 soil solution ratio and 40 sec shaking time (Bray and Kurtz, 1945). Total soil P (P$_{tot}$) was determined on subsamples of 0.25 mg soil with the addition of 5 mL concentrated H$_2$SO$_4$ and heating to 360° on a digestion block with subsequent stepwise (0.5 mL) additions of
H$_2$O$_2$ until the solution was clear (Thomas et al., 1967). Microbial nutrient pool sizes of P, C and N (P$_{\text{Chl}}$, C$_{\text{Chl}}$ and N$_{\text{Chl}}$) were estimated on the preincubated samples as by extraction, of chloroform-fumigated and unfumigated samples, with the Bray I extract (0.03 M NH$_4$F, 0.025 M HCl) (P$_{\text{Chl}}$) (Oberson et al., 1997) or K$_2$SO$_4$ (C$_{\text{Chl}}$ and N$_{\text{Chl}}$) (Vance et al., 1987). No k-factors (Brookes et al., 1982; Hedley and Stewart, 1982; McLaughlin et al., 1986) were used to calculate P$_{\text{mic}}$, C$_{\text{mic}}$ or N$_{\text{mic}}$ from measured P$_{\text{Chl}}$, C$_{\text{Chl}}$ and N$_{\text{Chl}}$ as there exist no proper estimates for these in acid tropical soils (Gijsman et al., 1997). P$_{\text{Chl}}$ was corrected for sorption of released P according to Oberson et al. (1997). Dithionite-citrate-bicarbonate extractable and oxalate extractable Fe and Al (Fe$_{\text{d}}$, Fe$_{\text{ox}}$, Al$_{\text{d}}$, Al$_{\text{ox}}$) were determined according to Mehra and Jackson (1960) and McKeague and Day (1966). The mineralogy of the soils was determined on total soil samples, pretreated with H$_2$O$_2$ to remove organic C, using X-ray diffraction analysis (XRD) (Table 4.1). The samples were ground under acetone in a tungsten carbide vessel of a vibratory disk mill (Retsch RS1) for 10 min. Longer grinding times were not applied due to the detrimental effect that further grinding can have on the crystallinity of minerals, especially Fe (hydr)oxides (Weidler et al., 1998). For the Cu K$\alpha$ radiation, the Bragg-Brentano geometry was chosen as an XRD routine setup. The measurements were carried out on a Scintag XDS 2000 equipped with a solid state detector from 2 to 52° 2θ with steps of 0.05° 2θ and counting times of 16 sec.

Sequential P Fractionation of Labelled Soils

Before starting the sequential P fractionation, the soil samples were preincubated in a climate chamber (24°C and 65% relative atmospheric humidity, no light) for two weeks in portions of 100 g at 50% of their water holding capacity (300 g water kg$^{-1}$ soil dry weight). Soil water content was controlled and adjusted every other day by weighing. Subsamples of preincubated soils were labelled in portions of 15 g with 120 MBq $^{33}$P kg$^{-1}$ which were added with 10 µl deionized water per g soil. The mass of P introduced with the $^{33}$P label can be neglected (<2.5 x 10$^{-3}$ µg P g$^{-1}$ soil, Amersham product specification, July 2000). Therefore, the term 'P concentration' always refers to $^{31}$P and specific activities (SA) are calculated as:
Soil P was fractionated sequentially, after three different incubation times after labeling (4h, 1 wk, and 2 wks), using three replicates per treatment following the modified method of Hedley et al. (1982), as described in Tiessen and Moir (1993), with HCO₃-saturated resin strips (BDH # 55164, 9 x 62 mm), followed by 0.5 M NaHCO₃ (referred to as Bic-P), 0.1 M NaOH, (these first three steps each with an extracting time of 16 h) and concentrated hot HCl at 80° C for 10 min. The step using diluted cold HCl was omitted, as Ca-phosphates are only present at very low levels or are absent in highly weathered acidic soils (Agbenin and Tiessen, 1995), as shown for the soils used in this study by Friesen et al. (1997). Residual P was extracted as described previously for determination of Pₜₒᵗ. The amount of soil extracted was doubled from 0.5 to 1 g using the original volumes of extractants (2 resin strips in 30 ml H₂O, 30 ml NaHCO₃, 30 ml NaOH, 15 ml concentrated HCl, 5 ml conc. H₂SO₄) in order to get higher ³²P-concentrations in the extracts. This was preferred to the alternative of higher label application as the radiation might affect microbial growth (Halpern and Stöcklin, 1977). After each extraction, the samples were centrifuged at 25000 x g for 10 min before filtering the solutions of the Bic- and the NaOH-extracts through 0.45 μm pore size millipore filters (Sartorius, cellulose acetate), and the hot HCl and residual P extract through Whatman No. 40 filter paper. Phosphorus concentrations in all extracts was measured after neutralization by the Murphy and Riley (1962) method. This method was used directly, after neutralization of the extracts, for the P recovered from the resin strips and for Pᵢ determination in the HCl extracts. Organic matter was first precipitated by acidification in the Bic- and the NaOH-extracts prior to Pᵢ determination (Tiessen and Moir, 1993). Total P (Pₜ) in the Bic-, the NaOH- and the HCl-extracts was measured after digestion of P₀ with potassium persulfate (Bowman, 1989). Organic P was calculated as the difference between total P and Pᵢ in the Bic-, NaOH- and hot HCl extracts.

To partition soluble ³²Pᵢ and ³³P₀ in the Bic-, the NaOH- and the hot HCl-extracts into separate solutions before counting, 5 ml of the extracts were shaken with acidified ammonium molybdate dissolved in isobutanol (Jayachandran et al., 1992). With this

\[
SA (Bq \mu g^{-1} P) = \frac{^{33}P}{^{31}P}
\]

(4.1)
method, P<sub>i</sub> is extracted into the isobutanol while P<sub>0</sub> remains in the aqueous phase. The complete recovery of P<sub>i</sub> in the isobutanol phase was verified with the addition of a standard amount of 33P in 0.5 M HCO<sub>3</sub>, 0.1 M NaOH and in 2.3 M HCl; recovery rates of added 33P in the isobutanol phase were between 97% and 103%, which was not significantly different from 100%. Counts in the aqueous phase were 1.1% (HCO<sub>3</sub>), 0.3% (NaOH) and 0.1% (HCl) of the original solutions showing that hardly any P<sub>i</sub> went into this phase. Determination of total P in the aqueous phase is not possible because the presence of the molybdate interferes with the analysis (Jayachandran et al., 1992).

