Factors controlling phosphate exchangeability and release in agricultural soils

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FACTORS CONTROLLING PHOSPHATE EXCHANGEABILITY
AND RELEASE IN AGRICULTURAL SOILS

A dissertation submitted to the
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for the degree of
Doctor of Natural Sciences

presented by
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Dr. S. Sinaj, co-examiner

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<tr>
<td>Al_d</td>
<td>g Al kg(^{-1})</td>
<td>amount of citrate-dithionite-bicarbonate extractable Al</td>
</tr>
<tr>
<td>Al_ox</td>
<td>g Al kg(^{-1})</td>
<td>amount of ammonium-oxalate extractable Al</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CEC</td>
<td>cmol(_e) kg(^{-1})</td>
<td>cation exchange capacity</td>
</tr>
<tr>
<td>C_p</td>
<td>mg P L(^{-1})</td>
<td>phosphate concentration in solution</td>
</tr>
<tr>
<td>D</td>
<td>mg P kg(^{-1})</td>
<td>amount of potentially releasable P in a flow-through reactor</td>
</tr>
<tr>
<td>E_t</td>
<td>mg P kg(^{-1})</td>
<td>amount of isotopically exchangeable P within t minutes</td>
</tr>
<tr>
<td>Fe_d</td>
<td>g Fe kg(^{-1})</td>
<td>amount of citrate-dithionite-bicarbonate extractable Fe</td>
</tr>
<tr>
<td>Fe_ox</td>
<td>g Fe kg(^{-1})</td>
<td>amount of ammonium-oxalate extractable Fe</td>
</tr>
<tr>
<td>I</td>
<td>mg P L(^{-1})</td>
<td>Intensity factor</td>
</tr>
<tr>
<td>IEK</td>
<td></td>
<td>isotopic exchange kinetics</td>
</tr>
<tr>
<td>L</td>
<td>mg P kg(^{-1})</td>
<td>plant available in isotopically exchangeable form</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>rate of decrease of radioactivity after 1 minute</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>probability level</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>phosphorus</td>
</tr>
<tr>
<td>P-AAEDTA</td>
<td>mg P kg(^{-1})</td>
<td>amount of ammonium acetate EDTA extractable P</td>
</tr>
<tr>
<td>P-CO(_2)</td>
<td>mg P kg(^{-1})</td>
<td>amount of CO(_2)-saturated water extractable P</td>
</tr>
<tr>
<td>P-NaHCO(_3)</td>
<td>mg P kg(^{-1})</td>
<td>amount of sodium bicarbonate extractable P</td>
</tr>
<tr>
<td>P(_t)-NaHCO(_3)</td>
<td>mg P kg(^{-1})</td>
<td>total amount of sodium bicarbonate extractable P</td>
</tr>
<tr>
<td>P(_o)-NaHCO(_3)</td>
<td>mg P kg(^{-1})</td>
<td>amount of sodium bicarbonate extractable organic P</td>
</tr>
<tr>
<td>P-w</td>
<td>mg P kg(^{-1})</td>
<td>amount of water extractable P</td>
</tr>
<tr>
<td>PBC</td>
<td>L kg(^{-1})</td>
<td>Phosphate Buffer Capacity factor</td>
</tr>
<tr>
<td>PBC([IEK])</td>
<td>L kg(^{-1})</td>
<td>PBC obtained from isotopic exchange data</td>
</tr>
<tr>
<td>PBC([Chem])</td>
<td>L kg(^{-1})</td>
<td>PBC obtained from chemical extraction data</td>
</tr>
<tr>
<td>P(_{min})</td>
<td>mg P kg(^{-1})</td>
<td>amount of soil inorganic P</td>
</tr>
<tr>
<td>P(_o)</td>
<td>mg P kg(^{-1})</td>
<td>amount of soil organic P</td>
</tr>
<tr>
<td>P(_{ox})</td>
<td>mg P kg(^{-1})</td>
<td>amount of ammonium-oxalate extractable P</td>
</tr>
<tr>
<td>P(_R)</td>
<td>mg P kg(^{-1})</td>
<td>amount of released P in a flow-through reactor</td>
</tr>
<tr>
<td>P(_S)</td>
<td>mg P kg(^{-1})</td>
<td>amount of sorbed P</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>OP</td>
<td>fertilization treatment with no P application</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>fertilization treatment with P application = crop off-take</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>[mg P kg(^{-1})] Quantity factor</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>[MBq ml(^{-1})] total introduced radioactivity</td>
<td></td>
</tr>
<tr>
<td>r(_t)</td>
<td>[MBq ml(^{-1})] radioactivity remaining in solution after t minutes</td>
<td></td>
</tr>
<tr>
<td>r(_\infty)</td>
<td>[MBq ml(^{-1})] radioactivity remaining in solution after infinite time</td>
<td></td>
</tr>
<tr>
<td>S.e.e.</td>
<td>standard error of the estimate</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>[Bq (^{33})P µg(^{-1}) (^{31})P] P specific activity</td>
<td></td>
</tr>
<tr>
<td>SSA</td>
<td>[m(^2) g(^{-1})] specific surface area</td>
<td></td>
</tr>
<tr>
<td>TOC</td>
<td>[g C kg(^{-1})] total organic carbon</td>
<td></td>
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Abstract

Inputs of phosphorus (P) in excess to plant needs have led to the accumulation of available P in the surface layer of most of the agricultural soils of developed countries. This has increased the load of P transferred from agricultural land to surface and ground waters through overland flow and leaching. High amounts of P discharged into water bodies trigger their eutrophication. In order to reduce the environmental impact of agriculture, regulations have been implemented to decrease the P content of agricultural topsoils by limiting new inputs via mineral and/or organic fertilizers. However, in order to properly implement the proposed fertilization schemes, their middle- and long-term effects on plant production, on soil P availability and on the potential risk of P losses must be evaluated.

Plants take up P as inorganic orthophosphate from the soil solution, which is supplied by P located on the soil solid phase. The reserves of soil P that can be transferred to the solution within a timeframe relevant for plant nutrition can be assessed with a number of laboratory methods. The isotopic exchange method allows quantifying the P ions located on the solid phase that can be transferred to the soil solution within a given time: therefore P exchangeability provides information on P availability.

The objective of this thesis was to investigate the factors influencing P exchangeability and the potential release from agricultural soils. The study was conducted on the surface horizon (0-20 cm) of cropped soils sampled from middle- and long-term field experiment where the effects of different fertilization schemes on plant production and on P availability were evaluated. Soils from two fertilization regimes were considered in this study: no P application (OP) and P applied yearly at rates equivalent to crops off-take (P).

Isotopic exchange kinetics (IEK) experiments allow the determination of the following parameters: (i) the ratio \( R/r_1 \) between the introduced radioactivity (R) and the radioactivity remaining in solution after 1 minute of exchange (\( r_1 \)); (ii) the rate of disappearance of radioactivity from the solution after 1 minute (n) and (iii): the equilibrium concentration of P in solution (\( C_p \)). These parameters are used to calculate isotopically exchangeable P as a function of exchange time (\( E_t \)). The effects of \( C_p \), soil properties, time and different fertilization treatments on \( R/r_1 \), n and \( E_t \) were tested by carrying out IEK experiments on soil samples incubated in the presence of increasing amounts of KH\(_2\)PO\(_4\). Results showed that, besides \( C_p \) and time, the
concentration of Fe and Al oxides, soil specific surface area and soil pH had a significant effect on P exchangeability and on the soil P buffer capacity (PBC) obtained from isotopic exchange data. The kinetic parameter R/ri was significantly correlated with the PBC. The results appeared to be valid for soils from similar agro-climatic conditions: when applied to another set of agricultural soils, the obtained relationships allowed a significant prediction of R/ri and of Ei within one minute (Eimin), which is considered a good indicator of P availability. For a given soil, fertilization did not significantly affect the influence of soil properties on P exchangeability.

The exchangeability of P extracted by a chemical method was evaluated by determining the specific activity (SA=33P/31P) of P extracted from a 33P-labeled soil by a given method and the SA of water extractable P. In the tested methods, P was extracted with (i) CO2-saturated water (P-CO2); (ii) ammonium acetate EDTA (P-AAEDTA); and (iii) NaHCO3 (P-NaHCO3). Our results showed that only exchangeable P was mobilized by an extractant with low ionic strength and rapidly buffered to the pH of the soil sample (P-CO2), but the amounts were lower than Eimin. On the other hand, 25 to 63% of P-AAEDTA and 66 to 92% of P-NaHCO3 was isotopically exchangeable, suggesting that slowly or no exchangeable forms of P can be solubilized by stronger extractants. The fraction of exchangeable P-AAEDTA showed a strong negative correlation with soil pH.

