Chapter 3
The Use of Tracers to Investigate Phosphate Cycling in Soil–Plant Systems

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3.1 Introduction

Phosphorus (P) is indispensable for all living organisms and cannot be replaced in most of its biological functions. Whereas agricultural production is limited in many areas by the lack of available P, excessive P inputs in other agro-ecosystems result in the pollution of surface waters (Frossard et al. 2009). Furthermore, there are indications that the current reserves of rock phosphates that can be mined at a relatively low cost to be processed into fertilizers will be exhausted within the next century (Cordell et al. 2009). P use must therefore become much more efficient in the future. Concepts and management practices for a better crop P use efficiency of P derived from soil or from fertilizer will be based on a better understanding and quantification of soil–plant processes at different spatial and temporal scales.

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The use of tracers is relevant for studying the release of phosphate ions (Pi is used in this chapter as an abbreviation for phosphate ions, i.e., for H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}) into the soil solution, where these are available to plants and microbes (Fig. 3.1) for at least two reasons. Firstly, the total P content of a soil is usually at least one order of magnitude larger than the amount of P that is rapidly cycling in the soil–plant system and two to three orders of magnitude larger than the amount of P present as Pi in the soil solution (Frossard et al. 1995). Secondly, Pi undergoes many abiotic and biotic reactions in the soil (Fig. 3.1), some occurring within a few seconds, others over several years, and is (re)distributed in a large number of pools (Fardeau 1996; Bünemann and Condron 2007).

Phosphorus has one stable isotope (^{31}P), making up virtually 100% of the total P on earth, and seven radioactive isotopes. Only two radioactive isotopes, ^{32}P and ^{33}P, can be used in soil–plant and soil–solution studies. These radioisotopes have a relatively short half-life (14.3 days for ^{32}P and 25.3 days for ^{33}P) and emit β^- radiation with a maximum energy of 1.71 and 0.25 MeV for ^{32}P and ^{33}P, respectively (Endt 1990). These radioisotopes are introduced at extremely low rates in natural systems from the atmosphere (Benitez-Nelson and Buesseler 1999). For research needs, they must be artificially produced either from sulfur in nuclear reactors or by neutron activation of ^{31}P. These radioisotopes have been used for decades to probe P pools in soils, to evaluate reactions in which P is involved, and to

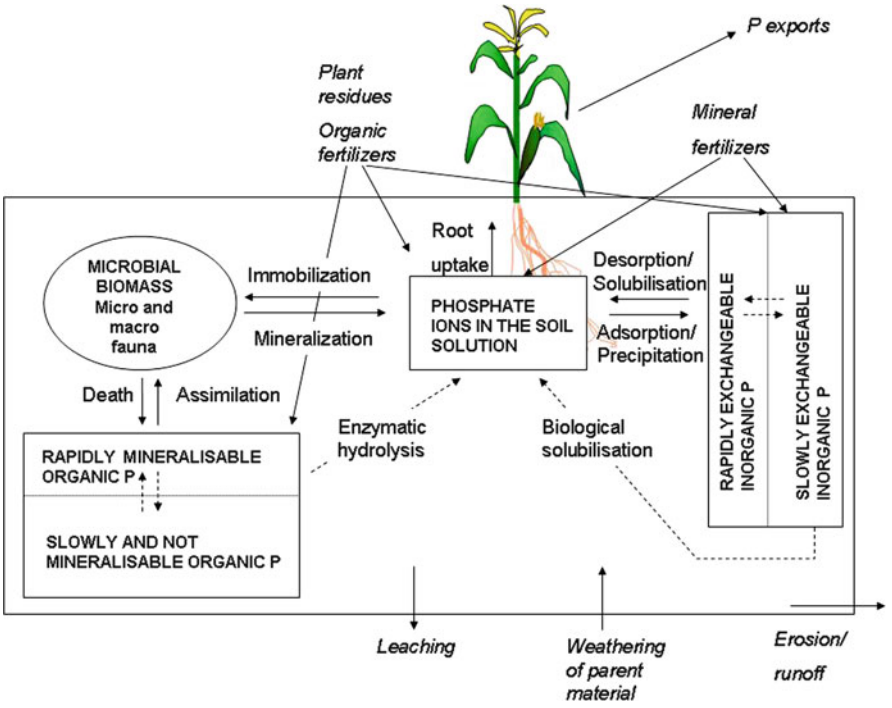

Fig. 3.1 The phosphorus cycle in the soil–plant system

trace the fate of P added as fertilizer from the soil to the plant (Dean et al. 1947; McAuliffe et al. 1947; Nelson et al. 1947; White et al. 1949; Wiklander 1950; Larsen 1952; Talibudeen 1957; Mattingly 1975; Fardeau 1996). But, given their relatively short half-lives, they can only deliver information on reactions for up to a few months, and since they are radioactive they are not easy to use under field conditions.

In soils, the P atom is always accompanied by oxygen (O) atoms in the form of phosphate (PO_4^{3-}, which is the dominant form in soils), phosphonate ($C\text{-}PO_3^{2-}$) or polyphosphate of different lengths [$(PO_3^{-})_n$] (Frossard et al. 2011). Recent publications have shown that variations in the natural abundance of the stable ^{18}O isotope bound to P (noted hereafter as $\delta^{18}O_P$) could deliver relevant information on P transformations and P sources in aquatic systems (Colman et al. 2005; McLaughlin et al. 2006a, b; Elsbury et al. 2009; Young et al. 2009; Jaisi and Blake 2010). Although some of these studies have addressed processes that are relevant to the soil–plant system, such as adsorption–desorption on oxides, precipitation of P-containing minerals, and uptake of phosphate by microorganisms (Jaisi et al. 2010; Liang and Blake 2007; Blake et al. 2005), this approach has not yet been successfully applied to soils. Among the problems slowing down progress in this direction are the lack of methods optimized for quantitatively isolating phosphate from the soil in a form that is amenable to $\delta^{18}O_P$ analysis, and the absence of plant studies.

The aims of this review are to present the use of P radioisotopes: (a) to probe pools and to study P transformations in soils, (b) to trace the fate of fertilizers in soil–plant systems, and (c) to assess the foraging strategies of arbuscular mycorrhizal fungi (AMF). In the last section, we will evaluate the potential of using the stable oxygen isotopes bound to P to study soil P dynamics. In each of these areas we will focus on the biological reactions controlling soil P transformations. These are (a) the uptake of P by soil microorganisms and plants (either through their roots or through mycorrhizal hyphae); (b) the release of Pi from the soil solid phase or from fertilizers, induced by the exudation of phosphatases and organic acids from roots or microorganisms; (c) the release of Pi from plant residues or organic fertilizers to the soil solution; and (d) the release of Pi from microorganisms and organic matter mineralization (Fig. 3.1).

3.2 Use of P Radioisotopes to Study Soil Processes and to Probe Soil P Pools

Many review articles have already been published on the use of P radioisotopes in soil science and plant nutrition (e.g., Wiklander 1950; Larsen 1952; Amer et al. 1969; Fardeau 1996; Di et al. 1997; Frossard and Sinaj 1997). Therefore, we will restrict our review to the principles of the main approaches used, their limits, and examples of their latest applications. We will show how the principles of isotopic

dilution can be used to assess the size of different soil P pools and to quantify the rate of transfer of P between these pools, first by the analysis of soil–solution systems and then by the analysis of soil–plant systems. Note that the methods presented in Sects. 3.2–3.4 of this manuscript can be used indifferently with both P radioisotopes (^{32}P or ^{33}P) and will give identical results because we assume that no significant isotopic fractionation occurs between ^{31}P, ^{32}P and ^{33}P (see the explanation in Sect. 3.2.3.2).

3.2.1 *Isotopic Dilution of P in Soil–Solution Systems: The Principles*

More than 60 years ago, McAuliffe et al. (1947) noted that the concentration of Pi labeled with ^{32}P (noted hereafter as ^{32}Pi) added to a soil–solution system decreased with time. They attributed this decrease to an exchange between ^{32}Pi in the solution and ^{31}Pi located on the solid phase of the soil. They interpreted their results by the presence of two P pools, one containing the fraction of readily exchangeable P and the other the fraction of less-exchangeable P. Later, Wiklander (1950) noted that after the addition of ^{32}P to a soil suspension that was at steady-state for Pi, the concentration of ^{32}Pi in solution decreased with time according to a simple power function (3.1). Fardeau (1996) confirmed that this equation was able to describe the decrease of radioactivity in soil–solution systems for many soils for isotopic exchange times as long as 3 months:

$$r_{(t)}/R = \left[r_{(1)}/R\right] t^{-n} \tag{3.1}$$

where R is the total radioactivity introduced in the soil–solution system as ^{32}Pi (or ^{33}Pi) (units are megabecquerel, MBq,), $r_{(t)}$ is the radioactivity remaining in the solution after t minutes of isotopic exchange, $r_{(1)}/R$ is the proportion of the total radioactivity remaining in the solution after 1 min of isotopic exchange, and n is a parameter describing the rate of decrease of radioactivity in the solution. Both $r_{(1)}/R$ and n vary with the concentration of Pi in the soil solution. Soils with a $r_{(1)}/R$ lower than 0.2 are usually considered to have a high sorbing capacity for Pi (Frossard et al. 1993). Parameters $r_{(1)}/R$ and n calculated from (3.1) for selected soils from Switzerland and Madagascar are shown in Table 3.1.