The radioactivity in each phase was determined with a liquid scintillation analyzer (Packard 2500 TR) using Packard Ultima Gold scintillation liquid in the ratio (extract to liquid) 1:5. The values were corrected for radioactive decay back to the day of soil labelling. All extracts were tested for possible quenching effects by adding defined 33P spikes. Quenching in the acid resin eluate could be prevented by dilution of 250 μl eluate with 750 μl deionized water for counting. The quench effect in the hot concentrated HCl extract could be avoided by counting solutions separated with acidified isobutanol because the separated phases were not affected by quenching. All other extracts were not affected by quench effects.

The recovery of the label calculated as sum of all fractions, including residual P, was never complete. Therefore, subsamples of the soil residue after final acid digestion were dried and weighed into scintillation vials. These subsamples were then counted after addition of 1 ml water and 5 ml of scintillation cocktail.

As fractionation data were compared to parameters measured with an isotopic exchange batch experiment described below, the influence of the experimental conditions of the isotopic exchange procedure on the 35P and 31P distribution in the different P fractions was checked. Six 1 g subsamples of each soil were shaken for 16 h on an overhead shaker with 9.9 ml deionized water. The samples were then stirred on a magnetic stirrer plate and labelled with the same amount as above of 33P per g soil introduced in 100 μl deionized water per 1 g sample. The samples were stirred for 100 min to simulate the conditions of an isotopic exchange experiment (Fardeau, 1996) and afterward left without further stirring at room temperature. The sequential soil P fractionation was started 4 hours after labelling by
adding two resin strips to each sample. Whether the soil was labelled in a 1:10 shaken soil suspension or at 50% of water holding capacity did not significantly influence either the $^{31}$P or the $^{33}$P concentrations in the different fractions 4 h after labelling (data not shown).

**Isotopic exchange kinetics**

The procedure of isotopic exchange kinetics was used to assess the exchangeability of P$_i$ in the soils sampled in the different land-use systems. The method was conducted as described by Fardeau (1996). Suspensions of 10 g of soil and 99 ml deionized water were shaken for 16 h on an overhead shaker to reach a steady state equilibrium for P$_i$. Then, at $t = 0$, 1 ml of carrier free H$_3^{33}$PO$_4$ tracer solution containing 1.2 MBq was added to each continuously stirred soil water suspension. Three subsamples were taken from each suspension after 1, 10 and 100 min, immediately filtered through a 0.2 μm pore size micropore filter, and the radioactivity in solution was measured by liquid scintillation as described previously. To determine the $^{31}$P concentration in the soil solution ($C_p$, mg P l$^{-1}$) 10 ml of the solution were filtered through a 0.025 μm filter (Schleicher & Schuell, NC 03) at the end of the experiment. The smaller filter pore size was used to exclude any influence of suspended soil colloids on $C_p$ determination (Sinaj et al., 1998). The P concentration in the filtrate was measured in a 1 cm cell using the Malachite green method (Ohno and Zibilske, 1991) with a Shimadzu UV-1601 spectrophotometer. Phosphorus concentrations in solutions from the SAV and GL treatment were close to the detection limit and they were measured again in samples concentrated by evaporation (5:1). This procedure resulted in $C_p$ values that were not significantly different from the non-concentrated solutions.

Assuming that at any given exchange time the specific activity (SA) of inorganic phosphate in the solution is equal to the SA of the total quantity of phosphate which has been isotopically exchanged, it is possible to calculate the amount of isotopically exchanged P ($E_t$, mg P kg$^{-1}$ soil). The amount of P exchangeable within one minute ($E_1$), indicating the immediately available P, is expressed as (Fardeau, 1996):

$$E_1 = R \times 10 \times C_p / r_1$$

(4.2)
where $R$ is the introduced radioactivity and $r_1$ is the radioactivity remaining in solution after 1 minute of isotopic exchange. The factor 10 results from the soil solution ratio of 1:10.

**Statistical analysis**

The effects of land-use systems and incubation time after labelling on P fraction size were tested by two-way ANOVA and Tukey's multiple range test over all treatments and times of fractionation. A separate one-way ANOVA was used to test the difference on label recovery and fraction size between samples labelled in soil water ratio 1:10 and samples labelled in incubated moist state 4 h after labelling. Percentage recovery data were log-transformed to meet the requirements of analysis of variance. Time and soil treatment influences for each repetition in time of sequential fractionation were tested by ANOVA.

**Results and discussion**

The mineralogy (Table 4.1) and Fe and Al (oxy)hydroxides contents (Table 4.2) of the surface soil from the four treatments was normal for this type of soil (Gaviria, 1993) and were almost identical among the different land-use systems (SAV, GL, CR, RGM). This implies that any difference seen in P dynamics among land-use systems was mainly due to the land-use system and not to differences in soil mineralogy.
Table 4.1 Mineralogy of the studied Colombian Oxisol under different agricultural systems, determined with X-ray diffraction.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quartz</th>
<th>Kaolinite</th>
<th>Anatase</th>
<th>Rutile</th>
<th>Gibbsite</th>
<th>Vermiculite</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>72</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>&lt;&lt;1</td>
</tr>
<tr>
<td>GL</td>
<td>67</td>
<td>23</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>&lt;&lt;1</td>
</tr>
<tr>
<td>CR</td>
<td>65</td>
<td>25</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>RGM</td>
<td>67</td>
<td>24</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>&lt;&lt;1</td>
</tr>
</tbody>
</table>

Table 4.2 Selected chemical and physical properties of the surface layer (0-20 cm) of the Colombian Oxisol under different agricultural systems. Values are the average of four analytical replicates, except Fe- and Al-contents (three replicates) e