A flow-through reactor method was developed in order to assess the potential of P release from fertilized and unfertilized soils in absence of growing crops. Such an approach was preferred over batch experiments because it was thought to better represent the specific field conditions. By measuring the release of P from 33P-labeled soils, the amount of soil P with the same isotopic composition of the released P (D-values) could be calculated and thus the total amount of potentially releasable soil P was evaluated. The amounts of released P (Pr) from OP soils were significantly lower than the ones from P soils. Pr represented only 7 to 13% of the calculated D-values in the P soils. Soil isotopically exchangeable P was the main source of released P.

In agricultural soils P availability should be evaluated from both agronomic and environmental points of view, i.e. taking into account the plant needs and the risk of losses to surface and subsurface waters. From the results of this study, we propose to consider two extractions to determine the indexes of soil P fertility: one weak extractant such as deionized water and a more aggressive extractant (AAEDTA or NaHCO3) to be chosen with respect to the properties of the analyzed soil. The combination of two methods should yield information on the reserve of plant-
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available P in the short and middle term, on the potential risk of P losses and on the P buffer capacity, which controls the dynamics of P between the solid phase and the solution. A water extraction, combined with the determination of basic characteristics, should allow the prediction of P exchangeability.
Riassunto

Le immissioni di fosforo (P) in eccesso al fabbisogno delle colture vegetali ha portato all'accumulo di P biodisponibile nel livello superficiale della maggior parte dei suoli agricoli dei paesi industrializzati. Questo ha incrementato il carico di P trasferito dalle terre agricole alle acque superficiali e di falda attraverso processi di ruscellamento superficiale e di lisciviazione. Alte quantità di P immesse nei corpi idrici ne innescano l'eutrofizzazione. Al fine di ridurre l'impatto ambientale dell'agricoltura, le normative mirano a ridurre il contenuto in P dei suoli agrari limitando nuove immissioni di fertilizzanti sia minerali che organici. Ciononostante, per poter implementare gli schemi di fertilizzazione proposti, è necessario valutare i loro effetti a medio e lungo termine sulla produzione, sulla disponibilità di P e sul rischio di perdite di P.

Le piante assimilano il P in forma di ortofosfato inorganico dalla soluzione del suolo, la quale a sua volta è alimentata dal P localizzato nella fase solida del suolo. Le riserve di P che il suolo può fornire alla soluzione in un lasso di tempo utile per la nutrizione vegetale possono essere stimate tramite una serie di metodi di laboratorio. Tra essi, il metodo dello scambio isotopico permette di quantificare gli ioni fosfato presenti nella fase solida del suolo che possono essere trasferiti alla soluzione entro un tempo determinato: di conseguenza la scambiabilità del P fornisce utili informazioni circa la disponibilità dell'elemento.

L'obiettivo di questa tesi è stato l'investigazione dei fattori che influenzano la scambiabilità del P ed il suo potenziale di rilascio dai suoli agrari. Lo studio è stato condotto sul livello superficiale (da 0 a 20 cm) di suoli agrari campionati da esperimenti di campo di medio e di lungo termine ove venivano valutati gli effetti di diversi schemi di fertilizzazione sulla produzione vegetale e sulla disponibilità del P. In questo studio si sono considerati suoli provenienti da due regimi di concimazione: nessuna applicazione di P (OP) e applicazione annuale di P pari all'asporto stagionale delle colture (P).

Esperimenti di cinetiche di scambio isotopico (IEK) consentono la determinazione dei seguenti parametri: (i) il rapporto $R/r_1$ tra la radioattività introdotta ($R$) e la radioattività residua in soluzione dopo un minuto di scambio ($r_1$); (ii) la diminuzione della radioattività in soluzione nel tempo dopo il primo minuto ($n$) e (iii): la concentrazione di equilibrio del P in soluzione ($C_P$). Questi parametri sono utilizzati per calcolare il P isotopicamente scambiabile in funzione del tempo di scambio ($E_t$). Gli effetti di $C_P$, delle proprietà del suolo, del tempo e dei differenti livelli
di fertilizzazione su R/r₁, n e Eₜ sono stati verificati conducendo esperimenti di cinetiche di scambio isotopico su campioni di suolo precedentemente incubati in presenza di diverse quantità di KH₂PO₄. I risultati hanno messo in luce che, oltre a Cₚ e al tempo, la concentrazione degli ossidi di Fe e Al, la superficie specifica del suolo e il pH del suolo hanno un effetto significativo sulla scambiabilità del P e sul potere tampone del suolo per il P (PBC) determinato dai dati ottenuti dallo scambio isotopico. Il parametro R/r₁ è significativamente correlato con il PBC. I risultati sembrano avere valore generale, per lo meno limitatamente a suoli provenienti da condizioni agro-climatiche simili a quelli testati: le relazioni ottenute, qualora applicate ad un altro set di suoli agrari, hanno permesso una significativa previsione sia di R/r₁ che di Eₜ entro un minuto (Eₘᵢₙ) che è considerato un buon indicatore della disponibilità di P. Rispetto a ciascun suolo, la fertilizzazione non ha influenzato significativamente l’effetto delle proprietà del suolo sulla scambiabilità del P.

La scambiabilità del P estratto tramite metodi chimici è stata valutata determinando l’asstività specifica (SA = ³²P/³¹P) del P estratto da un suolo marcato con ³²P da parte di un determinato metodo e la SA del P estratto in acqua distillata. Nei metodi testati, P è stato estratto con (i) acqua satura di CO₂ (P-CO₂); (ii) acetato ammonio EDTA (P-AAEDTA); e (iii) bicarbonato di sodio (P-NaHCO₃). I nostri risultati hanno mostrato che un estraeente a debole forza ioniaca e tamponato rapidamente al pH del campione di suolo (P-CO₂) è in grado di mobilizzare solo forme scambiabili di P, malgrado la quantità estratta è inferiore ai valori di Eₘᵢₙ. Per contro, tra il 25 e il 63% del P-AAEDTA e tra il 66 e il 92% del P-NaHCO₃ sono stati estratti in forma scambiabile. Questo suggerisce che forme lentamente o non scambiabili di P possono essere dissolte da estraeenti più forti. La frazione scambiabile del P-AAEDTA ha mostrato una forte correlazione lineare negative con il valore di pH del suolo.

Per valutare il potenziale di rilascio di P da suoli fertilizzati e non fertilizzati in assenza di raccolti, si è sviluppato un metodo consistente in un reattore a intraflusso. Questo approccio è stato preferito agli esperimenti in “batch” perché si è ritenuto che meglio rappresentasse le condizioni di campo specifiche. Misurando il rilascio di P da suoli marcati con ³²P, si è potuta calcolare la quantità di P con la stessa composizione isotopica del P rilasciato (valore D) al fine di stimare l’ammontare totale di P potenzialmente rilasciabile. Le quantità di P rilasciato (Pₖ) dai suoli OP sono state significativamente inferiori di quelle misurate nei suoli P. Nei suoli P, Pₖ ha
Riassunto 6

rappresentato soltanto tra il 7 ed il 13% del valori calcolati di D. Il P isotopicamente scambiabile è stata la sorgente principale del P rilasciato.