Equation (3.1) shows that Pi labeled with ^{32}P added in the soil–solution system are diluted in a large pool of ^{31}P. This can be interpreted as a homo-ionic exchange between the ^{32}Pi added in the solution and Pi located on the solid phase of the soil that can be desorbed (Fardeau 1996). The fact that this equation is valid for exchange times as long as 3 months shows that a much longer time is needed in most soils to reach a true isotopic equilibrium, which has been confirmed by sorption–desorption experiments (e.g., Torrent 1987). This is why Fardeau (1996), following the ideas of McAuliffe et al. (1947), proposed the

Table 3.1 Examples of results obtained from isotopic exchange kinetic experiments in Swiss (Cadenazzo, Ellighausen, Changins and Vaz) and Malagasy (Betafo, Ivory, Laniera, Lazaina) soils

Soil	Soil type	$r_{(1)}/R$ or m	n	C_P (mg P L^{-1})	T_m (min)	g_m (min^{-1})	F_m (mg P kg^{-1} min^{-1})	$E_{(1\ min)}$ (mg P kg^{-1})	$E_{(1\ day)}$ (mg P kg^{-1})	Total inorganic P (mg P kg^{-1})	Total P (mg P kg^{-1})
Cadenazzo[a]	Fluvisol	0.43	0.32	0.23	0.23	4.3	9.9	5.29	54	910	1,174
Ellighausen[a]	Cambisol	0.26	0.37	0.12	0.07	13.6	16.4	4.56	67	287	789
Changins[a]	Cambisol	0.15	0.41	0.04	0.02	40.9	16.4	2.64	52	199	590
Vaz[a]	Fluvisol	0.71	0.25	0.77	1.04	0.96	7.4	10.8	66	358	1,370
Betafo[b,c]	Andosol	0.002	0.57	0.0008	3.2×10^{-5}	3.1×10^{4}	2.5×10^{2}	3.93	117	218	864
Ivory[b,c]	Ferralsol	0.014	0.36	0.0019	2.0×10^{-5}	5.1×10^{4}	9.7×10^{2}	1.32	13	46	161
Laniera[b]	Ferralsol	0.016	0.37	0.013	3.8×10^{-5}	2.6×10^{4}	3.4×10^{3}	7.18	41	62	220
Lazaina[b]	Ferralsol	0.033	0.43	0.011	8.3×10^{-4}	1.2×10^{3}	1.3×10^{2}	3.05	24	36	165

The mean residence time of Pi in the solution (T_m), the mean turnover rate (g_m) and the mean flux of Pi between the solid phase and the solution (F_m) were calculated for all soils as described in Fardeau et al. (1991)

[a]These results come from Gallet et al. (2003a). The samples were taken in 1998, in the 0–20 cm horizon of a treatment that had not received phosphate fertilizers since 1989. The parameters [$r_{(1)}/R$ and n] describing the kinetics of isotopic exchange were calculated from (3.1)

[b]The parameters (m and n) describing the kinetics of isotopic exchange were calculated from (3.2) and the ratio $r_{(\infty)}/R$ was estimated as the ratio 10 C_P/total inorganic P

[c]The concentration of Pi in the solution was measured in these samples after a concentration of the phosphomolybdate complex in hexanol

characterization of this isotopic exchange through its kinetic parameters [$r_{(1)}/R$ and n] instead of measuring radioactive and stable Pi in the soil solution after a single exchange time. These parameters can be obtained from the isotopic exchange kinetic experiment. In this experiment, a known amount of Pi labeled with ^{32}P or ^{33}P (the tracer) is introduced into a soil–solution system (10 g of soil in 100 mL water) at steady-state equilibrium for Pi (the tracee). After different exchange times of up to 2 h, small volumes of the soil–water suspension are extracted with a syringe, filtered at 0.2 μm, and the tracer and the tracee in solution measured.

As (3.1) does not fit the decrease of radioactivity in the solution in all cases, Fardeau et al. (1985) adapted it as follows:

$$r_{(t)}/R = m\left(t + m^{1/n}\right)^{-n} + r_{(\infty)}/R \tag{3.2}$$

where m and n are parameters determined by nonlinear regression, and $r_{(\infty)}$ the amount of radioactivity that would remain in the solution at isotopic equilibrium. The term $r_{(\infty)}/R$ is operationally estimated as the ratio of the concentration of Pi present in the soil solution expressed in milligrams of P per kilogram of soil to the total inorganic soil P also expressed in milligrams of P per kilogram of soil (Fardeau 1993). The term $r_{(\infty)}/R$ can in some cases be neglected (Achat et al. 2009a, b). Both m and n vary with the concentration of Pi in the soil solution and other factors.

Equations (3.1) and (3.2) can be interpreted as a sum of many exponentials, which demonstrates that the tracer enters in a large number of compartments (Atkins 1969; Probert and Larsen 1972; Diesing et al. 2008). These equations reflect two approaches to the statistical description of the decrease of radioactivity in solution with time (Fardeau 1981). Therefore, especially when working with long-term kinetics (i.e., with isotopic exchange times longer than 1 day), it is necessary to evaluate which of these equations gives the best description of the radioactivity decrease in solution (Bünemann et al. 2007).

Fardeau et al. (1991) derived from (3.2) the mean turnover rate of Pi in the soil solution (g_m in min^{-1}), the mean residence time of Pi in the soil solution (T_m in min^{-1}), and the mean flux of Pi between the solid phase of the soil and the solution (F_m mg P kg^{-1} soil in min^{-1}). The principle underlying these calculations is not explained here, but some results for selected soils are presented in Table 3.1. These results suggest that the mean residence time of Pi in the soil solution is in most cases shorter than 1 min. The isotopic dilution of the tracer introduced in the soil is, in this experiment, mostly caused by its exchange with Pi located on the solid phase of the soil. It is important to note that this exchange refers to the swapping of intact phosphate groups and does not involve any breaking of the P–O bonds. The tracer can, however, also be diluted by a release of non-labeled Pi to the solution (e.g., from an added fertilizer) or as a result of soil organic P mineralization or soil inorganic P solubilization, as discussed below.

If we assume that ^{31}Pi, ^{32}Pi and ^{33}Pi have exactly the same behavior in the soil–solution system, then the specific activity of Pi in the soil solution, i.e., the ratio

between radioactivity and mass in the solution, is identical to the specific activity of the Pi that has been isotopically exchanged in the entire system and that is noted as $E_{(t)}$ hereafter (3.3):

$$r_{(t)}/(VC_{\mathrm{P}}) = R/E_{(t)} \tag{3.3}$$

where C_{P} is the concentration of Pi in the soil water extract (in mg P L^{-1}), V is the water to soil ratio (in L kg^{-1}), and $E_{(t)}$ is the amount of isotopically exchanged P within t minutes of exchange (in mg P kg^{-1} soil). This equation is only valid for exchange times shorter than the time that would be necessary to exchange the entire amount of soil inorganic P, including the Pi present in the soil solution.

The $E_{(t)}$ value is the sum of Pi in the solution and of Pi located on the solid phase of the soil that can exchange with Pi in the solution within a time t. It therefore yields the total amount of Pi that can potentially be taken up by a plant or a microbe within this time. In their critical review, Hamon et al. (2002) agree that authors assessing the availability of Pi can indeed use (3.3) to calculate the amount of isotopically exchangeable P. Hamon et al., however, state that researchers interested in measurement of the soil buffering capacity for Pi, or in assessing the effect of soil physicochemical properties on phosphate ion exchangeability, should specifically calculate the fraction of exchangeable phosphate present on the solid phase of the soil, leaving aside the fraction of Pi present in the soil solution. This calculation yields the amount of Pi present on the solid phase that can diffuse into the soil solution with time in response to a decrease in the concentration of Pi in the solution in the absence of microbial activity, a process that is known as "desorption" in environmental chemistry (Tan 1993). This amount of desorbable Pi has been named *Pr* (expressed in mg P kg^{-1} soil) by Ehlert et al. (2003). It is calculated using the following equation (Hamon et al. 2002; Ehlert et al. 2003; Stroia et al. 2007; Achat et al. 2009b):

$$\mathrm{Pr} = E_{(t)} - VC_{\mathrm{P}} = VC_{\mathrm{P}}\left[R/r_{(t)} - 1\right] \tag{3.4}$$

3.2.2 *Isotopic Dilution of Phosphate Ions in Soil–Solution Systems: Selected Applications*

3.2.2.1 Assessment of Availability of Phosphate Ions for Plants

Results of the isotope exchange kinetics experiment suggest that it is not possible to divide soil total inorganic P into an available P pool and a non-available P pool but, on the contrary, that most of the inorganic P can become available at some point in time. Although some inorganic P (a very small amount) will be very rapidly exchangeable with Pi in solution and therefore be very rapidly available, the vast majority of inorganic P will be very slowly exchangeable (Fardeau

1993). These results agree with those obtained earlier by Barrow (1974, 1983, 1991) and Barrow and Shaw (1975a, b) who, on the basis of long-term sorption experiments, concluded that inorganic P ions located on the solid phase of the soil are distributed along a continuum of solubility, some being in rapid equilibrium with Pi in the solution and some being in very slow equilibrium with Pi in the solution.