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total C</th>
<th>Total N</th>
<th>pH in water</th>
<th>Al-Saturation</th>
<th>Fe\textsubscript{d}</th>
<th>Fe\textsubscript{ox}</th>
<th>Al\textsubscript{d}</th>
<th>Al\textsubscript{ox}</th>
<th>Clay</th>
<th>Bulk density</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>27</td>
<td>1.64</td>
<td>4.8b</td>
<td>86.8b</td>
<td>26.7</td>
<td>3.6</td>
<td>7.8</td>
<td>2.0</td>
<td>35.0a</td>
<td>1.27</td>
</tr>
<tr>
<td>GL</td>
<td>29</td>
<td>1.55</td>
<td>4.9b</td>
<td>71.7a</td>
<td>26.4</td>
<td>3.6</td>
<td>7.7</td>
<td>2.0</td>
<td>39.3b</td>
<td>1.27</td>
</tr>
<tr>
<td>CR</td>
<td>26</td>
<td>1.45</td>
<td>4.3a</td>
<td>75.4a</td>
<td>26.2</td>
<td>3.7</td>
<td>7.6</td>
<td>2.0</td>
<td>39.9b</td>
<td>1.21</td>
</tr>
<tr>
<td>RGM</td>
<td>26</td>
<td>1.49</td>
<td>4.3a</td>
<td>76.3a</td>
<td>26.9</td>
<td>3.5</td>
<td>7.8</td>
<td>2.0</td>
<td>39.0b</td>
<td>1.24</td>
</tr>
</tbody>
</table>

\( ^a \) see Table 4.1

\( ^b \) Extraction with dithionite after Mehra and Jackson (1960)

\( ^c \) Extraction with oxalate after McKeague and Day (1966)

\( ^d \) source: CIAT (1999)

\( ^e \) Means followed by the same letter are not significantly different \((P=0.05)\) by Tukey’s multiple range test. The absence of letter in a column shows that no significant differences were observed between the treatments.
Table 4.3 Phosphorus status and calculated P balances of the studied Oxisol under different land-use systems. Total P calculated as sum of the sequential P fractionation (P_{sum}) or extracted directly with H_{2}O_{2} and H_{2}SO_{4}(P_{tot})

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bray II P$\dagger$</th>
<th>P_{sum}^b</th>
<th>ΔP_{sum}^c</th>
<th>P_{tot}^b</th>
<th>ΔP_{tot}^c</th>
<th>P-Balance^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>0.9a</td>
<td>165aA</td>
<td>0</td>
<td>172aA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GL</td>
<td>2.0b</td>
<td>190bA</td>
<td>25</td>
<td>213bB</td>
<td>41</td>
<td>28</td>
</tr>
<tr>
<td>CR</td>
<td>17.2c</td>
<td>290cA</td>
<td>125</td>
<td>293cA</td>
<td>121</td>
<td>92</td>
</tr>
<tr>
<td>RGM</td>
<td>35.5d</td>
<td>335dA</td>
<td>170</td>
<td>376dB</td>
<td>205</td>
<td>153</td>
</tr>
</tbody>
</table>

F-test (soil) *** *** ***

---

*a* see Table 1

*b* P concentrations followed by the same lower case letter (within columns) or upper case letter (comparison of P_{sum} and P_{tot} within rows) are not significantly different (P=0.05) according to Tukey’s test

*c* ΔP calculated as the difference between P_{sum} or P_{tot} of fertilized treatments - SAV

*d* Calculated by subtracting the P removed by grain and/or animals from the P applied with mineral fertilizer.

balance, these results suggest that most of the P added as fertilizer and not taken up by plants remained in the surface layers of the soils. Except for the CR treatment these results agree well with Oberson et al. (2001). In their study about half of the calculated positive P balance was recovered in total P. The sampling depth of 0-10 cm might explain this difference: soil tillage may have mixed P in the 0-10 cm soil layer with soil in the 10-20 cm layer, resulting in incomplete recovery of P in the 0-10 cm sampling depth.

**Isotopic Exchange Characteristics**

The effect of the four land-use systems on P\textsubscript{t} exchangeability in the surface layer of the Colombian Oxisol is illustrated in Table 4.4. The ratio r_{i}/R, which is inversely correlated to the P sorbing capacity of soils (Frossard et al., 1993), was below 0.05 for all treatments suggesting that these soils have a high P sorbing capacity (Frossard et al., 1993).
Furthermore, the \( r_t/R \)-values of the four treatments were positively correlated with \( P_{\text{tot}} \) \( (r^2=0.76, P<0.001) \). This suggests that the different land-use systems have resulted, through P fertilization and cropping, in different sorption rates of \( P_1 \) on soil minerals. Since in Oxisols P sorption is governed by Al and Fe (oxy)hydroxides (Fontes and Weed, 1996), these treatments probably induced different degrees of \( P_1 \) saturation on the (oxy)hydroxides such as gibbsite, which was identified in the soil from these treatments (Table 4.1).
Table 4.4 Parameters of isotopic exchange

<table>
<thead>
<tr>
<th>Treatment</th>
<th>r_l/R_e</th>
<th>C_p</th>
<th>E_1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg l^{-1})</td>
<td>(mg kg^{-1})</td>
<td></td>
</tr>
<tr>
<td>SAV</td>
<td>0.02a</td>
<td>0.0015a</td>
<td>0.7a</td>
</tr>
<tr>
<td>GL</td>
<td>0.03a</td>
<td>0.002b</td>
<td>0.6a</td>
</tr>
<tr>
<td>CR</td>
<td>0.04a</td>
<td>0.003c</td>
<td>0.8a</td>
</tr>
<tr>
<td>RGM</td>
<td>0.055b</td>
<td>0.015d</td>
<td>2.7b</td>
</tr>
<tr>
<td>F-test</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Values are the average of three replications.

a Values are the average of three replications.

b see Table 4.1

c ratio of radioactivity remaining in soil solution to radioactivity added at time 0 after 1 minute of isotopic exchange

d P concentration in the soil solution measured at soil:water ratio 1:10

e Quantity of P exchangeable within 1 minute

The P_i concentration in the soil solution (C_p) was close to the detection limit in the SAV, GL and CR treatments (Table 4.4). Although significantly different between all treatments, C_p was significantly increased only in the RGM treatment (P<0.001). In SAV, GL and CR, C_p was much lower than the critical concentration needed to sustain optimal growth for a large range of crops (Kamprath and Watson, 1980; Fox, 1981). The P_i concentration in the soil solution was not correlated with P_{tot}. The increase in C_p under RGM was therefore not only due to an increase in P_{tot} but also to other mechanisms. The strong increase in soil biological activity observed in land-use systems including legumes might partly explain the higher C_p value (Haynes and Mokolobate, 2001; Oberson et al., 2001). The variation in the amount of P_i isotopically exchangeable in one minute (E_1) followed the same trend as the variation in C_p.
P concentrations in different fractions of the sequential extraction