La disponibilità di P nei suoli agrari dovrebbe essere valutata sia dal punto di vista agronomico che da quello ambientale, ossia tenendo conto del fabbisogno delle colture e del rischio di perdite di P verso le acque superficiali e vadose. In base ai risultati di questo studio, si propone, al fine di determinare gli indici di fertilità in P dei suoli, di considerare due metodi di estrazione chimica: un estraente debole come l’acqua distillata e uno più aggressivo (AAEDTA o NaHCO₃) da scegliersi in funzione delle caratteristiche dei suoli da analizzare. La combinazione di due metodi dovrebbe permettere di conseguire informazioni valide sulla riserva di P disponibile sul medio e lungo termine, sul potenziale rischio di perdite di P e sul potere tampone del suolo per il P, il quale a sua volta controlla le dinamiche del P tra il suolo e la soluzione. Inoltre, un’estrazione in acqua distillata, combinata con la determinazione delle proprietà elementari del suolo, dovrebbe permettere la previsione della scambiabilità del P.
General Introduction
Phosphorus (P) is an essential nutrient for plant growth. P is taken up by the roots of growing plants as orthophosphate ions ($\text{HPO}_4^{2-}$ or $\text{H}_2\text{PO}_4^-$, depending on the pH) from the soil solution. Up to 99% of the phosphate taken up by crops derives from the solid phase of the soil (Morel et al., 2000). Due to the relatively low natural content of P in soils and to the high affinity of phosphate ions for the soil constituents, not all soils can supply enough P to maintain the desired production and P deficiency can become a limiting factor for plant growth. More than 60% of the soils in large areas of the tropical belt, of northern Europe and Asia and of most of the more densely populated areas of the North American continent are naturally P deficient (Fairhurst et al., 1999). In industrialized countries, inorganic and/or organic P fertilizers have been applied to agricultural soils to correct P deficiency, often in excess of plant requirements leading to an accumulation of P in the surface layers (Barberis et al., 1996). This enrichment has increased the potential for P losses from agricultural land to surface runoff and subsurface drainage. Although these losses are quantitatively small compared to the amounts of P added in agroecosystems (Edwards et al., 2000), they are of great significance in aquatic environment, where P is often the most limiting nutrient for algae (Correl, 1998) and where they can trigger eutrophication (Foy and Withers, 1995; Correll, 1998; Sharpley et al., 2000). It is therefore a common goal in the P-related research to reduce the risk of P losses while keeping an optimum level of soil available P to maintain plant production. This requires the understanding of the abiotic and biotic processes governing short- and long-term bioavailability of P in order to optimize the utilization of soil- and fertilizer-P by plants (Frossard et al., 2000). The study of soil P availability is central from the agronomic point of view, because it allows identifying the fertility status of soils and thus developing fertilization management (Walter et al., 1994; Tunney et al., 1997) and from the environmental point of view, as P availability can be used to assess the risk of P losses (Schoumans and Chardon, 2003).

P availability

Soil available P is defined as the amount of phosphate that can be released by the solid phase of the soil and arrive in the solution within a time-frame relevant for plant growth. It is characterized by three factors (Beckett and White, 1964): (i) the Intensity (I), which is the activity of phosphate ions in the soil solution; (ii) the Quantity (Q), which is the amount of soil P that can be released
to the solution; and (iii) the buffer capacity (PBC), i.e. the ability of the soil to maintain the Intensity constant on the addition of P to or its removal from the soil. P availability is controlled by a number of reactions detailed in Figure 1.1 and in the following section.

![Figure 1.1. Scheme of P cycling in a soil-solution-plant system (delimited by the dashed-line rectangle). Solid arrows indicate processes that govern P cycling; dashed arrows indicate P inputs in the system; dotted lines indicate P losses from the system. P\text{min}: soil inorganic P; P_0: soil organic P; A: fast, reversibly surface adsorption; B: desorption of adsorbed P; C: inward diffusion into particles or aggregates and sorption; D: outward diffusion from particles and aggregates; E: precipitation of Ca-, Fe- or Al-P compounds; F: dissolution; G: microbial immobilization; H: mineralization. The mobility of P within P\text{min} and P_0 decreases from top to bottom.]
Phosphorus in soils

Soils contain between 100 and 3000 mg P kg\(^{-1}\) (Frossard et al., 2000). The fraction of organic forms of P is variable and ranges between 30 and 65% (Harrison, 1987). In the soil solution P is present as orthophosphate ions (which is the form taken up by plant roots and by mycorrhizal hyphae) and also as dissolved forms of organic P. Upon fresh addition of soluble fertilizer P, a fraction of the added P reacts with the solid phase of the soil. The initial reaction is considered to be a fast, reversible adsorption on the charged surfaces of metal (mostly Fe and Al) oxy-hydroxides and, to a lesser extent, on clay minerals and organic matter complexes. In calcareous soils, phosphate can be sorbed as well on the surface of Ca and Mg carbonates (Holford, 1997). The fast adsorption occurs as a ligand exchange reaction between the phosphate ions in solution and OH or H\(_2\)O groups located on the surfaces of the oxides and clays. The initial fast reaction is followed by slow reactions as the diffusion of P within aggregates to sorption sites, the solid state diffusion of P inside e.g. oxides (Barrow, 1983a), and the conversion of metal P complexes into a phosphate precipitate characterized by a very low mobility (Van der Zee and Van Riemsdijk, 1991). Precipitation processes can occur at high phosphate concentration: Ca-phosphates can form in calcareous soils (Castro and Torrent, 1998; Tunesi et al, 1999) and in soils heavily fertilized with manure (Sharpley et al., 2004), whereas Fe- and Al-phosphates can form in acid soils (Pierzinsky et al., 1990). When phosphate is removed by plant uptake and/or by leaching, the solution is replenished by an initially fast desorption of P reversibly adsorbed on the soil surfaces, followed by the dissolution and/or desorption of intra-aggregates and intra-particles P-compounds and the diffusion of the mobilized phosphate towards the solid-liquid interface. The rate of this diffusion processes is inversely related to the time allowed to the previous sorption reaction (Barrow, 1983b). To a lesser extent dissolution of P-bearing minerals can replenish the phosphate concentration in the soil solution. Holford (1997) suggests that it may become the main supply process in later stages of plant growth, when large decreases of phosphate concentration have occurred. The root- and micro-organism-induced acidification of the rhizosphere (Marschner, 1995) may also trigger the dissolution of the less stable Ca-P minerals. The main biotic processes controlling the release of P to the solution are the mineralization of organic P forms by soil micro-organisms and the hydrolysis of organic P compounds by phosphatase exo-enzymes (Oberson and Joner, 2005). The forms of soil organic P and their contribution to the nutrition of plants and to phosphate transfer to water bodies are still badly
known (Turner et al., 2005). However, this thesis is focused only on the availability of inorganic P.

**Laboratory methods to evaluate P availability**

Routine measurements of soil P availability include extractions with water (van der Paauw, 1971), dilute chemicals, acids or bases (Kamprath and Watson, 1980). In order to establish soil fertility classes, the values of extractable P must be related to yield responses and/or P uptake by crops. Such chemical methods give an estimation of the amount of soil P that can be released to the solution (the Quantity factor). However, the results are not only a function of the content of available P in a soil, but also of the nature of the extracting solution and of its interaction with the soil constituents (Fardeau et al., 1988; Kato et al., 1995). It is accepted that water or dilute CaCl₂ extract only a small fraction of soil available P, while stronger extractants solubilize a fraction of soil available P and some non available P (Fardeau et al., 1988; Kato et al., 1995). Two extraction methods are routinely used in Switzerland: one employs CO₂-saturated water (Dirks and Scheffer, 1930) and the other extracts P with ammonium-acetate EDTA (FAL et al., 1996, modified from Cottenie et al., 1982).

Sorption and desorption reactions have been studied extensively, since they control the mobility of phosphate ions between soil and solution. The determination of sorption isotherms allows studying the Quantity/Intensity relationship, thus obtaining information on the PBC and on the amounts of sorbed P that equilibrate with the solution. The inverse process, desorption of P from the solid phase, has gained interest in the studies of P availability and can be carried out in water, dilute CaCl₂ or in the presence of P sinks. Anion exchange resins (Sibbesen, 1978), Fe-oxide impregnated paper (Van der Zee et al., 1987; Chardon et al., 1996) or dialysis membrane tubes filled with Fe oxide (Freese et al., 1995) have been used as sinks to remove desorbed P from the solution, thus promoting further desorption and allowing a kinetic approach to the transfer of phosphate from soil to solution. Disadvantages of the sink methods can be grouped in: (i) changes in efficiency of the sink with time; (ii) difficulties in separating the sink from the soil suspension when mixed; (iii) the need to work at a low soil:solution ratio (generally lower than 1:10), a factor that strongly deviates from the ratio in the field (1:0.3) and influences P desorption (Sharpley et al., 1981).
As isotopic exchange methods allow assessing the amount of P located in a soil that can reach the soil solution within a given exchange time without disturbing the soil/solution system (Fardeau, 1996; Frossard and Sinaj, 1997; Di et al., 1997), they also give information on soil P availability. One of these methods, the isotopic exchange kinetics method, gives simultaneously access to the three factors that characterize P availability (I, Q and PBC) (Fardeau, 1996). Published results obtained in pot experiments with different plant and soil combinations have shown that soil isotopically exchangeable P was the main source for plant nutrition (Morel and Plenchette, 1994; Frossard et al., 1994; Bühler et al., 2003).