The isotopic exchange kinetic experiment provides information on the concentration of P in the soil solution (C_P), on the amount of P that is potentially available to plants [$E_{(t)}$] and on the soil buffer capacity, which expresses the changes in desorbable Pi when the concentration of P in the solution varies (dPr/dC_P; Hamon et al. 2002; Stroia et al. 2007). The concentration of Pi in the solution (C_P) and E values calculated for 1 min and 1 day are shown in Table 3.1 for selected soils from Switzerland and Madagascar. Soils with a $E_{(1\ min)}$ lower than 5 mg P kg^{-1} soil are considered to be phosphate-limiting for crops (Gallet et al. 2003a). The buffer capacity is, however, not straightforward to compute because the parameters used for the calculation of desorbable Pi are C_P-dependent (Morel et al. 1994; Achat et al. 2009a).

Morel et al. (2000) and Stroia et al. (2007) working on soils, and Nemery et al. (2005) working on sediments, succeeded in modeling the changes in desorbable Pi for a wide range of concentrations of Pi in the soil solution and for different exchange times using a kinetic Freundlich equation (3.5):

$$Pr = vC_P{}^w t^p \tag{3.5}$$

where v, w and p are parameters determined by nonlinear regression. This equation is valid when desorbable Pi is lower than the difference between total inorganic soil P and soil solution P. These parameters are obtained by conducting isotopic exchange kinetic experiments over short periods in the same soil with increasing concentrations of Pi in the solution. An example of results obtained with this approach is given in Fig. 3.2.

Using this kinetic Freundlich equation parameterized in batch experiments combined with a mass balance model, Stroia et al. (2007) successfully showed that the removal of 1 kg P ha^{-1} would be buffered by desorbable Pi over a few weeks in a Luvisol and a Brunisol. Their approach showed that the changes in Pi concentration in the solution were larger in the Brunisol than in the Luvisol. In the Luvisol, their modeled final C_P value was almost identical to the final C_P value derived from the field experiment for the removal of 1 kg P ha^{-1}. In the Brunisol, however, the final modeled C_P value was three times higher than the final C_P value derived from the field experiments. This shows that the approach taken by Stroia et al. (2007) is promising for prediction of the changes in C_P for different P balances, but it should be completed by taking into account other mechanisms that can affect the concentration of Pi in the soil solution, such as the transfer of P to deeper horizons or the uptake of Pi by soil microbes.

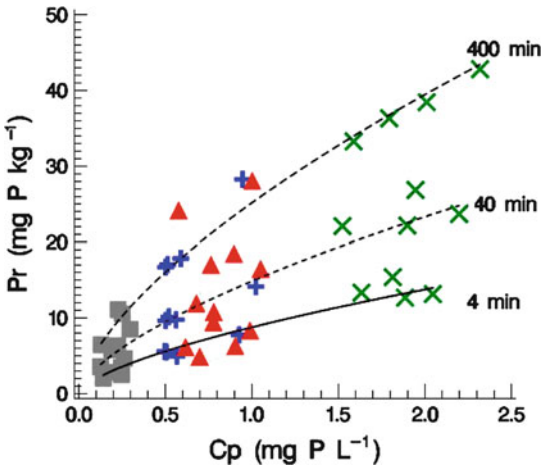

Fig. 3.2 Experimental and modeled values of desorbable Pi (Pr) of soil samples as a function of Pi ion concentration in solution (C_P) and time (Morel 2002). Soils were collected after 17 years of experimentation from the plough layer of a long-term field experiment established on a neutral, non-carbonaceous, sandy loamy soil. It was located close to the village Mant in the south-west of France. Four treatments were repeated four times in blocks. Phosphate was added as supertriple phosphate. The *symbols* represent experimental values: *squares* no P; *plus symbols* on average 27 kg P ha^{-1} applied every year; *times symbols* on average 79 kg P ha^{-1} applied every year; *triangles* on average 52 kg P ha^{-1} applied every 2 years. *Lines* show values after 4, 40, and 400 min of isotopic exchange calculated by the following Freundlich equation: $Pr = 6.4 \times {C_P}^{0.65} \times t^{0.23}$ (48 observations, $r^2 = 0.96$)

3.2.2.2 Quantification of Phosphate Mineralized from Soil Organic Matter and from Soil Microorganisms

Isotopic dilution has been used to quantify the amount of P mineralized from soil organic matter and from microbial P (Walbridge and Vitousek 1987; Oehl et al. 2001a; Bünemann et al. 2007; Achat et al. 2009b). The basic principle of the approaches followed by these authors is sketched in Fig. 3.3.

Let us consider a soil in which isotopically exchangeable P has been labeled with ^{32}Pi or ^{33}Pi but in which organic P has not yet been labeled (this applies to the first days after labeling when no fresh organic matter has been introduced). The release of ^{31}Pi induced by the mineralization of organic P will dilute the labeled exchangeable Pi, resulting in an increase in isotopically exchangeable Pi [$E'_{(t)}$]. This increase will be stronger than the increase over time caused solely by physicochemical reactions [$E_{(t)}$]. The difference between these two values [$E'_{(t)} - E_{(t)}$] will deliver the amount of Pi that has been released from soil organic matter through mineralization within a given time t.

The $E'_{(t)}$ value is measured in a biologically active soil labeled with ^{32}Pi or ^{33}Pi during an incubation experiment, whereas the $E_{(t)}$ value reflecting the physicochemical reactions can be estimated from the results of a short-term isotopic exchange kinetics experiment extrapolated to an exchange time equivalent to the

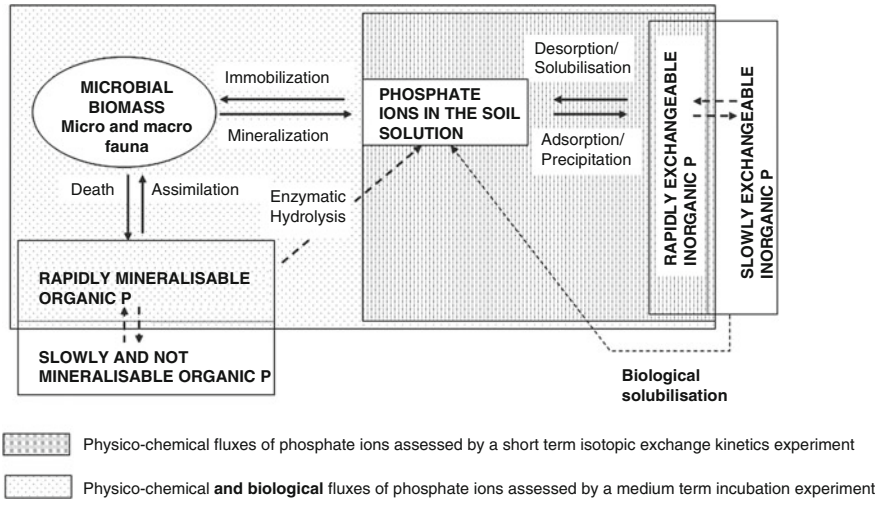

Fig. 3.3 Organic P mineralization is calculated by subtracting the amount of isotopically exchangeable Pi related solely to physicochemical reactions obtained from a short-term batch experiment and extrapolated for a period of, e.g., 14 days, from the amount of isotopically exchangeable Pi measured after a 14-day incubation experiment in which physicochemical and biological reactions were allowed to take place (adapted from Achat et al. 2009b)

duration of the incubation experiment (López-Hernández et al. 1998). Although the principle is simple, its application is more delicate because it requires that all compartments connected to the Pi in the soil solution have a constant isotopic composition. Oehl et al. (2001a) and Bünemann et al. (2007) evaluated each of the steps involved in this approach and made recommendations for the measurement of soil organic P mineralization that should be followed in order to obtain meaningful results. The experiment should be done under steady-state equilibrium for Pi and carbon, i.e., in the absence of rapidly degradable organic matter, at constant and low respiration rate and at constant C_P. They recommend restricting the incubation to 14 days to avoid re-mineralization of organic P compounds that would have been labeled. For each soil, the agreement of measured and extrapolated $E_{(t)}$ values must be checked against the sterilized soil in a batch experiment and in an incubation experiment. The gross P mineralization rate is to be determined by comparing the $E_{(t)}$ values extrapolated from a short-term batch experiment to the $E'_{(t)}$ values measured in a 7–14-day incubation experiment using pre-incubated non-sterile soil. The amount of P immobilized in the microbial biomass during the incubation must then be determined from the amount of microbially held radiolabeled P and ^{31}P, so as to calculate the net P mineralization (gross P mineralization − microbial immobilization). Finally, the detection limit for P mineralization has to be determined for each soil. The work of Bünemann et al. (2007) yielded gross mineralization rates of 0.9–1.2 mg P kg^{-1} soil day^{-1} and net mineralization rates of 0.5–0.9 mg P kg^{-1} soil day^{-1}. The gross mineralization rate measured over 24 h

in this study was equivalent to 8–11% of the amount of Pi exchangeable over 24 h [$E_{(24\ h)}$]. Oehl et al. (2004) studied gross mineralization rates of organic P as affected by different cropping systems in a long-term field experiment and showed that gross mineralization reached rates that were equivalent to 5–9% of the amount of Pi exchangeable within 24 h. Although these rates might seem low, they might contribute substantially to plant nutrition in low-input agro-ecosystems or natural ecosystems.