The positive P balances of the fertilized GL, CR and RGM treatments resulted in significantly higher P concentrations (P<0.001) compared to the savanna soil in all fractions except the organic fractions and residual P (Table 4.5). This agrees with the results of Friesen et al. (1997) and Oberson et al. (2001), who fractionated P forms according to the same method in the same field experiment, and studies conducted using other tropical soils (Beck and Sanchez, 1994; Linquist et al., 1997). Our results show that resin-P, Bic-P, and NaOH-P increased with P fertilizer input, with the NaOH-P fraction being the main sink for the applied P. The function of the NaOH-P fraction can be explained by the adsorption of P through ligand exchange with hydroxyl groups (Sposito, 1989) located on the surface of Fe and Al (oxy)hydroxides (Ainsworth et al., 1985; Parfitt, 1989; Torrent et al., 1992) and by the desorption of P from the surface of (oxy)hydroxides in the presence of 0.5 M NaOH (Houmane et al., 1986; Cross and Schlesinger, 1995).

During the continuous 2-week incubation of the soil samples, resin-P, and Bic-P, fractions increased significantly (P<0.05) between the first and second fractionation date for all treatments (between 4 and 14 mg kg⁻¹ for the sum of resin-P, and Bic-P,). There was no significant decrease in any fraction although total extractable P tended to decline (between 8 and 18 mg kg⁻¹) for all soils (Table 4.5). The absence of a significant movement of P out of P fractions may be due to the high variability of the results, especially for the organic fractions where coefficients of variation for Bic-P ranged from 13 to 70% and for NaOH-P from 7 to 45%. Since P is determined by the difference between P and P for a given extract there are multiple sources of error. High variability of repeated measuring of Bic-P and NaOH-P were reported by Magid and Nielsen (1992). Problems in the determination of P are mentioned in Tiessen and Moir (1993), especially the possibility that P is precipitated along with the organic matter upon acidification and erroneously determined as P (P-P). On the other hand, P compounds could be hydrolyzed in the acidic solution during the measurement of P during the colorimetric essay (Condron et al., 1990; Gerke and Jungk, 1991).

Increases in resin-P, and Bic-P, between 4 h and 1 wk of incubation suggest that mineralisation of P led to the release of labile P from P fractions. As the first
fractionation was started 4 h after labelling, the disturbance by mixing the soil with the label and the increased humidity may have stimulated microbial activity inspite of the preincubation. A temporary stimulation of the microbial activity by mixing when labelling the soil was indicated in microbial turnover studies conducted on soils from the same field experiment (Oberson et al., 2001). This assumption seems likely, as there were little changes in fraction sizes between the second and the third fractionation indicating a stabilization of the system.
Table 4.5 Distribution of P in various fractions of the modified Hedley fractionation in different agricultural systems with and without P application on an Oxisol, at three times of incubation after mixing the soils for label application.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation time</th>
<th>Resin P&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Bicarbonate P&lt;sub&gt;t&lt;/sub&gt;, P&lt;sub&gt;o&lt;/sub&gt;</th>
<th>NaOH P&lt;sub&gt;t&lt;/sub&gt;, P&lt;sub&gt;o&lt;/sub&gt;</th>
<th>Hot HCL P&lt;sub&gt;t&lt;/sub&gt;, P&lt;sub&gt;o&lt;/sub&gt;</th>
<th>Residual P&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Total P</th>
<th>Total P&lt;sub&gt;o&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>4 hours</td>
<td>0.9 g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 g</td>
<td>12.4</td>
<td>22 de 46</td>
<td>37 b 6.1 ab</td>
<td>44 ab</td>
<td>172 ef 65</td>
</tr>
<tr>
<td>GL</td>
<td>4 hours</td>
<td>2.0 ef</td>
<td>2.8 fg</td>
<td>11.8</td>
<td>27 de 56</td>
<td>34 b 8.6 a</td>
<td>43 b</td>
<td>185 ef 76</td>
</tr>
<tr>
<td>CR</td>
<td>4 hours</td>
<td>4.8 d</td>
<td>9.7 def</td>
<td>15.0</td>
<td>102 b 48</td>
<td>56 a 9.1 a</td>
<td>49 ab</td>
<td>298 cd 72</td>
</tr>
<tr>
<td>RGM</td>
<td>4 hours</td>
<td>10.0 b</td>
<td>21.4 bc</td>
<td>6.7</td>
<td>100 bc 62</td>
<td>65 a 5.2 abc</td>
<td>47 ab</td>
<td>321 abc 74</td>
</tr>
<tr>
<td>SAV</td>
<td>1 week</td>
<td>2.0 ef</td>
<td>4.3 fg</td>
<td>5.7</td>
<td>20 e 42</td>
<td>36 b 4.1 bc</td>
<td>42 b</td>
<td>157 f 52</td>
</tr>
<tr>
<td>GL</td>
<td>1 week</td>
<td>2.4 e</td>
<td>6.4 efg</td>
<td>10.0</td>
<td>33 d 47</td>
<td>38 b 3.3 bc</td>
<td>43 ab</td>
<td>184 ef 61</td>
</tr>
<tr>
<td>CR</td>
<td>1 week</td>
<td>8.0 c</td>
<td>14.3 cde</td>
<td>14.3</td>
<td>89 c 47</td>
<td>53 a 2.5 bc</td>
<td>50 ab</td>
<td>279 d 64</td>
</tr>
<tr>
<td>RGM</td>
<td>1 week</td>
<td>16.4 a</td>
<td>29.8 a</td>
<td>12.8</td>
<td>119 a 40</td>
<td>63 a 3.3 bc</td>
<td>54 ab</td>
<td>338 ab 56</td>
</tr>
<tr>
<td>SAV</td>
<td>2 weeks</td>
<td>2.0 ef</td>
<td>4.1 fg</td>
<td>6.3</td>
<td>20 e 42</td>
<td>36 b 4.1 bc</td>
<td>48 ab</td>
<td>164 f 52</td>
</tr>
<tr>
<td>GL</td>
<td>2 weeks</td>
<td>4.2 d</td>
<td>6.4 efg</td>
<td>10.3</td>
<td>33 d 49</td>
<td>38 b 2.9 bc</td>
<td>62 a</td>
<td>207 e 62</td>
</tr>
<tr>
<td>CR</td>
<td>2 weeks</td>
<td>7.5 c</td>
<td>16.6 cd</td>
<td>11.0</td>
<td>90 bc 56</td>
<td>58 a 1.2 c</td>
<td>61 ab</td>
<td>305 bcd 68</td>
</tr>
<tr>
<td>RGM</td>
<td>2 weeks</td>
<td>15.8 a</td>
<td>27.5 ab</td>
<td>15.9</td>
<td>118 a 45</td>
<td>63 a 4.3 bc</td>
<td>62 a</td>
<td>354 a 65</td>
</tr>
</tbody>
</table>

Treatment *## *** n.s. *** n.s. *** ** n.s. *** n.s.
Time *** *** n.s. n.s. n.s. n.s. *** * n.s.