**Isotopic methods**

Radioactive and stable isotopes are a useful tool to investigate the dynamics of nutrients and pollutants in soil/plant systems. Sheppard (1962) developed a compartmental model theory and applied it to trace pharmacokinetics reactions in humans. Such an approach can be transferred to other multicompartmental systems as soils. We define a compartment as a unit in which an element acts kinetically as a distinct, homogeneous and well mixed amount. The volume in which one or more compartments are contained is a pool. A soil can be modeled as a sum of compartments interconnected with each other, where each connection is defined by the rate of transfer of the element out of and in a given compartment. A tracer can be used to estimate the mass of element in each compartment and the transfer rates between compartments. The tracer is injected in an accessible compartment of the system and must be ideal, that is: (i) must be detectable by an observer; (ii) its injection must not perturb the system; (iii) must be indistinguishable with respect to the studied element (the tracée). Furthermore, the studied system must be well mixed and in steady state with respect to the tracée and samples must be taken in the same compartment where the tracer has been injected (Cobelli et al., 2000; Frossard, 2003).

The radioisotopes $^{32}$P and/or $^{33}$P have been used as tracers in P studies. The earliest applications of isotopes to P availability studies date back to the middle of the 20th century. McAuliffe et al. (1948) and Wiklander (1950) measured the isotopically exchangeable phosphate in soil/solution systems spiked with radioactive $^{32}$P (E-values). Larsen (1952), by looking at the isotopic composition or specific activity (SA = $^{32}$P/$^{31}$P) of P taken up by barley grown in a soil fertilized by $^{32}$P-labelled calcium phosphates, calculated the content of isotopically exchangeable phosphate in the soil that was plant-available (L-values). Since the supply of nutrients from the
soil to the solution is a kinetic process, the kinetics of isotopic exchange were studied and modeled (Fardeau et al., 1985) with an isotopic exchange kinetics (IEK) experiment. This last approach allowed Fardeau (1996) to calculate E-values as a function of time (E_t) up to 3 months from parameters obtained from a short term (60 or 100 minutes) IEK experiments. Fardeau (1993) proposed to describe soil available P as a mamillary model presenting a central compartment containing the amount of P isotopically exchangeable within 1 minute connected to other compartments containing P exchangeable between 1 min and 24 h (which corresponds to the order of magnitude of active P uptake by a single root or a mycorrhizal hyphae), P exchangeable between 24 h and 3 months (which corresponds to the maximum period of nutrient uptake by the entire root system of an annual crop), and the fraction of P that can not be exchanged within 3 months which is of little relevance to plant nutrition. One limitation of the IEK method is that the mobilization of organic P is not addressed, since it is driven by mineralization processes and not by exchange. However, indirect observation of organic P mineralization rates can be obtained with this method (Oehl et al., 2001). Besides P, the IEK approach has been applied to study the availability of other nutrients such as K (Poss et al., 1991), S (Vannier et al., 1993), Ca and Fe (Schrärer, 2003) or heavy metals such as Zn (Sinaj et al., 1999; Sinaj et al., 2004; Diesing, 2004) and Cd (Gray et al., 2004). More details on the experimental procedures and on the meaning of the information obtained from this method are given and discussed in Chapter 2.

**P losses from agriculture**

A growing body of research is focusing on the pathways and the modalities of P transfer from agricultural soils to surface and subsurface waters (see e.g. the review written by Haygarth and Jarvis, 1999). Erosion and surface run-off are often considered to be the main pathways of P losses from arable land (Sharpley and Rekolainen, 1997; Heathwaite and Dils, 2000). However, leaching through the soil profile has recently gained interest (Sims et al., 1998; Stamm et al., 1998; Sinaj et al., 2002). Leaching can be quantitatively less important than run-off, but it is of major concern because P can be transported in dissolved and therefore available form in concentrations that may cause eutrophication (Turner and Haygarth, 2000). High P availability in the surface horizon of agricultural soils enhances the risk of leaching losses (Heckrath et al., 1995), especially during the absence of growing plants.
Motivations of the study

As in most western European countries, many agricultural soils in Switzerland present high to very high concentrations of available P (Frossard et al., 2004). The eutrophication of some lakes on the Swiss plateau has been related to diffuse losses of P from agricultural land with a high animal density (Gächter et al., 1996, Stadelmann et al., 2002). It is suggested that the only way for eutrophic lakes to return to a mesotrophic state is to reduce the discharge of P from the agroecosystems located in their watersheds (Wehrli and Wüst, 1996). In this perspective, the Swiss authorities have proposed that farmers should calculate P fertilization as a function of: (i) crop requirements determined by the quantity of P in the harvested crops; (ii) P availability in the topsoil, determined by a chemical extraction and corrected using the clay content of the soil as an indicator of the buffer capacity (Walter et al., 1994). In addition, as part of the framework of the “Direct payment to farmers”, farmers must calculate a P balance sheet at the farm level (P production/plant needs) that can not exceed 110% of the plant needs (Ordonnance sur les paiements directs versés dans l’agriculture, Titre 1, Chapitre, 3, Section 1, Art. 6, 1998; source www.admin.ch/ch/f/rs/910_13/index.html).

These practices need to be implemented on a scientific basis. This includes the study of the response of crops and of P availability to changes in P inputs and the evaluation of the ability of crops to use old reserves of P. On the other hand, P availability must be properly assessed and the methods employed to determine it must be evaluated. Finally, the effects of the fertilization practice on the potential risk of P losses should be assessed.

This sets the ground for the project on which this thesis is based. The project was divided into two parts. One studied the effects of three fertilization regimes (no application and application equal or in excess to crops exportation) on plant production and on P availability in soils sampled in field experiments under crop rotations and under grassland and the availability of P fertilizers added in the past to plants (Gallet et al., 2003a and b). The second part (this dissertation) was a methodological study aiming at a better understanding of processes controlling P exchangeability and at proposing an approach for the estimation of the risk of P losses from agricultural soils. Both field and laboratory approaches were needed to achieve the aims of this project, since in nature the changes in nutrients dynamics and the response of crops to these changes occur within spatial and temporal scales that are not reliably reproducible in laboratory or greenhouse conditions. The entire project was based on samples taken in six field experiments established by
the Swiss Federal Research Station for Agroecology (FAL, Reckenholz, Canton of Zurich) in 1989, and in the field experiment established in 1971 by the Swiss Federal Research Station for Plant Production (RAC, Changins, Canton of Vaud).

Objectives, hypotheses and structure of the thesis

The main objective of this thesis was to study the factors that influence P exchangeability and the potential of P release to the environment from agricultural soils.

In Chapter 2, we tested the hypothesis that phosphate exchangeability measured by the isotopic exchange kinetic method could be described by both the concentration of P in the soil solution and soil chemical and/or physical characteristics controlling P sorption. Until now contradictory results have been reported in the literature, which hinder a proper understanding of the processes controlling the rate of P exchangeability and its prediction with models. To test whether our observations were limited to our studied soils or were of general value, we attempted to use our findings to predict P exchangeability in another independent set of soils sampled under similar agro-climatic conditions.

Since having methods that accurately describe P availability is a prerequisite for giving a proper recommendation for P fertilization, the exchangeability of the quantity of P extracted by selected routine chemical methods was tested in Chapter 3. Our hypothesis was that a relatively weak extractant operating at low ionic strength and with a low pH buffering capacity would not mobilize large amounts of non-exchangeable P, whereas stronger extractants would dissolve significant fractions of slowly or non-exchangeable P.

Finally, in Chapter 4, an attempt was undertaken to estimate the potential P losses from a few samples. The conditions we set were targeting losses by leaching in the absence of plant uptake, that is, in the absence of a sink for P. We assumed that experiments with a flow-through reactor system using soil repacked in a column, through which dilute CaCl₂ could flow would reproduce conditions closer to the field situation than a batch experiment. Furthermore using soil samples labeled with radioactive ³³P, we hypothesized that the specific activity of P released from the
reactor would be a characteristic of the entire population of P ions present in the soil reactor that could potentially be released from the soil.
Influence of soil properties on phosphorus isotopic exchange kinetics in agricultural temperate soils
Abstract