Comparison of the specific activity of soil solution Pi and P held in the microbial biomass was also used to study the kinetics of microbial P uptake and cycling at either constant or changing size of the microbial biomass (Oehl et al. 2001b). This application revealed rapid microbial P turnover that was affected by the cropping system (see Oberson et al. 2011). The addition of glucose and nitrogen resulted in almost complete re-mineralization of microbial P after 70 days of incubation.

Achat et al. (2009b) further adapted the approach proposed by Bünemann et al. (2007) to a sandy forest soil with a very low total P content (31 mg P kg^{-1}), of which 77% was in "dead" soil organic matter, 17% in the microbial biomass, and 6% as inorganic P. The authors measured phosphate fluxes during a 154-day incubation experiment. They estimated the amount of desorbable Pi, the gross mineralization of microbial P, and the gross mineralization of P in dead soil organic matter. The gross mineralization of total organic P (defined by Achat et al. as the sum of P in dead soil organic matter and in the soil microorganisms) was calculated using the difference $E'_{(t)} - E_{(t)}$. The mineralization of microbial P was calculated from the P uptake in the microbial biomass (microbial ^{33}P and P) and from the net decrease in microbial P. The gross phosphate release from dead soil organic matter was calculated as the difference between the gross mineralization of total organic P minus the mineralization of microbial P, or was estimated from the gross carbon mineralization. Using this approach, Achat et al. (2009b) concluded that the gross mineralization rate of total organic P and the increase in Pi concentration in the soil solution were essentially related to the mineralization of microbial P while the release of P from dead soil organic matter and from mineral surfaces remained low. This is to our knowledge the first time that these different fluxes have been quantified in the same experiment. This work shows also that although long-term incubations are difficult to conduct, they can be interesting if one wants to homogeneously label the soil microbial biomass (Achat et al. 2010). This should allow measuring the rate of organic P mineralization from dead organic matter over longer periods (i.e., going beyond the period of 7–14 days proposed above), which would be more realistic for the study of P uptake by plants in agro-ecosystems.

Similar research (assessment of soil organic P and microbial P mineralization and immobilization rates) needs to be done in soils with a high sorbing capacity for Pi since they often have a low to very low P availability. Such analyses could also be considered in order to monitor soil quality, e.g., for soils under different cropping systems or for soils that have received various rates and types of organic or inorganic pollutants.

3.2.3 *Isotopic Dilution of Phosphate Ions in Soil–Solution Systems: Limits and Answers*

Important considerations for correctly interpreting data from isotopic exchange kinetic experiments have been covered in earlier publications (Atkins 1969; Fardeau 1981; Cobelli et al. 2000). These points are the following: (a) the introduction of the tracer (^{32}Pi or ^{33}Pi) should not modify the mass balance of the tracee (^{31}Pi) (i.e., the tracer should add no mass, therefore it should be added carrier-free); (b) the tracer and the tracee must have the same behavior in the soil–solution system, which means that the tracer must be added in the chemical form of the tracee to be studied (here as Pi); (c) the tracer and the tracee must be measured in the same, accessible, compartment (e.g., the soil solution); and (d) the soil–solution system must be at steady-state equilibrium for the tracee when the tracer is introduced. In Sect. 3.2.3.1 and 3.2.3.2, we discuss several potential problems of isotopic dilution techniques and show solutions.

3.2.3.1 Measuring Very Low Concentrations of Phosphate Ions in Solution

Measuring isotopically exchangeable phosphate is extremely difficult in soils that contain very low concentrations of Pi in the soil solution (Salcedo et al. 1991; Hamon and McLaughlin 2002; Bühler et al. 2003; Maertens et al. 2004). This case is often met in highly weathered tropical soils where the very low concentration of available Pi strongly limits plant growth. Different strategies have been proposed to measure these very low concentrations. Phosphate can be concentrated by evaporating the aqueous extract (Bühler et al. 2003), by adding into the aqueous extract a resin that is then eluted in a smaller volume (Salcedo et al. 1991; Hamon and McLaughlin 2002), or by adding a molybdate solution in a high volume of soil water extract and concentrating the phosphomolybdate complex in isobutanol (Pons and Guthrie 1946; Jayachandran et al. 1992a). Two of us (Lalajaona Randriamanantsoa and Christian Morel) recently adapted a standard method (NF EN 1189: Qualité de l'eau. Dosage du phosphore, AFNOR) used to measure low concentrations of Pi in environmental waters for measuring low Pi concentrations in soil water extracts. The method consists in developing the blue phosphomolybdate complex in a high volume of soil extract and subsequently concentrating it in hexanol. When combined with a measurement in a 10-cm cell, this last approach yields a detection limit of 0.3 μg P L^{-1} and a quantification limit of 0.8 μg P L^{-1}, which are lower than those previously reported, e.g., by Bühler et al. (2003). This method needs, however, to be miniaturized because it currently needs large volumes of soil extracts. Another approach to solve this problem is to extract the labeled soil–solution system with a HCO_3^--saturated resin as proposed by Maertens et al. (2004). Because Schneider and Morel (2000) reported that the difference in isotopically exchangeable phosphate measured before and after soil extraction with a HCO_3^--saturated resin was identical to the amount of Pi extracted by the resin, we can assume that the Pi extracted by the HCO_3^--saturated resin and

in the soil solution belong to the same compartment. But, before generalizing, this remains to be tested on other soils using isotopic tracers.

3.2.3.2 Are P Radioisotopes Irreversibly Fixed in Soils That Sorb Very High Amounts of Phosphate Ions?

Wolf et al. (1986) suggested that an unknown but significant fraction of the added ^{32}Pi would be irreversibly fixed in soils that sorb very high amounts of Pi, thereby reducing the fraction of radioactivity really participating in the isotopic exchange. They concluded that isotopic dilution would not be a useful tool for assessing phosphate availability in these soils. In other words, they assumed that ^{31}Pi and ^{32}Pi would have a different behavior in the soil–solution system, ^{32}Pi being preferentially sorbed on soil. To our knowledge there is no published information on the sorption of the different P isotopes on soil particles.

We checked the hypothesis that no significant isotopic discrimination would occur between ^{32}Pi and ^{33}Pi during isotopic exchange kinetic experiments in the presence of a strong adsorbant for Pi. This hypothesis sounds reasonable because at a pH close to 5 (e.g., typical for Ferralsols) the mass difference between 1 mol of $H_2{}^{32}PO_4^-$ and 1 mol of $H_2{}^{33}PO_4^-$ would be 1/98 g. If this hypothesis can be confirmed, then we will deduce that there will be little isotopic discrimination between ^{31}Pi and ^{32}Pi and between ^{31}Pi and ^{33}Pi.

A concentration of 0.25 mM of KH_2PO_4 was added in 100 mL of H_2O or 0.05 M $NaNO_3$ that was bathing 1 g of synthetic goethite prepared according to the protocol of Schwertmann and Cornell (2000). The pH of the solutions was adjusted to 4.5 at the beginning of the experiment. The phosphate–goethite suspensions were shaken overnight. The following day, carrier-free ^{32}Pi and ^{33}Pi were added simultaneously to the suspensions and isotopic exchange kinetics experiments were performed. The experiment was conducted in four replicates. The concentration of Pi in the solution measured after concentrating the phosphomolybdate complex in hexanol was 1.2 μg P L^{-1} in water and 1.7 μg P L^{-1} in $NaNO_3$ (Table 3.2). Figure 3.4 shows that the proportion of radioactivity remaining in the water–phosphate–goethite system decreased slightly more rapidly with ^{33}Pi than with ^{32}Pi, whereas almost identical kinetics were observed in the presence of $NaNO_3$. The $r_{(1)}/R$ parameter was lower with ^{33}Pi than ^{32}Pi in water, whereas the n values were identical. In $NaNO_3$ the n value was lower with ^{32}Pi, whereas the $r_{(1)}/R$ values were identical for both radioisotopes. The $E_{(t)}$ values varied accordingly (Table 3.2). The differences observed in $r_{(1)}/R$ and n could be ascribed to a slight preferential adsorption of the heavier isotope on the goethite. These differences could also be due to the presence of small quantities of ^{32}P-labeled pyro- or polyphosphates in the ^{32}Pi solution, which would not exchange at the same rate as Pi (McBeath et al. 2009). Such chemical contamination has been observed earlier with ^{32}P that had been produced by neutron activation performed at too-high temperatures (Jean-Claude Fardeau, personal communication). Altogether, these values remain very similar. Therefore, although a slight discrimination between the P radioisotopes can occur, especially