---

* ** *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

<sup>a</sup> values within a column followed by the same letter do not differ significantly (P=0.05) according to Tukey's test.

<sup>b</sup> see Table 4.1
Distribution of $^{33}P$ among $P$ fractions and dynamics over time

The fraction of $^{33}P$ recovered in the resin-P$_i$ fraction 4 h after labelling varied between 22% in SAV and 60% in RGM (Figure 4.1). The $^{33}P$ recovery in this fraction was positively correlated to $P_{tot}$ ($r^2=0.87, P<0.001, 4$ h after labelling). The corresponding decrease with time of $^{33}P$ in the resin fraction in RGM and CR coincided with an increase in label recovery in Bic-P$_i$ and NaOH-P$_i$, while in SAV and GL the decline in resin $^{33}P$ was accompanied by an increase in $^{33}P$ in NaOH-P$_o$ (GL also NaOH-P$_i$), HCl-P$_i$, and residual-P. For SAV and GL, the label recovered in resin-P$_i$ decreased only slightly but significantly between the 1$^{st}$ and the 2$^{nd}$ wk, and the label recovery in Bic-P$_i$ did not change significantly in this time. The amount of $^{33}P$ in NaOH-P$_i$ was almost stable over the entire incubation time with a small but significant increase between the 1$^{st}$ and the 2$^{nd}$ wk for GL. This shows that in SAV and GL the label was rapidly exchanged between these fractions and that equilibrium with the (labelled) soil solution was reached. In contrast, $^{33}P$ in the Bic-P$_i$ and the NaOH-P$_i$ of CR and RGM was still increasing after one wk while resin-$^{33}P_i$ continued to decrease, showing that the exchange between these fractions was incomplete.

The data for $^{33}P_o$ were, because of the determination after the separation of from $P_i$ with the isobutanol method, not conditioned by the inherent problems in determination of the $P_o$ fractions in the Hedley fractionation scheme as described previously. Only small amounts of the label were found in organic fractions after 4 h, but there were already significant differences in NaOH-$^{33}P_o$ ($P<0.001$) in the order:

SAV (4%) ≈ GL (2%) > CR (0.4 %) ≈ RGM (0.1 %).

This might be due to differences in microbial activity as observed by Oberson et al. (2001) in the same field experiment. Microbial biomass in the incubated soils, indicated by measured $P_{Cul}$, $C_{Cul}$ and $N_{Cul}$ values, was significantly different between the soils (Table 4.6), despite the fact that the samples had been stored in an air-dried condition for more than three years before being used in this study. The assumption that recovery of the label in organic fractions was actually due to active processes and not to any analytical artifact is supported by the observed increases of NaOH-$^{33}P_o$ and HCl-$^{33}P_o$ for all soils over time. The total recovery of 20% (SAV) or 14% (GL), respectively, of the label in organic fractions two wks after labelling shows that these compartments have to be taken into account to
understand the fate of P in these very low-P soils (Tiessen et al., 1984; Beck and Sanchez, 1994; Linquist et al., 1997).
Fig. 4.1 Percentage of label recovery in the different fractions of the sequential P extraction and in the sum of all fractions at 4 hours, 1 and 2 weeks after labeling soil (Means of three replicates ± SD)
Table 4.6 Size of soil microbial biomass nutrient pool in different agricultural systems after 20 days of incubation of the formerly air-dried soils. Values are the averages of three replicates.

<table>
<thead>
<tr>
<th>treatment</th>
<th>C&lt;sub&gt;Chl&lt;/sub&gt;</th>
<th>N&lt;sub&gt;Chl&lt;/sub&gt;</th>
<th>P&lt;sub&gt;Chl&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>88.7a</td>
<td>13.7a</td>
<td>1.6a</td>
</tr>
<tr>
<td>GL</td>
<td>80.8a</td>
<td>13.5a</td>
<td>1.2ab</td>
</tr>
<tr>
<td>CR</td>
<td>72.9a</td>
<td>8.5b</td>
<td>0.7b</td>
</tr>
<tr>
<td>RGM</td>
<td>48.2b</td>
<td>6.1b</td>
<td>0.5b</td>
</tr>
</tbody>
</table>

** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

**a** Means followed by the same letter are not significantly different (P=0.05) by Tukey's multiple range test.

**b** see Table 4.1

The proportion of label in the hot HCl and residual P fractions increased significantly with incubation time in all soils. This contradicts the prevailing opinion of recalcitrance of P in these fractions (Guo and Yost, 1998; Neufeldt et al., 2000). While the total P content in the residual fraction varied significantly with time (Table 4.5), this was not the case for hot HCl extractable P, while hot HCl extractable Po tended to decrease. This suggests that the movement of the label to these fractions was not due to net P-movement but to exchange processes.

Total $^{33}$P label recovery

At all sampling times during the incubation study, in total between 67% and 94% of the applied $^{33}$P label could be recovered in the sum of all P fractions (Fig. 4.1). This sum was generally in the order SAV<GL<CR<RGM. Incomplete recoveries can be explained by the fact that the method used to assess total P or residual P was not efficient enough to extract all of the soil P. Comparative studies have shown that total P can only be reliably extracted by alkali fusion (Syers et al., 1967; Bowman, 1988), which could not be used in this work.
The analysis of soil residues after the acid extraction of residual P (Table 4.7) indicated that significant amounts of the label remained unextracted, these being higher for SAV and GL than CR and RGM. Although counting of $^{33}$P bound to solid phases is generally possible, problems of phase, impurity, self absorption of scintillations by the soil particles or color quenching effects (Gibson, 1980) are difficult to correct, as these influences might be highly variable between samples. However, the recovery of standard additions of $^{33}$P to our soil residues was complete and the correlation of the measured radioactivity in the different soil treatment residues with the sample weight was linear (data not shown), thus confirming the qualitative information obtained from the counting of the soil residues.