The transfer of phosphorus (P) from the solid phase of the soil to its solution can be assessed by the isotopic exchange kinetic method. The importance of soil properties on isotopic exchange kinetic remains however badly known and prevents the prediction of P exchangeability. In this study, the influence of P concentration in the soil solution (C_P) and of selected soil properties controlling P sorption on the kinetics of P isotopic exchangeability was investigated. A range of C_P was obtained by incubating samples of the surface layer (0-20 cm) of 10 agricultural soils sampled under crop rotations in the presence of increasing concentrations of water-soluble P. Isotopic exchange kinetic experiments were carried out on these soils to assess the ratio R/r_1 which is the total radioactivity added in the soil solution system divided by the amount of radioactivity remaining in the solution after 1 minute of exchange, n which describes the rate of decrease of radioactivity after one minute, and C_P the concentration of P in the soil solution that are used to calculate the amount of P isotopically exchangeable with time (E_t). The parameters R/r_1 and n depended on C_P, on the amounts of Fe oxy-hydroxides in the soil and on soil pH. These relationships allowed us to predict short term (1 and 60 minutes) E_t-values and to obtain an estimate of soil P buffer capacity (PBC) which was significantly (p<0.01) correlated with the PBC expressed as the ratio between NaHCO_3-extractable P and C_P. We used our findings to predict the exchangeability of P in a larger set of soils either cultivated (30 soils) or under permanent grassland (23 soils) using their pH, oxides content and C_P as inputs in the model. Predicted and measured values were highly significantly correlated for all samples. However predicted values matched measured values only in the case of R/r_1 and E_1min of cropped soils whereas the values predicted for n and E_60min underestimated the measured values. Predicted values of R/r_1, n, E_1min and E_60min did not match measured values in the grassland soils. In conclusion relationships between the isotopic exchange kinetic parameters and soil properties should be studied in soils showing a wider range of properties and sampled under different land-use before being applied for modeling purposes.
Introduction
The quantity of phosphate ions in the soil solution represents about 1% of the total amount of phosphorus (P) annually taken up by crops (Morel et al., 2000). Thus the evaluation of soil P availability must take into account: the P concentration in the solution, the amount of P that can be transferred to the solution within a timeframe relevant to plant growth, and the ability of the soil solid phase to control this transfer. These three factors have been described by Beckett and White (1964) as the Intensity (I), the Quantity (Q) and the P buffer capacity (PBC), respectively. Isotopically exchangeable P is calculated from the specific activity \( SA = \frac{^{33}P}{^{31}P} \) of P measured in the solution of soils spiked with radioactive P after a given time of isotopic exchange (E-values, Wiklander, 1950) or in plants grown in soils that had been labeled with radioactive P (L-values, Larsen, 1952). Publications comparing E and L values showed that isotopically exchangeable P is the main source of P for a large number of plant species (Fardeau and Jappé, 1976; Frossard et al., 1994; Morel and Plenchette, 1994; Bühler et al., 2003). The isotopic exchange kinetics (IEK) experiment allows assessing 3 parameters that are used to calculate E-values (mg kg\(^{-1}\)) for any given exchange time ranging between 1 minute and 3 months according to the following equation that is valid in most temperate agricultural soils (Fardeau, 1996):

\[
E_t = 10C_F \left( \frac{R}{r_1} \right) t^n
\]  

[2.1]

where \( R/r_1 \) is the ratio of the total radioactivity introduced in the soil/solution system (R, MBq ml\(^{-1}\)) over the radioactivity remaining in the solution (\( r_1 \), MBq ml\(^{-1}\)) after 1 minute of isotopic exchange; \( n \), describes the rate of disappearance of \(^{33}\)P from the solution, due to the continuing exchange process between \(^{33}\)P and \(^{31}\)P after one minute and \( C_F \) is the concentration of P in the soil solution (mg L\(^{-1}\)) which is assumed to remain constant throughout the experiment. The factor 10 is the solution:soil ratio used in the IEK experiments. The IEK allows assessing the three factors proposed by Beckett and White (1964): \( E_t \) is the Quantity, \( C_F \) is the Intensity and their relationship \( (dE_t/dC_F) \) is the PBC (Morel et al., 2000).

The physico-chemical meaning of \( R/r_1 \) and \( n \) remains however poorly understood. On one side several studies showed that \( R/r_1 \) was related to soil properties which control P sorption, such as Fe oxy-hydroxides and clay content (Frossard et al., 1993; Tran et al., 1988), to clay mineralogy (Frossard et al., 1992a), to the PBC (Frossard et al., 1992b) and to the degree of phosphate saturation proposed by Van der Zee and Van Riemsdijk (1988) (Sinaj et al., 2002). Fardeau (1981) and Morel et al. (1989) designated \( r_1/R \) as the P fixing capacity of the soil as they noticed
that P added in a water soluble form rapidly disappeared from the solution of soils with a low \( r_t/R \) ratio. However, these are empirical relationships and \( R/r_t \) can only give indirect information on P sorption capacity, since it is assumed that radioactive P undergoes only exchange and does not become specifically sorbed onto soil surfaces (Fardeau, 1996). On the other side, Morel et al. (1994), Morel et al. (2000) and Schneider and Morel (2000) suggested that \( E_t \) and the \( n \) value were controlled uniquely by \( C_P \) and exchange time \( (t) \), \( C_P \) being determined by the P balance (input/output) of the soil. They proposed the following equation to calculate \( E_t \):

\[
E_t = v C_P^{w} t^{-q} \ln(C_p) \tag{2.2}
\]

where \( v, w, p \) and \( q \) are coefficients determined for each soil. Morel et al. (1994) showed that different fertilization rates and crop management (crop-fallow rotation or continuous crop) did not significantly affect these coefficients, unless they induced small but significant changes in soil properties such as soil pH. Whereas this approach is useful when applied to a given soil type, it has its limits when different soil types are considered. If valid over all soil types, this approach would assume that a given input of P would result in the same increment in \( E_t \) values in two soils having the same initial \( C_P \) but different sorption properties due for instance to different iron oxides content. This is however not likely to be the case as the PBC depends on soil properties controlling P sorption (Holford, 1997). The hypothesis of the present paper is that the parameters describing the isotopic exchange of P in a soil solution system are correlated to both \( C_P \) and soil properties controlling P sorption/desorption reactions as the concentration in iron oxides, soil pH, and/or clay content (Parfitt, 1978; Van der Zee et al., 1988; Salcedo et al., 1991; Holford, 1997). The objective of this paper was first to identify, on a set of 10 soil samples, the relationships between \( R/r_t, n, E_t \)-values and the PBC as estimated by isotopic exchange data and selected soil properties controlling P sorption and \( C_P \) and then to evaluate whether these relationships could be applied to a larger set of soils to predict their \( R/r_t, n, E_t \) and PBC. Relationships between \( R/r_t, n, E_t \) and PBC on the one side and \( C_P \) and selected soil properties on the other side, were obtained by conducting IEK experiments on 10 soils that had been incubated with increasing P additions so as to vary \( C_P \) without changing soil properties.
Material and Methods

Soils

Two sets of soils (A and B) were used for this study. Relationships between soil properties and Cp and R/r1, n, E, PBC were obtained from experiments conducted with the soils of set A, and the validity of these relationships were tested on set B.

The soils of set A were sampled in 1998 in six field experiments testing different strategies of crop P fertilization established either in 1989 by the Swiss Federal Research Station for Agroecology, Zurich (Cadenazzo, Rümlang, Ellighausen, Oensingen, FAL) or in 1971 by the Swiss Federal Research Station for Plant Production, Nyon (Changins). Samples were taken from the 0-20 cm horizon. Two P treatments (no P application since the beginning of the experiment (0P) and P applied as triple superphosphate in quantities equal to P uptake by the crops (P)) were sampled in Rümlang, Ellighausen, Oensingen and FAL. Only the 0P treatment was sampled in the Cadenazzo and Changins sites. Further details on the experimental design, the crop rotations, sampling and on the effects of P fertilization strategies on crop yield and soil P availability are given in Gallet et al. (2003b). The soils from set B were sampled in 2002 in farms located in the watershed of Lake Baldegg (Canton of Lucerne, Switzerland) (Sinaj, personal communication). This set was composed of 30 cultivated soils and 23 grassland soils. All cultivated soils were sampled in the 0-20 cm horizon while the grassland soils were sampled in the 0-10 cm layer. Samples were air-dried, plant debris removed by hand and the soils sieved at 2 mm before conducting the analyses and the experiments. All routine soil analyses (pH in H2O, total organic carbon, texture, and cation exchange capacity) were performed according to FAL et al. (1996). Ammonium oxalate-extractable Fe and Al (Fe_{ox}, Al_{ox}) were extracted according to Loeppert and Inskeep (1996) and citrate-dithionite-bicarbonate extractable Fe and Al (Fe_{d} and Al_{d}) were determined according to Mehra and Jackson (1960). The specific surface area (SSA, m² g⁻¹) was determined by the BET method as described in Weidler et al. (1998).