Table 3.2 Parameters of the isotopic exchange kinetic experiment [$r_{(1)}/R$ and n], the concentration of Pi (C_P) in the solution measured after pre-concentrating the phosphomolybdate complex in hexanol, and the amount of Pi isotopically exchangeable within 1 min and 1 day [$E_{(1\ min)}$, $E_{(1\ day)}$] calculated for water–phosphate–goethite suspensions and $NaNO_3$–phosphate–goethite suspensions

Suspension	$r_{(1)}/R$	n	C_P (μg P L^{-1})	$E_{(1\ min)}$ (mg P kg^{-1})	$E_{(1\ day)}$ (mg P kg^{-1})
^{32}Pi–water	0.01 ± 0.0002	0.30 ± 0.06	1.2 ± 0.01	10.6 ± 0.9	82.4 ± 9.7
^{33}Pi–water	0.008 ± 0.0001	0.30 ± 0.02	1.2 ± 0.01	12.8 ± 1.2	103 ± 22
^{32}Pi–$NaNO_3$	0.007 ± 0.00004	0.36 ± 0.03	1.7 ± 0.02	25.9 ± 0.6	321 ± 69.9
^{33}Pi–$NaNO_3$	0.007 ± 0.00007	0.40 ± 0.01	1.7 ± 0.02	23.4 ± 0.1	426 ± 38.4

The water–phosphate–goethite suspensions (^{32}Pi–water and ^{33}Pi–water) and $NaNO_3$–phosphate–goethite suspensions (^{32}Pi–$NaNO_3$ and ^{33}Pi–$NaNO_3$) contained 7.75 mg P g^{-1} goethite simultaneously spiked with ^{32}Pi and ^{33}Pi. Means of four replicates are given with their standard deviation. The parameters $r_{(1)}/R$ and n were calculated by fitting the decrease of radioactivity with time shown in Fig. 3.4 with (3.1)

when the concentration of Pi in the solution is very low, we conclude that this is not the most important issue to be solved when conducting isotope exchange kinetics studies in soils that contain low concentrations of Pi in the soil solution. The most important problem is the determination of these low P concentrations (see Sect. 3.2.3.1).

3.2.4 *Application of Isotopic Dilution Techniques to Soil–Plant Systems*

The above-presented principles can be applied to soil–plant systems. In this approach (Larsen 1952), plants are grown on a soil in which exchangeable Pi have been labeled with radioactive Pi. If the fractions of stable and radioactive P taken up by the plants remain small compared to the amounts of stable and radioactive P present in the soil, then the measurement of the specific activity of P in the aerial parts of the plant is equal to the specific activity of the pool of soil exchangeable P in which the added radioactivity has been diluted (3.6), which is called the L (L as in Larsen) value:

$$SA_{plant} = SA_{soil} = R/L \tag{3.6}$$

where SA_{plant} is the ratio between the amount of radioactive P recovered in the plant (in Bq kg^{-1} soil) and the amount of ^{31}P taken up by the plant coming from the soil (in mg P kg^{-1} soil), and SA_{soil} is the ratio between the total amount of radioactive P (R) added as carrier-free labeled Pi to the soil (in Bq kg^{-1} soil) and L, the amount of Pi that has undergone isotopic exchange within the duration of plant growth (in mg P kg^{-1} soil).

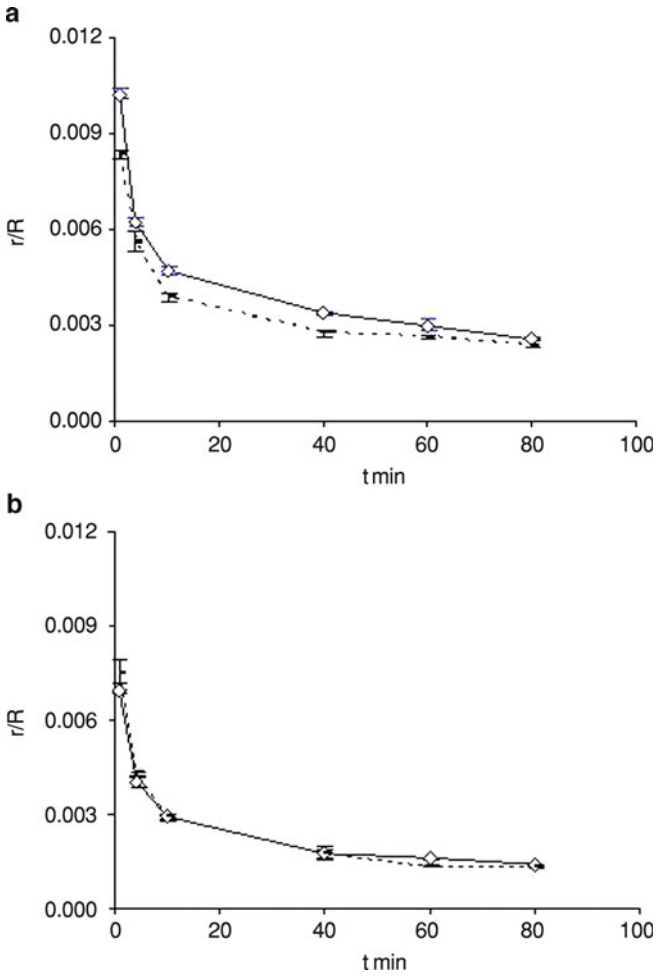

Fig. 3.4 Changes in the proportion of ^{32}P (*continuous line* and *diamonds*) and ^{33}P (*dashed line*) remaining in the solution (r/R) with time (t, expressed in minutes) in (**a**) water–phosphate–goethite suspension and (**b**) $NaNO_3$–phosphate–goethite suspension. *Standard deviations* are shown for each point. Both suspensions had an initial concentration of Pi of 0.25 mmol, were shaken overnight, and were then simultaneously spiked with ^{32}Pi and ^{33}Pi. The $r_{(1)}/R$ and n parameters derived from these curves are shown in Table 3.2

A major problem of this method is to distinguish between the amount of P in the aerial plant parts taken up from the soil or the soil–fertilizer mixture and the amount of P derived from the seed or the planting material (Russell et al. 1957; Truong and Pichot 1976; Brookes 1982). Because seed P or P in the planting material is not labeled, a high transfer of seed P to the aerial plant parts results in an L value that is overestimated. This problem is particularly acute when plants with large seeds (maize, legumes) are grown in soils that contain very little available phosphate

(Pypers et al. 2006). An elegant method to account for the transfer of seed P to the aerial parts has been published by Pypers et al. (2006). They achieved this by growing maize and cowpea (*Vigna unguiculata*) in sand in the presence of increasing concentrations of Pi labeled with ^{32}P. Comparison of the specific activity of P in the plant and in the nutrient solution allowed calculation of the amount of P transferred from the seed to the shoot for any plant P level. Afterwards, regression equations can be used to derive the amount of P derived from the seed for any amount of P accumulated in the shoots. However, the parameters of these regression equations must be determined anew for each plant species and genotype. Another approach is to use a plant with very small seeds (i.e., containing very little P) that can be cut several times, such as *Agrostis tenuis* (Truong and Pichot 1976). With this approach, the amount of P in the plant derived from the seed will be negligible from the third cut on and the results can be used to calculate the *L* value.

For most plants, growing in the presence or absence of AMF, the measured amount of isotopically exchangeable phosphate is similar to that extrapolated from short-term isotopic exchange kinetic experiments conducted in soil–solution systems for an exchange time equivalent to the duration of plant growth (Fardeau and Jappe 1976; Frossard et al. 1994; Morel and Plenchette 1994; Bühler et al. 2003). This confirms that isotopically exchangeable phosphate is the main source of P for these plants. For other plants, however, *L* values can be higher than *E* values. This has been observed, e.g., for rape by Hedley et al. (1982), white lupin (Braum and Helmke 1995), and cowpea (Pypers et al. 2006) in soils containing very little available phosphate. This points to the fact that these plants are able to access P pools other than the isotopically exchangeable phosphate pool. A large body of work reviewed by Neumann and Martinoia (2002) has since confirmed that white lupin, when grown under P-limiting conditions, is able to exude protons, citric acid, and phosphatase from its cluster roots and, therefore, is able to dissolve insoluble calcium phosphate and to hydrolyze organic P compounds.

3.3 Use of P Radioisotopes to Trace the Fate of P Sources in the Soil–Plant System

3.3.1 Labeling the P Source (Direct Labeling Technique)

Labeled P fertilizers have been used in agriculture for over 60 years (Dean et al. 1947; Nelson et al. 1947; Spinks and Barber 1947). At the time, these experiments were called the "Green Cheese Experiments," showing that they were considered with some skepticism. It is interesting to see how famous the majority of these "first" experimenters became!