Table 4.7 Radioactivity measured in soil solid residues by scintillation counting after extraction of residual P by sequential P fractionation starting 1 week after soil labelling

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Bq g$^{-1}$ soil (decay corrected)$^a$</th>
<th>% of initial label</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>2251 (111)</td>
<td>4.4%</td>
</tr>
<tr>
<td>GL</td>
<td>1843 (357)</td>
<td>3.6%</td>
</tr>
<tr>
<td>CR</td>
<td>427 (215)</td>
<td>0.8%</td>
</tr>
<tr>
<td>RGM</td>
<td>348 (140)</td>
<td>0.7%</td>
</tr>
</tbody>
</table>

$^a$ Average of three replications, standard error in brackets decay corrected to the day of soil labelling

Altogether the results suggest that the transfer of $^{33}$P among the different fractions determined by the sequential extraction was strongly dependent on the degree of saturation of soil Al and Fe (oxy)hydroxides with P,$_i$, and therefore on the bonding energy of P,$_i$ to the soil minerals. It is indeed known that a high P,$_i$ saturation of metal oxide surfaces causes a more negative charge on the surface and prevents the specific sorption of further P,$_i$ ions (Ryden et al., 1977; Bowden et al., 1980). In the low P treatments (SAV and GL), most P,$_i$ would be sorbed with such a high energy that its exchangeability would be very limited. Specific sorption of $^{33}$P to the surface of Al and Fe (oxy)hydroxides of these soils, although unlikely (Frossard et al., 1994), cannot be excluded (Barrow, 1991). In contrast, in the P
rich soils (CR and RGM), annual P additions may have resulted in the build up of larger quantities of P$_1$ that was exchangeable with $^{33}$P.

Specific activities in the fractions determined by the sequential extraction

The highest specific activities (SA) observed in the incubation experiment were obtained in the resin extract after 4 h of incubation (Table 4.8). This is consistent with the assumption that the amount of P desorbed from the soil by resin is in very rapid exchange with P$_1$ in the soil solution, as suggested by other studies (Amer et al., 1955; Bowman and Olsen, 1979; Tran et al., 1992; Schneider and Morel, 2000). The subsequent decrease in SA of resin-P$_1$ reflected the process of isotopic exchange between $^{33}$P and stable P$_1$ located on the soil’s solid phase (Fardeau, 1996). The order of the SAs in the P$_1$ fractions after 4 h of incubation followed the extraction sequence (resin-P$_1$>Bic-P$_1$>NaOH-P$_1$>HCl-P$_1$>residual P$_1$), showing that the strongest reactants extracted either large quantities of slowly exchangeable P or a large quantity of P in which only a small part was rapidly exchangeable. After 2 wks the SAs of resin-P$_1$, Bic-P$_1$ and NaOH-P$_1$ became closer, suggesting that equilibrium with respect to P transfer between these fractions was being approached. The SAs of resin-P$_1$, Bic-P$_1$ and NaOH-P$_1$ were not significantly different in SAV and GL while the SA of resin-P$_1$ was still significantly higher than the SA of Bic-P$_1$ and NaOH-P$_1$ in CR and RGM. These observations show that it is not possible to discuss the exchangeability of a certain P fraction without reference to a defined time of exchange (Fardeau, 1996).

Although the SAs of the NaOH-P$_0$ and HCl-P$_0$ fractions were relatively low they showed that, depending on land-use, these fractions were connected through active processes with the soil solution, most probably through microbial activity (Oehl et al., 2001b). This indicates that the determination of plant available P with short-term isotopic exchange experiments might lead to errors since the dynamics of organic P forms are excluded.
Table 4.8 Specific activities ($^{33}$P/$^{31}$P) in isotopic exchange soil solution and in extracts of the Hedley sequential fractionation in the labelled Oxisols derived from different agricultural systems at different times after labelling.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Resin P</th>
<th>NaOH-Pi</th>
<th>NaOH-Po</th>
<th>HCl-Pi</th>
<th>HCl-Po</th>
<th>Residual P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>kBq mg P&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4 hours</td>
<td></td>
<td></td>
<td></td>
<td>1 week</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td></td>
<td>SAV</td>
<td>32.9 A</td>
<td>5.9  aC</td>
<td>3.3 bB</td>
<td>16.4  cB</td>
<td>9.4  cB</td>
<td>32.9 A</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>24.5 AB</td>
<td>2.7  aB</td>
<td>2.2 bB</td>
<td>6.4  aA</td>
<td>3.1  cA</td>
<td>24.5 AB</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>13.8 BC</td>
<td>1.3  cC</td>
<td>1.3 cC</td>
<td>5.3  abA</td>
<td>3.1  cA</td>
<td>13.8 BC</td>
</tr>
<tr>
<td></td>
<td>RGM</td>
<td>7.9  A</td>
<td>0.6  cB</td>
<td>0.6  cB</td>
<td>3.1  aC</td>
<td>3.1  aC</td>
<td>7.9  A</td>
</tr>
<tr>
<td></td>
<td>F-test:</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>1 week</td>
<td>SAV</td>
<td>5.1 abA</td>
<td>2.7  aB</td>
<td>2.2 bB</td>
<td>6.4  aA</td>
<td>3.1  cA</td>
<td>5.1 abA</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>6.4  A</td>
<td>0.6  cB</td>
<td>0.6  cB</td>
<td>3.1  aC</td>
<td>3.1  aC</td>
<td>6.4  A</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>5.3  abA</td>
<td>1.1  cC</td>
<td>1.1  cC</td>
<td>3.1  aC</td>
<td>3.1  aC</td>
<td>5.3  abA</td>
</tr>
<tr>
<td></td>
<td>RGM</td>
<td>3.1  bC</td>
<td>0.6  cB</td>
<td>0.6  cB</td>
<td>3.1  aC</td>
<td>3.1  aC</td>
<td>3.1  bC</td>
</tr>
<tr>
<td>2 weeks</td>
<td>SAV</td>
<td>2.1  A</td>
<td>1.6  aB</td>
<td>1.4  aC</td>
<td>1.6  aC</td>
<td>1.6  aC</td>
<td>2.1  A</td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>2.1  B</td>
<td>1.4  aC</td>
<td>1.1  abB</td>
<td>2.6  A</td>
<td>2.6  A</td>
<td>2.1  B</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>2.6  A</td>
<td>1.1  abB</td>
<td>0.7  bB</td>
<td>0.7  bB</td>
<td>0.7  bB</td>
<td>2.6  A</td>
</tr>
<tr>
<td></td>
<td>RGM</td>
<td>1.9  A</td>
<td>0.8  bBC</td>
<td>0.5  bC</td>
<td>0.5  bC</td>
<td>0.5  bC</td>
<td>1.9  A</td>
</tr>
</tbody>
</table>