Total P was assessed by extracting 2 g of soil with 0.5M H₂SO₄ after ignition of the samples at 550°C for 1 hour. The amounts of P extracted from the soils of set A with this method were not significantly different (p<0.001) from total P determined with a microwave digestion method (Gallet, 2001). Some chemical and physical characteristics of the soils are presented in Table 2.1 (set A), and in Table 2.2 (set B).
<table>
<thead>
<tr>
<th>Soil/Treatment</th>
<th>Soil type</th>
<th>pH</th>
<th>TOC (H2O) g kg⁻¹</th>
<th>Clay Al₄⁺</th>
<th>Feox₀⁺ cmol kg⁻¹</th>
<th>Al/Fedd</th>
<th>C.E.C. cmol kg⁻¹</th>
<th>SSA g m⁻²</th>
<th>Total P mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümplang P</td>
<td>Calcaric Cambisol</td>
<td>7.7</td>
<td>18.6</td>
<td>0.96</td>
<td>2.07</td>
<td>11.12</td>
<td>17.2</td>
<td>6.3</td>
<td>982</td>
</tr>
<tr>
<td>Rümplang OP</td>
<td>Calcaric Cambisol</td>
<td>7.6</td>
<td>17.4</td>
<td>0.97</td>
<td>2.07</td>
<td>12.33</td>
<td>15.7</td>
<td>6.8</td>
<td>804</td>
</tr>
<tr>
<td>FALP</td>
<td>Eutric Gleysol</td>
<td>7.0</td>
<td>26.1</td>
<td>2.47</td>
<td>2.96</td>
<td>29.3</td>
<td>9.0</td>
<td>9.5</td>
<td>1139</td>
</tr>
<tr>
<td>FAL OP</td>
<td>Eutric Gleysol</td>
<td>7.1</td>
<td>24.9</td>
<td>2.43</td>
<td>3.00</td>
<td>30.9</td>
<td>9.5</td>
<td>9.5</td>
<td>1007</td>
</tr>
<tr>
<td>Oensingen P</td>
<td>Gleyi-calcaric Cambisol</td>
<td>7.3</td>
<td>23.2</td>
<td>1.31</td>
<td>5.10</td>
<td>12.05</td>
<td>14.82</td>
<td>13.9</td>
<td>1015</td>
</tr>
<tr>
<td>Oensingen OP</td>
<td>Gleyi-calcaric Cambisol</td>
<td>7.2</td>
<td>22.5</td>
<td>1.34</td>
<td>5.39</td>
<td>14.50</td>
<td>12.99</td>
<td>14.6</td>
<td>997</td>
</tr>
<tr>
<td>Eilighausen P</td>
<td>Gleyic Cambisol</td>
<td>6.5</td>
<td>19.1</td>
<td>1.34</td>
<td>5.39</td>
<td>15.00</td>
<td>21.9</td>
<td>8.5</td>
<td>1015</td>
</tr>
<tr>
<td>Eilighausen OP</td>
<td>Gleyic Cambisol</td>
<td>6.4</td>
<td>20.3</td>
<td>1.31</td>
<td>5.29</td>
<td>18.11</td>
<td>26.3</td>
<td>8.6</td>
<td>789</td>
</tr>
<tr>
<td>Changins OP</td>
<td>Eutric Fluvisol</td>
<td>6.0</td>
<td>11.6</td>
<td>0.84</td>
<td>4.23</td>
<td>8.41</td>
<td>12.1</td>
<td>3.9</td>
<td>1204</td>
</tr>
</tbody>
</table>

- FAO classification
- Total organic carbon
- Ammonium oxalate-extractable Al and Fe
- Citrate-dithionite-bicarbonate-extractable Al and Fe
- Cation exchange capacity
- Specific surface area
### Table 2.3: Ranges of soil properties, IEK parameters and isotopically exchangeable P within 1 minute for the soils of set B.

<table>
<thead>
<tr>
<th>Soils</th>
<th>pH (H₂O)</th>
<th>TOC</th>
<th>Cₜₚ</th>
<th>Clay</th>
<th>Alox</th>
<th>Fe²⁺</th>
<th>Aℓₜ</th>
<th>Total P</th>
<th>Cp</th>
<th>Rp/r</th>
<th>Rᵣᵣ</th>
<th>n</th>
<th>Eᵣᵣᵣ min</th>
<th>mg P kg⁻¹</th>
<th>mg P L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivated Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0-20 cm) min</td>
<td>5.4</td>
<td>24.0</td>
<td>133</td>
<td>0.45</td>
<td>0.98</td>
<td>0.49</td>
<td>4.77</td>
<td>730</td>
<td>0.038</td>
<td>1.45</td>
<td>0.16</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>7.4</td>
<td>53.0</td>
<td>330</td>
<td>1.74</td>
<td>1.74</td>
<td>2.12</td>
<td>15.23</td>
<td>1679</td>
<td>1.963</td>
<td>7.04</td>
<td>0.40</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>6.5</td>
<td>35.7</td>
<td>240</td>
<td>1.25</td>
<td>1.25</td>
<td>3.20</td>
<td>10.28</td>
<td>1028</td>
<td>0.485</td>
<td>2.87</td>
<td>0.27</td>
<td>10.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.5</td>
<td>35.5</td>
<td>230</td>
<td>1.24</td>
<td>1.24</td>
<td>3.11</td>
<td>1.36</td>
<td>943</td>
<td>0.382</td>
<td>2.34</td>
<td>0.28</td>
<td>8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grassland Soils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(0-10 cm) min</td>
<td>5.9</td>
<td>50.0</td>
<td>100</td>
<td>0.67</td>
<td>1.78</td>
<td>0.54</td>
<td>2.84</td>
<td>780</td>
<td>0.175</td>
<td>1.29</td>
<td>0.16</td>
<td>6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>7.3</td>
<td>138.0</td>
<td>371</td>
<td>2.05</td>
<td>5.10</td>
<td>2.43</td>
<td>18.80</td>
<td>3036</td>
<td>2.324</td>
<td>3.96</td>
<td>0.31</td>
<td>38.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>6.5</td>
<td>66.3</td>
<td>266</td>
<td>1.23</td>
<td>1.23</td>
<td>3.22</td>
<td>9.32</td>
<td>1504</td>
<td>1.183</td>
<td>2.00</td>
<td>0.23</td>
<td>20.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.4</td>
<td>65.0</td>
<td>250</td>
<td>1.17</td>
<td>1.17</td>
<td>3.00</td>
<td>8.82</td>
<td>1407</td>
<td>1.220</td>
<td>1.74</td>
<td>0.23</td>
<td>20.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ammonium acetate EDTA extractable P (P-AAEDTA) was extracted in a 1:10 suspension for 1 hour by a mixture of 0.5M ammonium acetate, 0.5M acetic acid and 0.02M EDTA. Sodium bicarbonate extractable P (P-NaHCO₃) was extracted by 0.5M NaHCO₃ at pH = 8.5 for 30 minutes with a soil:solution ratio of 1:20. P-NaHCO₃ was extracted at room temperature (20 to 25°C) whereas P-AAEDTA was extracted at 23°C as recommended by FAL et al. (1996). Orthophosphate concentration in the extracts was determined by colorimetry with the malachite green method (Ohno and Zibilski, 1991) after filtration through a 0.2 μm acetate cellulose membrane filter (Sartorius).

**Isotopic exchange kinetic (IEK) and isotopically exchangeable P (E) experiments**

IEK experiments were carried out on non-amended air-dried samples of the soils of set A and B and on moist samples of set A that had been incubated with increasing P quantities. The IEK method has been described in detail in several papers (e.g. Fardeau, 1996; Frossard and Sinaj, 1997). Here only an outline of the method is given. Ten grams of air-dried soil were weighed in 250 ml plastic bottles and equilibrated with 99 ml of deionized H₂O for 16 hours at 22°C on a rotating shaker. Before the beginning of IEK, the bottles were placed on a magnetic stirring plate and stirred at 300 rpm to maintain a homogeneous suspension. At time t = 0, 1 ml containing 0.1-1 MBq ml⁻¹ of carrier-free ³³P ions (as H₃³³PO₄ in HCl, specific activity at delivery >3.6 TBq ³³P mg⁻¹ ³¹P, Hartmann Analytic, GmbH) was injected in the soil suspensions and about 5 ml samples were taken with a polyethylene syringe 1, 10, 30 and 60 minutes after ³³P addition. The suspensions were rapidly filtered through a 0.2 μm cellulose-acetate membrane filter (Sartorius) and ³³P was measured by scintillation counting in the filtrates. Solution P concentration (C_p) was measured with the malachite green colorimetric method (Ohno and Zibilski, 1991) after the 60 minutes sampling.