Phosphate transfer from mineral or organic sources (e.g., mineral P fertilizer, plant residues, microbial bodies, organic compounds) to a plant can easily be studied

when the source of P is homogenously labeled with radioactive P. Measuring the amount of radioactive and stable P in a plant allows, knowing the specific activity of the source of P, the calculation of the amount of P in the plant that is derived from the fertilizer. The proportion of P in the plant that is derived from a P fertilizer (%Pdff) can be calculated with (3.7) when using the direct labeling technique:

$$\%\text{Pdff} = 100(\text{SA}_{+\text{P}}/\text{SA}_{\text{f}}) \tag{3.7}$$

where %Pdff is the percentage of P in the plant that is derived from the labeled fertilizer, $\text{SA}_{+\text{P}}$ is the specific activity of P in the aerial parts of the plant that has received the P fertilizer and SA_{f} the specific activity of P in the fertilizer.

This approach has been used many times. We mention here only a few examples: the mineralization of ^{32}P-labeled organic P compounds and their subsequent use by plants or transfer to other soil pools (Martin and Cartwright 1971; Harrison 1982; Kapoor and Haider 1982; Jayachandran et al. 1992b), the transfer of P added as plant residues to plants and/or to the soil microbial biomass (McLaughlin and Alston 1986; Friesen and Blair 1988; McLaughlin et al. 1988; Thibaud et al. 1988; Armstrong and Helyar 1993; Bünemann et al. 2004), and the transfer of P from ^{32}P-labeled inorganic soluble or insoluble P sources to plants (Boniface et al. 1979; McLaughlin and Alston 1986; Armstrong and Helyar 1993; McBeath et al. 2009). The main limit of this approach is the need for the source to be homogeneously labeled with P. There are, however, many fertilizers such as farmyard manure, compost, and rock phosphate that cannot be readily homogeneously labeled with radioactive P isotopes. In order to do so, it would be necessary to bombard them with neutrons (Kucey and Bole 1984), but this would lead to the production of other radioisotopes (^{24}Na, ^{45}Ca, among others) making the product difficult to handle and to changes in the chemical properties of the substrate. Besides, especially because of the short half-lives of ^{33}P and ^{32}P, this approach is limited to experiments lasting a few months and cannot be used, e.g., to quantify the residual value of P fertilizers for crops. For these types of experiments it is necessary to use the indirect labeling approach, which is described in the next section.

3.3.2 Labeling the Plant-Available Soil Phosphate (Indirect Labeling Technique)

This approach is based on the isotope dilution technique presented earlier in this chapter (Sect. 3.2.1). The release of P from a fertilizer to a plant or to any soil P pool can be studied by introducing a non-labeled fertilizer into a soil in which the isotopically exchangeable phosphate has been labeled (Kucey and Bole 1984; Morel and Fardeau 1989a). The proportion of P in the plant that is derived from a P fertilizer can be calculated with (3.8) when using the indirect labeling technique:

$$\%\text{Pdff} = 100(1 - \text{SA}_{+\text{P}}/\text{SA}_{0\text{P}}) \tag{3.8}$$

where %Pdff is the percentage of P in the plant that is derived from the fertilizer, SA_{+P} is the specific activity of the plant grown in the presence of the P fertilizer, and SA_{0P} is the specific activity of the plant grown in the absence of the P fertilizer.

This approach has been used, e.g., to assess the transfer of P added as rock phosphate, sewage sludge, compost, and animal manure to plants (Bolan et al. 1987; Hedley et al. 1988; Fardeau et al. 1988; Zapata and Axmann 1995; Kato et al. 1995; Frossard et al. 1996; Sinaj et al. 2002; Oberson et al. 2010). The indirect labeling approach has also been used for calculating the residual value of P fertilizers by comparing a control that was not fertilized to soils that were regularly fertilized (Bowman et al. 1978; Gallet et al. 2003b; Morel and Fardeau 1989b; Oberson et al. 2010).

This indirect approach supposes that the specific activity of the soil exchangeable phosphate is identical in the non-amended and in the amended soil. This basic hypothesis is very difficult to verify. Precautions must be taken when using this indirect approach, especially when the radioactive P is added to the soil at the same time as organic fertilizers. Indeed, these organic fertilizers could cause a substantial microbial immobilization of the radioactive P or could stimulate the mineralization of non-labeled soil organic P, both processes leading to a decrease in the specific activity in the soil solution of the fertilized treatment and therefore to an overestimation of the use of the P derived from the organic fertilizer by the test crop. This phenomenon, which is called "pool substitution," has been recognized for a long time by researchers working on nitrogen (Jenkinson et al. 1985; Hood-Nowotny 2008) but has not yet been addressed explicitly in P studies. Hood-Nowotny (2008) suggests circumventing this problem for nitrogen studies by labeling the soil well in advance with a ^{15}N mineral fertilizer combined with a carbon addition so as to incorporate as rapidly as possible the ^{15}N in the soil microbial biomass, and to add the non-labeled fertilizer only when the ^{15}N excess in the soil solution has reached equilibrium, i.e., several weeks after labeling. The major difference between N and P in this respect is that N dynamics are largely determined by microbial processes, whereas P dynamics are dominated by physicochemical processes in most soils. Oberson et al. (2010) suggest minimizing pool substitution for P by incubating the soil labeled with radioactive P under optimal temperature and humidity conditions for 1–2 weeks before adding the organic P source. Of course, it is very important when using this approach to properly account for the transfer of P from seeds to the aerial plant parts during plant growth (see Sect. 3.2.4). Finally, in principle, this indirect approach can also be used to derive the %Pdff of a non-labeled P source in soil P pools that have been previously labeled with radioactive P.

3.4 Using P Radioisotopes to Assess Foraging Strategies of AMF

Phosphorus radioisotopes have been used for several decades for measuring the acquisition of soil P by mycorrhizal fungi and comparing it to that of roots (Mosse et al. 1973; Powell 1975; Jakobsen et al. 1992). Early studies comparing the specific

P activities in plants colonized by AMF and in non-mycorrhizal plants growing in labeled (and uncompartmented) soils indicated usage of the same P pool by the mycorrhizal and non-mycorrhizal plants (Mosse et al. 1973; Blal et al. 1990; Bolan 1991), in contrast to the ectomycorrhizal and ericoid mycorrhizal associations (Read et al. 2004). Although a few studies scrutinizing the acquisition of P from organic sources by the AMF provided some evidence for differences in the utilization of certain organic P forms between plants colonized by AMF and non-mycorrhizal plants (Jayachandran et al. 1992b; Joner and Jakobsen 1995), this seems to be related to the presence of other soil microbes rather than to the P-mineralization capacity of the AMF themselves (Joner and Jakobsen 1995; Joner et al. 2000; Jansa et al. 2011).

Labeling of soil patches (compartments) inaccessible to roots but accessible to mycorrhizal hyphae through fine mesh-walls (20–35 μm) further contributed to understanding the kinetics of P uptake and the transfer via a mycorrhizal pathway to the plants (Fig. 3.5). Different studies employed systems differing in the construction and mode of labeling. The pros and cons of these different approaches for expanding the knowledge of AMF physiology and functional diversity and their role in plant P nutrition, as well as for studying remaining unanswered questions, are briefly outlined below.

With respect to the construction of the experimental system, important differences can be seen between the studies in terms of (a) the presence of a buffer zone between the root and labeling compartments, (b) the size of the root-free zone as compared to the root zone, and (c) the positioning of the labeling compartment (below or alongside of the root compartment). Large root-free compartments (Jansa et al. 2003; Mikkelsen et al. 2008) allow tracking of P transfer via mycorrhizal hyphae over large and defined distances, but have been criticized for possible bias due to unrealistic constraints imposed on the root development and exaggerated contrasts between AMF and non-mycorrhizal treatments. These studies showed that different AMF species acquire P at distances of several millimeters up to 15 cm from the roots (Jakobsen et al. 1992; Jansa et al. 2003; Smith et al. 2004). Small root-free compartments (vials with mesh lids) containing labeled soil and buried in pots or in the fields (Schweiger and Jakobsen 1999; Smith et al. 2004) are claimed to provide a more realistic comparison of the non-mycorrhizal and mycorrhizal treatments, but are impractical for kinetic studies in which isotopes are either administered by injection or placed at different distances from the roots. These setups do not usually include a buffer zone, which results in some radioactive P transfer to non-mycorrhizal plants due to root hair exploration of the root-free zone (Schweiger et al. 1999).

Two modes of P labeling have been employed – either mixing the isotope with the soil or injecting it into the soil or soilless substrate (Nielsen et al. 2002; Wang et al. 2002; Smith et al. 2004; Jansa et al. 2005). Whereas greater homogeneity of label distribution can be achieved with the first approach, the second (injection) approach allows measuring the efficiency of P uptake by an already established hyphae network (Jansa et al. 2005).