F-test: n.s. * *** *** *** n.s. ***

* Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

All values are the average of three replicates. Decay corrected to the day of soil labelling.

ANOVA was calculated separately for each time, means followed by different lower case letters within one column at one time are significantly different (P<0.05) by Tukey’s test. The same is valid for means within one row followed by different upper case letters.
Conclusions

The effect of contrasting land-use systems on soil P fractions extracted by a modified Hedley et al. (1982) P sequential fractionation procedure was assessed in an Oxisol during a 2-week incubation on soils labelled with carrier free $^{33}$P. The quantities of $^{31}$P and $^{33}$P recovered in the different fractions were strongly dependent on the total P content of the soil, which was affected by the amount of P added as fertilizer and removed by plant P uptake.

In the two treatments fertilized annually with P and with a large positive P input-output balance, most of the P was stored in the resin-P, Bic-P, and NaOH-P, fractions. The use of carrier free $^{33}$P confirmed that, under all land-use systems studied, these soil P fractions contained most of the exchangeable P and that $^{33}$P was transferred from the soil solution first to the resin fraction and then to the Bic-P, and NaOH-P, fraction. This suggests that, when this Oxisol is regularly fertilized, P is stored in these three fractions while plants might take up P from the same fractions. In the two other treatments, which had either never been fertilized or had been fertilized only once at the beginning of the field trial, the transfer of $^{33}$P in these three fractions (i.e. resin-P, Bic-P, and NaOH-P) was less clear, suggesting that the soil P was much less exchangeable. In these soils, however, the transfer of $^{33}$P into organic P fractions was more important (up to 20% of the label was found in the organic P fractions two wks after labelling). As the pool sizes of these organic fractions did not change significantly over time of incubation, the label recovery indicates relatively quick cycling processes, probably as a result of microbial activity. In low P Oxisols, these processes are relevant and should be considered when estimating soil P availability for plants.

Acknowledgements

I thank to Dr. P.G. Weidler (ETH Zürich, ITÖ) for the XRD measurements, Mrs Roesch (ETH Institute for Plant Science) for measuring the Al and Fe concentrations and the field staff at CORPOICA-CIAT Carimagua research station for taking soil samples. This research was funded by ZIL (Swiss Centre for International Agriculture) and SDC (Swiss Development Cooperation).
General Conclusions
The general objective of this research project was to contribute to the knowledge about soil P availability, plant P uptake and P dynamics in tropical low P acid soils by the application of P isotopic exchange methodologies. These methodologies are widely used in temperate well P supplied soils, but rather few studies exist on the application in low P tropical soils. A short review of the existing literature is given in chapter 1 of this thesis. Most of them reported fundamental problems for the application of the methodology to low P acid soils (Amer et al. 1969, Wolf et al. 1986, Salcedo et al., 1991). A main purpose of this work was to test the isotopic exchange, according to the method of Fardeau (1996) and the newest state of knowledge, on soils posing the problems discussed in these published studies.

The application of the isotopic exchange methods, either in soil/solution suspension in a batch experiment (E value determination) or with Agrostis capillaris grown as test plant on labelled soils (L value determination) was tested on soils from two sites in Colombia (chapter 2).

**E values**

The results showed that the determination of the low \( C_p \) value remains a main limitation of the isotopic exchange method to determine E values. The limit of quantification at the 95 % probability level of the malachite green colorimetric method using a 1 cm cell was determined as 3.6 \( \mu g \text{ l}^{-1} \). As in highly P sorbing soils \( C_p \) values are often below this value, either cells with a longer light pathway or a concentration step on the soil solution have to be used. Both measures require relatively large samples of filtrated soil solution which are difficult to obtain.

A second main problem was shown to consist in the extrapolation of E values to several weeks or months from values measured until 100 minutes of isotopic exchange. On the soil with the lowest total P content (SAV), the function of the measured decrease of radioactivity in the soil solution reached an asymptotic value within a few weeks. This shows that the parameter of \( r_m/R \) in Equation (2.2), expressing the ratio of the applied label which remains in the soil solution at infinite time, is important already for short times of
isotopic exchange, whereas it might almost be neglectable in well P supplied soils (Fardeau et al., 1985). Due to this high impact on the extrapolation of r/R according to the calculation of Fardeau (1996), errors in the estimated r'/R, which is in turn depending on C_p and P_i, but also in the parameter n derived from the first 100 minutes of isotopic exchange, might lead to an incorrect estimation of E_t values. On the other hand, an extrapolation of the short time kinetic using a non linear fitting procedure to determine n, r'/R and r'/R was shown to result in very low precision, due to the limitation of an extrapolation on the base of four data points measured at t < 100 minutes, and can not be recommended. Therefore, the precision of the extrapolation of E_t is not high enough to use extrapolated E values as baseline to determine differences in the range of few mg P kg^{-1} soil as for organic P mineralisation or as reference value to deduce special P uptake mechanisms of plants from higher L than E values.

On the other hand, the E_t values calculated according to Fardeau (1996) using the P_i calculated as sum of the P_i fractions obtained from the sequential P extraction either with or without the residual P until 8 or 12 weeks of isotopic exchange were positively and highly significantly correlated to the L values determined with Agrostis capillaris, and both values were in the same order of magnitude. There was also a significant positive correlation between E and L values and other parameters of P availability, as soil P extractions, plant P uptake and biomass production. All these findings suggest that the method of isotopic exchange can be used, for soils with a C_p value > 4 μg l^{-1}, to assess plant P availability. Additionally, the single parameters describing the isotopic exchange in the batch experiment give valuable information about the P sorption capacity (r/R and n) and the intensity factor of soil P availability (C_p). This multiple information got in one experimental procedure is one of the main advantages of the isotopic exchange measurement in comparison to simple chemical extractions as Bray II or to P extraction with resins.