The added ³³P in solution decreases with time, due to isotopic exchange with stable P on the solid phase, according to the following equation (Fardeau et al., 1985)

\[
\frac{r_t}{R} = (\frac{r_t}{R}) \cdot [t + (\frac{r_t}{R})^{1/n}]^{n} + r_o/R \tag{2.3}
\]

where R, r_t and n have the same meaning as in Eq. [2.1], r_t and r_o (MBq ml⁻¹) are, respectively the radioactivity remaining in solution after t minutes and infinite time. The parameter n is calculated as the slope of the linear regression between log(r_t/R) and log(t). The ratio r_o/R, which
quantifies the maximum possible dilution of the tracer, is approximated to the ratio between water soluble P (10\( \cdot \)C\(_P\) mg P kg\(^{-1}\)) and soil inorganic P (P\(_{\text{min}}\) mg P kg\(^{-1}\)) (Fardeau, 1996):

\[
\frac{r_o}{R} = 10 \cdot \frac{C_P}{P_{\text{min}}}
\]  

[2.4]

For the typical timeframe of IEK experiments, the \((r_t/R)^{1/n}\) and \(r_o/R\) terms can be considered as negligible and therefore the experimental short term kinetic data can be fitted to a simple power function of decrease of \(r_t/R\) with time:

\[
\frac{r_t}{R} = \frac{r_i}{R} \cdot t^n
\]  

[2.5]

The amount of soil isotopically exchangeable P (E\(_t\), expressed in mg P kg\(^{-1}\) soil) is calculated assuming that (i) \(^3\)P and \(^3\)P ions have the same fate in the system and (ii) at any given time t the specific activity (SA = \(^3\)P / \(^3\)P) of the phosphate ions in soil solution (\(r_t/10C_P\)) equals the SA of the pool of isotopically exchangeable P ions in the whole system (R/E\(_t\)):

\[
\frac{r_t}{C_P} = \frac{R}{E_t}
\]  

[2.6]

which, solved for E\(_t\), gives:

\[
E_t = 10 \cdot C_P \cdot (R/r_t)
\]  

[2.7]

and, for one minute of exchange,

\[
E_{t_{\text{min}}} = 10 \cdot C_P \cdot (R/r_t)
\]  

[2.8]

The E\(_{t_{\text{min}}}\) pool contains phosphate ions in the soil solution plus ions located on the solid phase with the same mobility as the ions in the solution (Fardeau et al., 1985; Salcedo et al., 1991). They are totally and immediately available to crops without chemical transformation (Fardeau, 1996).

**Phosphate sorption isotherms**

Phosphate was added to the soils of set A in order to obtain a range of C\(_P\). Fifty grams of dry soil were incubated in plastic pots for 21 days at 22°C in the dark with 0.3 ml g\(^{-1}\) of deionized water containing (NH\(_4\))\(_2\)HPO\(_4\) at concentrations corresponding to additions of 0, 30, 60, 120 and 360 mg P kg\(^{-1}\) soil. The pots were closed with a plastic cap to limit evaporation during incubation. P sorption was evaluated after the incubation. Sorbed P (P\(_S\), mg P kg\(^{-1}\)) was calculated as the difference between the amount of added P and the amount of water-extractable-P determined in the 1:10 soil:water suspension during the IEK experiments. For each soil, data were fitted to a Freundlich equation:

\[
P_S = i \cdot C_P^j
\]  

[2.9]
where $i$ is the amount of sorbed P when $C_p$ equals 1 mg P L$^{-1}$ and $j$ describes the rate of increase of $P_s$ with increasing $C_p$.

**IEK on P-amended soils and relationships between the kinetic parameters and $C_p$**

After the incubation, IEK were carried out on moist samples using the equivalent of 10 g soil dry weight. For each soil, the relationships between $C_p$, $R/r_1$ and $n$ were expressed as:

\[ R/r_1 = a \cdot C_p^{-b} \]  \[ n = f \cdot C_p^{-g} \]

where $a$, $b$, $f$, and $g$ are fitting coefficients. Morel et al. (1994) proposed a logarithmic function to describe the variation of $n$ with $C_p$, although in certain cases they found that a power function showed a better fit (Morel et al., 1996). We considered that Eq. [2.11] fitted our data better than a logarithmic function.

$E_t$ values were calculated by replacing $R/r_1$ and $n$ by their expressions as a function of $C_p$ in equation Eq. [2.1]:

\[ E_t = 10 \cdot a \cdot C_p^{-a} \cdot t^{f \cdot C_p^{-g}} \]

PBC for a given time $t$ was calculated as $\frac{dE_t}{dC_p}$ according to Eq. [2.13]

\[ PBC = 10 \cdot a \cdot (1-b) \cdot C_p^{-b} \cdot t^{f \cdot C_p^{-g}} - 10 \cdot a \cdot C_p^{-b} \cdot t^{f \cdot C_p^{-g}} \cdot f \cdot g \cdot C_p^{-(f+g)} \cdot \ln(t) \]

**Statistical analyses**

All experiments were performed in duplicate. Means were compared with the one-way ANOVA procedure of StatGraphics Plus 3.1 (Statistical Graphics Corp.) and statistically significant differences between means were determined with Duncan’s multiple range test at the 95% ($p<0.05$) or 99% ($p<0.01$) probability level. The $a$, $b$, $f$, and $g$ coefficients and their relationships with soil properties were assessed with linear, non linear and multiple regression procedures of StatGraphics. Significance of the regressions is given at the 95% (*) or 99% (**) probability level throughout the text. When models based on our findings were applied to predict measured values, the accuracy of the prediction was tested with the one-sample t test procedure of StatGraphics. We tested the null hypothesis that the average of the ratios between predicted and measured values (for each given modeled parameter) was not significantly different from 1 at $p<0.01$. The output is a $p$-value which indicates the probability level at which the alternative hypothesis (i.e. the average of the ratios is different from 1) should not be rejected.
Results and Discussion

IEK parameters and isotopically exchangeable P in the non-amended soils of set A

The kinetic parameters $R/r_1$ and $n$ ranged, respectively, between 1.8 and 9.5 and between 0.28 and 0.39 (Table 2.3). According to Fardeau (1981) 5 of these soils present a high “P fixing” capacity ($R/r_1>5$) while 2 have a low “P fixing” capacity ($R/r_1<2.5$). $E_{1\text{min}}$ values ranged between 2.4 and 12.8 mg P kg$^{-1}$. In most of the $0P$ soils (with the exception of Cadenazzo and Ellighausen), $E_{1\text{min}}$ was below the critical limit of 5 mg P kg$^{-1}$ suggested by Gallet et al. (2003b), under which P availability can become a limiting factor for plant production. As already reported in the literature (Tran et al., 1988; Frossard et al., 1993), $R/r_1$ was positively correlated ($p<0.01$) with the dithionite-citrate-bicarbonate extractable iron ($Fe_d$) and the specific surface area (SSA) ($p<0.01$) and negatively to $C_P$ ($p<0.01$) (Table 2.5). The parameter $n$ showed a weaker but significant positive correlation with $Fe_d$ ($p<0.05$) and was negatively correlated to $C_P$ ($p<0.01$) (Table 2.5).

Table 2.3. The isotopic exchange kinetic parameters and isotopically exchangeable P within one minute of the soils of set A (means and standard error of the mean).

<table>
<thead>
<tr>
<th>Soil/Treatment</th>
<th>$C_P$ mg P L$^{-1}$</th>
<th>$R/r_1$</th>
<th>$n$</th>
<th>$E_{1\text{min}}$ mg P kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümlang $P$</td>
<td>0.184 ± 0.004</td>
<td>3.7 ± 0.19</td>
<td>0.31 ± 0.00</td>
<td>6.8 ± 0.22</td>
</tr>
<tr>
<td>Rümlang $0P$</td>
<td>0.040 ± 0.001</td>
<td>5.9 ± 0.18</td>
<td>0.38 ± 0.01</td>
<td>2.4 ± 0.10</td>
</tr>
<tr>
<td>FAL $P$</td>
<td>0.319 ± 0.005</td>
<td>4.0 ± 0.10</td>
<td>0.28 ± 0.00</td>
<td>12.6 ± 0.20</td>
</tr>
<tr>
<td>FAL $0P$</td>
<td>0.089 ± 0.001</td>
<td>5.4 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>4.8 ± 0.46</td>
</tr>
<tr>
<td>Oensingen $P$</td>
<td>0.093 ± 0.002</td>
<td>7.8 ± 0.23</td>
<td>0.36 ± 0.00</td>
<td>7.2 ± 0.23</td>
</tr>
<tr>
<td>Oensingen $0P$</td>
<td>0.042 ± 0.003</td>
<td>9.5 ± 0.46</td>
<td>0.38 ± 0.02</td>
<td>4.0 ± 0.15</td>
</tr>
<tr>
<td>Ellighausen $P$</td>
<td>0.354 ± 0.008</td>
<td>2.4 ± 0.11</td>
<td>0.31 ± 0.01</td>
<td>8.4 ± 0.39</td>
</tr>
<tr>
<td>Ellighausen $0P$</td>
<td>0.166 ± 0.001</td>
<td>3.0 ± 0.01</td>
<td>0.34 ± 0.00</td>
<td>5.0 ± 0.06</td>
</tr>
<tr>
<td>Changins $0P$</td>
<td>0.033 ± 0.000</td>
<td>8.1 ± 0.34</td>
<td>0.39 ± 0.00</td>
<td>2.7 ± 0.04</td>
</tr>
<tr>
<td>Cadenazzo $0P$</td>
<td>0.301 ± 0.006</td>
<td>1.8 ± 0.03</td>
<td>0.32 ± 0.01</td>
<td>5.3 ± 0.06</td>
</tr>
</tbody>
</table>
Figure 2.1. P sorption isotherms for the studied soils. Points are experimental data (with standard deviation). Dotted (OP) and dashed (P) lines are the plots of Eq. [2.9] with the coefficients $i$ and $j$ presented in Table 2.4.
**Psorption isotherms**