Values between 0% and 30% of the added P radioisotope have been recorded in the literature as proportions of the transported radioactivity via mycorrhizal fungal

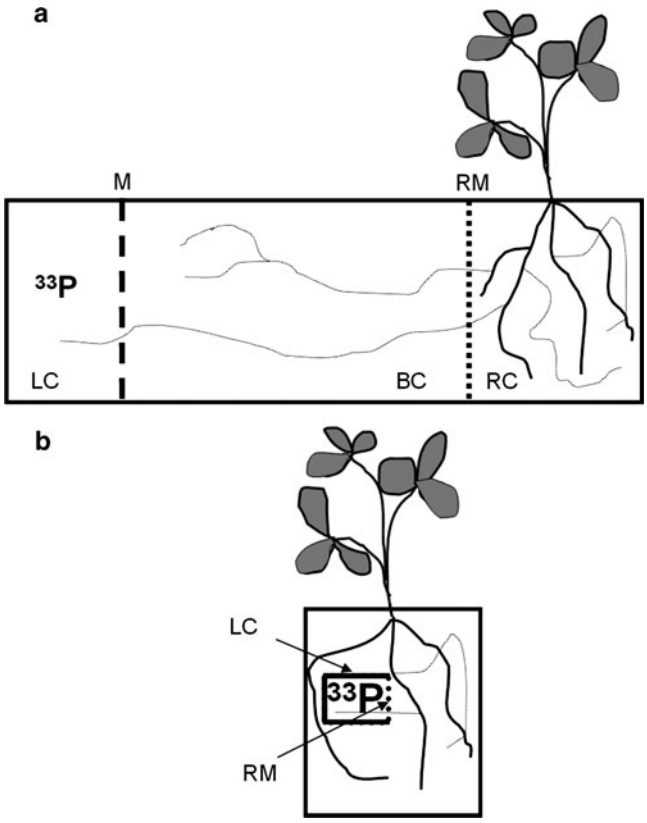

Fig. 3.5 Construction of compartmented cultivation systems for studying acquisition of P via AMF hyphae. The cuvette system (**a**) containing a root compartment (*RC*), and large buffer (*BC*) and labeling (*LC*) compartments (both could vary in size) is suitable for studying mycorrhizal P transfer over a defined distance and for administration of the radioisotope-labeled P by injection. The pot system (**b**) containing a small hyphal compartment, usually without a buffer zone, is claimed to offer less biased determination of the importance of the mycorrhizal acquisition pathway in plant P acquisition from the soil (adapted from Nagy et al. 2005). *RM* root exclusion mesh (20–35 μm), *M* mesh wall (500 μm), *fine lines* AMF hyphae

mycelium from the root-free soil to the roots (Jakobsen et al. 1992; Smith et al. 2000, 2004; Jansa et al. 2003, 2005). Absolute numbers depend on the plant and fungal identities, level of fungal development, substrate properties, time between isotope introduction and harvest, and other features of the experimental system. It has, however, been rather difficult to use this information for quantification of the contribution of the fungus to plant P uptake, although attempts to do this have been made (Smith et al. 2004; Jakobsen et al. 2005). Two major obstacles hinder progress along these lines: (a) quantification of active AMF mycelium has so far only been made by measuring hyphal length density using staining and microscopy, which may not be appropriate due to demonstrated difficulties in distinguishing living and dead hyphal stretches (Gamper et al. 2008), and (b) the specific activity

of the soil phosphate pool available to plants and fungi has so far only been assessed in bicarbonate extractions of substrate samples at a single time point (Smith et al. 2004). This could be improved by applying the principles of isotope dilution outlined above, and by quantifying the gene expression of the different P transporters, both in the plant roots and in the AMF hyphae (Maldonado-Mendoza et al. 2001; Grace et al. 2009).

In the future, more attention should be paid to performing functional studies with monoxenic cultures of different AMF, especially those including the whole plant as a host (Voets et al. 2009), to look at realistic source–sink relationships. Little use has been made of the availability of the two P radioisotopes, which could be concomitantly applied to track different P-acquisition pathways in the same system (Cavagnaro et al. 2005). Direct labeling of different organic compounds in combination with monoxenic cultures will provide greater precision in measuring the accessibility of the different organic P forms to the AMF. Isotope labeling studies could in the near future also be coupled to the quantification of expression of fungal gene transporters in a similar manner as done previously for the plant P transporters involved in assimilation of P delivered by the AMF (Nagy et al. 2005).

Finally, this niche labeling can also be used in experiments to study P uptake by the root system, e.g., of specific plant species in mixed stands (with intercropped plants), or to study P uptake from deep soil horizons, as done by Göransson et al. (2006).

3.5 Can the Isotopic Composition of Oxygen Bound to Phosphorus Be Used to Study Biological P Transformations in Soil–Plant Systems?

3.5.1 What Do We Know Already?

Oxygen has three stable isotopes ^{16}O, ^{17}O, and ^{18}O, which are present at 99.759%, 0.037%, and 0.204%, respectively, in the earth's atmosphere. The two most abundant oxygen isotopes ^{16}O and ^{18}O are used in phosphorus studies (Longinelli 1965). The natural abundance of ^{18}O bound to P ($\delta^{18}O_{P\text{-sample}}$, expressed in parts per thousand, ‰) is calculated according to (3.9):

$$\delta^{18}O_{P-\text{sample}} = 1{,}000\left\{\left[\left(^{18}O_P/^{16}O_P\right)_{\text{sample}}/\left(^{18}O/^{16}O\right)_{\text{reference}}\right] - 1\right\} \qquad (3.9)$$

where $(^{18}O_P/^{16}O_P)_{sample}$ represents the ratio of ^{18}O to ^{16}O in phosphate in a given sample, and $(^{18}O/^{16}O)_{reference}$ the ^{18}O to ^{16}O ratio in a reference sample. For oxygen isotopes in phosphate, the reference material used is the Vienna Standard Mean Ocean Water (VSMOW). Several papers have dealt with the oxygen isotope geochemistry of phosphate in adsorption–desorption processes, mineral precipitation,

and biological processes, e.g., uptake, intra- and extracellular enzymatic catalysis (Liang and Blake 2007; Blake et al. 2005; Jaisi et al. 2010). In the rest of this section we give a short account of the main findings of these studies, and then in Sect. 3.5.2 we discuss future research to be done to use this tracer to study biological P transformations in soil–plant systems.

In the absence of biological activity and at ambient temperature, isotope exchange between the oxygen present in Pi and water is slow and negligible (Winter et al. 1940; Longinelli 1965; Kolodny et al. 1983; Luz and Kolodny 1985). Studies on precipitation of authigenic P-bearing minerals showed that fractionation between ^{16}O-phosphate and ^{18}O-phosphate linked to this process is small, in the order of about 1‰ (Liang and Blake 2007). Also, sorption of phosphate to Fe oxides does not lead to a significant isotopic fractionation between the sorbed phosphate and the phosphate in solution (Jaisi et al. 2010).

When biological activity is present, however, oxygen exchange between phosphate and water becomes significant and is characterized by both kinetic and equilibrium fractionations, but is dominated by equilibrium isotope fractionation effects (Kolodny et al. 1983; Paytan et al. 2002; Blake et al. 2005). Rapid microbial turnover of phosphate, controlled by reversible intracellular processes such as pyrophosphatase-catalyzed hydrolysis (Blake et al. 2005), is responsible for a temperature-dependent equilibration with ambient water. Such rapid biologically mediated turnover has been demonstrated in natural aquatic systems (Paytan et al. 2002; Colman et al. 2005). The enzyme pyrophosphatase catalyzes the isotopic exchange of oxygen between water and phosphate within a few hours at high enzyme concentrations in laboratory experiments (Blake et al. 2005), and completely overprints the initial $\delta^{18}O_P$ of Pi taken up by microorganisms (Blake et al. 1998). The equation relating the equilibrium between $\delta^{18}O_P$, $\delta^{18}O$ water ($\delta^{18}O_W$), and temperature (Longinelli and Nuti 1973) is shown below:

$$T(^{\circ}\mathrm{C}) = 111.4 - 4.3\left(\delta^{18}O_P - \delta^{18}O_W\right) \tag{3.10}$$

In contrast to intracellular processes, extracellular enzymatic hydrolyses are generally irreversible. Phosphodiesterase catalytic activity results in incorporation of one oxygen atom from ambient water into the phosphate group of a diester P (R–O–PO_2^-–O–R') to produce a monoester P (R–O–PO_3^{2-}). Phosphomonoesterase activity in turn will result in the incorporation of a second oxygen atom from ambient water into the phosphate group released from a phosphomonoester (Blake et al. 2005; Liang and Blake 2009). Furthermore, the effect of the phosphodiesterases on the $\delta^{18}O_P$ of monoester P may depend on the substrate, because ^{16}O is preferentially incorporated into nucleotides released from DNA, whereas ^{18}O is preferentially incorporated into nucleotides released from RNA (Liang and Blake 2009). This substrate and enzyme specificity might be useful to distinguish between different pathways of organic P metabolism in the soil–plant system. The original $\delta^{18}O_P$ signature of organic P compounds might therefore be partially retained when enzymatic catalysis occurs in the extracellular medium and when P is not entirely recycled by microorganisms, i.e., when P is present in high concentrations or when

P is not the primary limiting factor for microbial growth. Finally, studies on *Escherichia coli* showed that this organism preferentially takes up ^{16}O-phosphate into its cells (Blake et al. 2005).