L values used for the identification of germplasm with adaptation to low P conditions

For the determination of L values (chapter 2 and 3), it was shown that it is not possible to get reliable results if the plant P uptake is lower than the P content in the seed. This shows a
fundamental problem of this method for the aspired purpose to identify crop or forage germplasm with specific adaptation to low P conditions. A compromise is required between, on the one hand, the attempt to reassure the P limiting conditions for plant growth and on the other hand the need to get a relative high P uptake by the plant to overcome the problem of seed P influence. In this work, it was also shown that the often suggested addition of carrier $^{31}$P with the label to get higher plant biomass and P uptake and to prevent specific sorption of the label (Truong and Pichot, 1976; Brookes, 1982) did not allow determining the uptake of soil P. Obviously, the addition of 10 mg P kg$^{-1}$ soil was high enough to modify the system. The nearly identical values of the specific activities of the plants and the applied carrier indicate that the carrier P was the main source for the P taken up by the plant.

The determination of L values might therefore be most suitable for plants with very small seeds or which can be cut several times. As the L value is derived from the specific activity of the P taken up by the plant, differences in L values between plants indicate that the specific activity of the P in the soil solution was diluted by the solubilisation or mineralisation of sparingly available inorganic or organic P forms. This was shown in this thesis for *Brachiaria decumbens*. With this forage grass the determined L value was higher than the total P$_i$ extracted with the sequential P fractionation method. This strongly suggests P uptake from mineralised organic P or solubilised recalcitrant P$_i$ forms.

*The estimation of the availability of residual P fertilizer by isotopic exchange techniques*

The comparison of increases in E values or L values (chapter 2) with the increase in total P due to fertilization on the two studied sites allowed to coarsely calculate the percentage of the residual fertilizer which remained isotopically exchangeable and therefore plant available on the long-term. The obtained high percentage of 50 to 70 % is in agreement with the observations of Friesen et al. (1997) who found high effectiveness of P inputs and low rates of fixation into more stable forms, using the sequential P fractionation method in one of the field trials (Culticore in Carimagua) also used for the present study. This shows a useful application of the isotopic exchange technique, as there is no other possibility to directly determine the availability of residual fertilizer P.
Sequential P fractionation of labelled soil

Oxisols from one site already studied in chapter 2, with different amounts of residual fertilizer P input, were labelled and afterwards extracted according to the sequential P fractionation methods described by Tiessen and Moir (1993) (chapter 4). By this, the assignment of chemical P fractions to pools of different availability, as well as the effect of land-use systems and related P fertilizer inputs on size of P fractions and their isotopic exchangeability was investigated. In two soils with annual fertilizer application and with large positive P input-output balance, most of the $P_i$ was stored in the resin-$P_i$, Bic-$P_i$, and NaOH-$P_i$ fractions. The recovery of the applied carrier free $^{33}P$ label in the different fractions at different times after labelling the soil showed that these $P_i$ fractions also contained most of the exchangeable P and that the $^{33}P$ was transferred from the soil solution first mainly to the resin-$P_i$ fraction and then to the Bic-$P_i$ and NaOH-$P_i$ fraction. This suggests also that there might not only occur isotopic exchange between soil solution and solid phase but that there are also exchange processes between sites with different properties on the soil solid phase.

In the soils without P fertilization (SAV) or only a single small P input (GL), the transfer of the $^{33}P$ to the resin-$P_i$, Bic-$P_i$, and NaOH-$P_i$ was less clear, reflecting the smaller size of these fractions than in the fertilized soils, and therefore also the lower amount of isotopically exchangeable $P_i$. In contrast, the transfer to organic fractions was more important, indicating the reactivity of organic P forms and their possible significance for plant P uptake. It might therefore be useful to further investigate the transformation of organic P compounds by the help of tracer techniques.

Outlook

In this work it was shown that the determination of E values can give valuable information about the P availability on soils with a $C_p$ value $> 4 \mu g l^{-1}$ and about the availability of residual fertilizer and that L values could be used, considering some restrictions, to compare plants for their specific ability to take up P from sparingly available P forms.
These two methods could therefore be used in future to get either information about the P availability in differently fertilized and managed systems (E value) and to contribute to the identification of plants with traits of adaptation to low P conditions (L value).

The growing world population and the need for increased food production requires either higher production on already cultivated land or the exploration of new land resources by cultivation of marginal lands or deforestation. A great potential for increased agricultural production lies in agro-ecosystems dominated by acid low P soils (von Uexküll and Mutert, 1995). However, there will also be higher needs for nutrient inputs, with P and N being the most limiting elements (Buresh et al., 1997). P inputs can only be given by fertilization, in contrast to N that can be fixed from the atmosphere and integrated into the system by planting N-fixing legumes. Application of processed mineral P fertilizers cannot be relied upon to reasonably redress P depletion because of economic and logistic reasons and because P is a limited resource. It would thus be desirable to identify plants accessing P forms that are not normally available to other plants. This would lead to an incorporation of this P in labile forms in the nutrient cycles of cropping systems, and thereby potentially reduce the need for application of soluble P fertilizers. These unavailable P forms could include native soil P but also sparingly available forms in unprocessed fertilizers such as rock phosphate. The adaptation to low P conditions could either be improved by classic plant breeding or genetic manipulation of the identified nutrient acquisition mechanisms (Johansen et al., 1995) to favorite mechanisms and traits that confer greater P acquisition through root architecture, mycorrhizal associations and exudates in the rhizosphere. Research on the genetic level have led to the identification of the regulation of ion transporters (Daram et al., 1998; Raghothama, 1999) and the encoding of excreted enzymes or exudates (Bassam et al., 1990). The determination of L values with such germplasm can give important information about the forms of P taken up from soil. However, it is recognized that more effective mining of total soil nutrient reserves is only a medium-term solution and that long-term sustainability will require compensation of nutrients removed from the system.

Altogether, the future improvement of the management of low P acid soils has most likely to be based on the combination of new technology, as genetic plant modification, with other
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