These findings show that the final $\delta^{18}O_P$ signature of Pi derived from monoester P and diester P and of microbially cycled P is a function of the $\delta^{18}O$ of water, the temperature, the speciation and initial $\delta^{18}O_P$ of the phosphates coming into the ecosystem, the abundance of available P, and the biological processes that take place within the ecosystem itself (Blake et al. 2001). Under P-limited conditions, inducing a complete turnover of phosphate (e.g., oligotrophic ocean surface waters), $\delta^{18}O_P$ can be used to track biological processes (e.g., alkaline phosphatase hydrolysis; Paytan et al. 2002; Colman et al. 2005). Under excess P conditions (coastal waters, estuaries, and continental water bodies) $\delta^{18}O_P$ can be used to characterize P sources (McLaughlin et al. 2006a, b; Elsbury et al. 2009). In fact, incomplete turnover of the phosphate pool may result in partial inheritance of the isotope signature of the original P source, which can be tracked in the environment. Elsbury et al. (2009) found $\delta^{18}O_P$ values of dissolved Pi in Lake Erie varying between 10‰ and 17‰, i.e., that were out of the predicted equilibrium with ambient conditions, which would vary between 13‰ and 15‰. By comparing $\delta^{18}O_P$ values of dissolved Pi in lake water to $\delta^{18}O_P$ values in its tributaries, the authors suggest that the rivers supply Pi with a light isotope composition, whereas Pi released from the sediments in the bottom waters following anoxic events have a heavy $\delta^{18}O_P$. Young et al. (2009) showed that the $\delta^{18}O_P$ signature varies widely in materials that could act as sources of P for water. The analyses conducted by Young et al. (2009) yielded a mean $\delta^{18}O_P$ for effluents from wastewater treatment plants of 11‰, a mean $\delta^{18}O_P$ for mineral P fertilizers of 20‰, and a mean $\delta^{18}O_P$ for vegetation and detergents of 17‰.

To date, very few studies have attempted to understand $\delta^{18}O_P$ variations in soils (Ayliffe et al. 1992; Mizota et al. 1992; McLaughlin et al. 2006a). All conclude that anomalous $\delta^{18}O_P$ values in soils compared to parental material might be attributed to biological activity. Because the pioneering study conducted by Mizota et al. (1992) specifically used $\delta^{18}O_P$ to understand soil P dynamics as affected by pedogenesis, we summarize their findings hereafter. Mizota et al. (1992) measured the $\delta^{18}O_P$ of P associated to Ca and Al in volcanic soils from Java and East Africa. They compared $\delta^{18}O_P$ values obtained from soils with those of volcanic ash, animal bones, and local phosphate deposits formed at high temperature. Their results showed a trend of increasing $\delta^{18}O_P$ values from young toward more weathered soils. They interpreted the low $\delta^{18}O_P$ of young soils by the presence of P-bearing apatite produced at high temperatures, and attributed the high $\delta^{18}O_P$ of older soils to intense biogenic recycling.

Two other studies have been conducted with ^{18}O-enriched compounds. Kok and Varner (1967) studied the changes in ^{18}O enrichment in water and showed that no O exchange occurred between H_2O and PO_4 in sterile soils, whereas this exchange was strongly accelerated in biologically active soils or in the presence of microorganisms. Furthermore, using KH_2PO_4 doubly labeled with ^{32}P and ^{18}O, Larsen et al. (1989) showed that ^{18}O disappeared very rapidly from the Pi when KH_2PO_4

was added to a soil on which ryegrass was growing, whereas ^{18}O remained in Pi when KH_2PO_4 was added to a sterile soil–water suspension. Larsen et al. (1989) concluded that the loss of ^{18}O from Pi could be used as a measure of soil biological activity, which is in line with the conclusions drawn by Kok and Varner (1967) for soils and by Blake et al. (2001) for aquatic systems.

3.5.2 *What Should Be Done to Apply This Approach to Soil–Plant Systems?*

Many points remain to be clarified before $\delta^{18}O_P$ can be confidently used in soils. The first problem is to isolate phosphate from any other compound containing O and to convert it to silver phosphate for oxygen isotope analysis. This is a challenge in soils, where organic matter is often present in high concentrations and distributed in many forms. Wiedemann-Bidlack et al. (2008) showed that the presence of organic matter strongly affects the measurement of $\delta^{18}O_P$ in apatites and suggested measures to eliminate this contamination. Tamburini et al. (2010) recently adapted a method based on the use of multiple mineral precipitations that does not require extreme pH adjustments of the solutions for the production of pure Ag_3PO_4 amenable to $^{18}O_P$ analysis from HCl extracts of soil or fertilizer. They successfully applied this method to soils rich in organic matter and to fertilizers, showing that the $\delta^{18}O_P$ of HCl-extractable P could be related to the source of P in P-rich soils or to biological activity in low-P soils. Because phosphate in soils is distributed in many pools (e.g., adsorbed on Fe and Al oxides, precipitated on Ca minerals, in organic compounds), another challenge for soil studies is to perform $\delta^{18}O_P$ measurements on different P fractions of a sequential extraction, as was done partially for marine sediments (Jaisi and Blake 2010). Moreover, it is necessary to check for possible exchange of oxygen between water and phosphate for each extraction step by introducing ^{18}O-labeled water into the extraction solutions. This step is particularly important for soils because P forms other than phosphate (e.g., condensed phosphate, pyro- and polyphosphate, phosphate mono- and diester, or phosphonate) can be present in the extracts. In fact, inorganic hydrolysis of such compounds could lead to incorporation of oxygen from the aqueous medium into the newly formed phosphate, thus altering the original isotopic signal. Finally, ultraviolet digestion can be used to determine the $\delta^{18}O_P$ of extracted organic P, as shown by Liang and Blake (2006).

Once these extractions and preparation issues are solved, it will be necessary to assess the oxygen isotope fractionation associated with the most important processes controlling P transformations in the soil–plant system. For instance, whereas a lot is known about partial dissolution of P-bearing minerals like apatite (e.g., rates of dissolution, pH dependency of the process, effects of chelating agents and organic acids), only a few studies have explored the oxygen isotope geochemistry of this process (Lécuyer et al. 1999; Blake et al. 1998). Plants have many types of

P transporters (Bucher 2007) and we do not know whether these transporters preferentially take up ^{16}O-enriched phosphate (like *E. coli*, see Blake et al. 2005), enriching the residual phosphate in the soil solution with ^{18}O.

Another effect to be investigated is the effect of evapotranspiration. Because evapotranspiration leads to an ^{18}O-enrichment in the leaf-water (Helliker and Ehleringer 2000), we can assume that this will also affect the $\delta^{18}O_P$ in the leaves. Finally, it is necessary to investigate how microbial uptake and mineralization processes affect the $\delta^{18}O_P$ of soil available P and soil microbial P.

Information on the $\delta^{18}O_P$ of P sources in P-rich soils will help to trace P fluxes at the ecosystem level (Elsbury et al. 2009; McLaughlin et al. 2006a). In P-limited soil systems, where biological P turnover is high, the dependence of $\delta^{18}O_P$ on water $\delta^{18}O$ and equilibration temperature might be used to study and reconstruct temperature and rainfall gradients over time.

In conclusion, whereas the measure of $\delta^{18}O_P$ in different soil P pools could provide information on the biological transformations of P, and maybe also on the transfer of P in terrestrial ecosystems, there is still much to do to render this approach operational.

3.6 Concluding Remarks and Research Needs

This review shows the importance of using tracers to understand both the physico-chemical and biological transformations of P in soil–plant and soil–solution systems, i.e., to understand "Phosphorus in Action"!

Although P radioisotopes have been used for decades, the last decade has seen some significant improvements in these isotopic approaches, e.g., in the measurement of low P concentration in solution and by taking into account the P derived from the seed in plant P nutrition. Thanks to the use of radioisotopes, significant progress has been made in assessing and modeling relationships between Pi in the solution and Pi bound to soil constituents that can be desorbed to replenish the solution with time under gradients of Pi concentration. Progress has also been made in measuring the mineralization of soil organic P and the immobilization of P in the soil microorganisms, as well as in assessing the foraging strategies of AMF. The most challenging research areas remain (a) to extend the modeling efforts so as to be able to predict the rate of soil solution replenishment with Pi as a function of soil properties and management, (b) to extend the measurement of organic P mineralization to soils that contain very little available P and that have a high sorbing capacity for Pi, and (c) to assess the importance of pool substitution in assessing the fate of P from organic exogenous sources in soil–plant systems.

The measurement of the $\delta^{18}O_P$ in different soil and plant pools bears an interesting potential for studying the biological transformations of P in soil–plant systems and in water bodies, for a better understanding of the origin of the P that triggers their eutrophication. But, a lot of research remains to be done before this tool can become operational.

Finally, other tracers might be of interest, especially for studying the long-term impact of mineral and organic P fertilizers in agro-ecosystems. Rare earth elements, heavy metals, and radionuclides are known to be present in variable concentrations in phosphate deposits and in organic fertilizers, and could in the future be used to record the accumulation of P fertilizers in soils and other parts of the environment (Hu et al. 1998; Otero et al. 2005; de Kok and Schnug 2008). An example of this approach is given by Bertrand et al. (2003), who used the Cd:P ratio of mineral fertilizers to show that most of the P found in the upper horizons of Australian calcarosols was derived from mineral P fertilizer applications.

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