The sorption curves obtained for the 10 soils of the set A are presented in Figure 2.1. At high $C_p$, the high values of sorbed P may result, besides from surface sorption, from precipitation processes especially in neutral to calcareous soils (Castro and Torrent, 1998; Tunesi et al., 1999). The coefficients $i$ and $j$ of the Freundlich equation (Eq. [2.9]) fitted to the data are shown in Table 2.4. The $i$ (i.e. the amount of sorbed P at a $C_p$ of 1 mg P L$^{-1}$) and $j$ of the $0P$ soils were significantly ($p<0.05$) higher than those of $P$ soils, showing that 9 years of omitted fertilization increased the P sorption capacity of these soils. The $i$ values showed significant positive correlations with $R/n$ and $n$ ($p<0.01$), with $F_e$ ($p<0.01$) and SSA ($p<0.05$) of all soils of set A (Table 2.5) confirming earlier results (Tran et al., 1988; Frossard et al., 1993).

<table>
<thead>
<tr>
<th>Soil/Treatment</th>
<th>$i^a$</th>
<th>$j^a$</th>
<th>$r^2$</th>
<th>S.e.e. $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümlang $P$</td>
<td>57.69a</td>
<td>0.87b</td>
<td>0.95</td>
<td>* 0.291</td>
</tr>
<tr>
<td>Rümlang $0P$</td>
<td>86.19b</td>
<td>0.69a</td>
<td>0.94</td>
<td>* 0.297</td>
</tr>
<tr>
<td>FAL $P$</td>
<td>44.93a</td>
<td>0.93b</td>
<td>0.98</td>
<td>* 0.205</td>
</tr>
<tr>
<td>FAL $0P$</td>
<td>77.91b</td>
<td>0.79a</td>
<td>0.93</td>
<td>* 0.325</td>
</tr>
<tr>
<td>Oensingen $P$</td>
<td>68.03a</td>
<td>0.85b</td>
<td>0.93</td>
<td>* 0.331</td>
</tr>
<tr>
<td>Oensingen $0P$</td>
<td>89.57b</td>
<td>0.78a</td>
<td>0.89</td>
<td>* 0.429</td>
</tr>
<tr>
<td>Ellighausen $P$</td>
<td>46.08a</td>
<td>0.93b</td>
<td>0.96</td>
<td>* 0.257</td>
</tr>
<tr>
<td>Ellighausen $0P$</td>
<td>63.12b</td>
<td>0.82a</td>
<td>0.96</td>
<td>* 0.241</td>
</tr>
<tr>
<td>Changins $0P$</td>
<td>84.35</td>
<td>0.73</td>
<td>0.97</td>
<td>* 0.224</td>
</tr>
<tr>
<td>Cadenazzo $0P$</td>
<td>38.10</td>
<td>0.82</td>
<td>1.00</td>
<td>** 0.065</td>
</tr>
</tbody>
</table>

$^a$ Different letters between the estimates of $i$ and $j$ indicate a statistically significant difference ($p<0.05$) between two treatment of the same soil

$^b$ Standard error of the estimate
Table 2.5. The correlations between the IEK parameters (R/ri and n) and the coefficient i of Eq. [2.9] with soil properties and Cp in the 10 soils of set A

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>$r^2$</th>
<th>S.e.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R/ri = 0.010 \cdot Fe_d^{2.49}$</td>
<td>0.95**</td>
<td>0.13</td>
</tr>
<tr>
<td>$R/ri = 0.63 \cdot SSA - 0.83$</td>
<td>0.79**</td>
<td>1.29</td>
</tr>
<tr>
<td>$n = 0.01 \cdot Fe_d + 0.22$</td>
<td>0.49*</td>
<td>0.03</td>
</tr>
<tr>
<td>$R/ri = 1.46 \cdot Cp^{-0.53}$</td>
<td>0.74**</td>
<td>0.30</td>
</tr>
<tr>
<td>$n = 0.27 \cdot Cp^{-0.12}$</td>
<td>0.87**</td>
<td>0.04</td>
</tr>
<tr>
<td>$i = 4.80 \cdot Fe_d^{1.04}$</td>
<td>0.59**</td>
<td>0.21</td>
</tr>
<tr>
<td>$i = 23.10 \cdot SSA^{0.46}$</td>
<td>0.46*</td>
<td>0.24</td>
</tr>
<tr>
<td>$i = 30.72 \cdot R/ri^{0.48}$</td>
<td>0.75**</td>
<td>0.16</td>
</tr>
<tr>
<td>$i = 456.70 \cdot n - 90.7$</td>
<td>0.84**</td>
<td>7.98</td>
</tr>
</tbody>
</table>

Relations between $R/ri$, $n$ and $C_p$ and soil properties in the incubated P amended soils

The relationships between $C_p$ measured in the incubated P amended soils and the respective $R/ri$ and $n$ values are shown in Figure 2.2a ($R/ri$) and 2.2b ($n$) for all soils and all P additions.

Figure 2.2. The relationships between $R/ri$ and $C_p$ (a) and between $n$ and $C_p$ (b). The regression equations are $R/ri = 2.80 \cdot Cp^{0.27}$ ($r^2 = 0.54**$) and $n = 0.163 \cdot Cp^{0.264}$ ($r^2 = 0.93**$) (all soils and all P additions are shown).
Soil properties and P exchangeability

Both parameters decreased with increasing \( C_P \). Significant correlations were observed between \( C_P \) and \( R/r_1 \) using Eq. [2.10] and between \( C_P \) and \( n \) using Eq. [2.11]. Eq. [2.10] explained only 54% of the overall data variability, but it explained between 91 and 100% of the data variability when it was applied to each individual soil (Table 2.6), demonstrating that besides \( C_P \) some soil properties should be taken into account to properly describe \( R/r_1 \).

Table 2.6. The estimates of the coefficients \( a, b, f \) and \( g \) of Eq. [2.10] and [2.11]. For a given soil, the differences of the coefficients between \( 0P \) and \( P \) treatments are indicated at \( p<0.05 (*) \) and \( p<0.01 (**) \). ns = not significant.

<table>
<thead>
<tr>
<th>Soil/Treatment</th>
<th>( R/r_1 = a \cdot C_P^{-b} )</th>
<th>( N = f \cdot C_P^{-2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( a )</td>
<td>( b )</td>
</tr>
<tr>
<td>Rümlang ( P )</td>
<td>3.81</td>
<td>0.22</td>
</tr>
<tr>
<td>Rümlang ( 0P )</td>
<td>3.64</td>
<td>* 0.12</td>
</tr>
<tr>
<td>FAL ( P )</td>
<td>2.65</td>
<td>0.27</td>
</tr>
<tr>
<td>FAL ( 0P )</td>
<td>2.97</td>
<td>* 0.30</td>
</tr>
<tr>
<td>Oensingen ( P )</td>
<td>3.39</td>
<td>0.32</td>
</tr>
<tr>
<td>Oensingen ( 0P )</td>
<td>3.88</td>
<td>ns 0.40</td>
</tr>
<tr>
<td>Ellighausen ( P )</td>
<td>2.26</td>
<td>0.17</td>
</tr>
<tr>
<td>Ellighausen ( 0P )</td>
<td>2.38</td>
<td>* 0.17</td>
</tr>
<tr>
<td>Changins ( 0P )</td>
<td>2.63</td>
<td>0.27</td>
</tr>
<tr>
<td>Cadenazzo ( 0P )</td>
<td>1.39</td>
<td>0.12</td>
</tr>
</tbody>
</table>

On the other side Eq. [2.11] explained 93% of the overall data variability suggesting that variations in the parameter \( n \) were almost fully explained by variations in \( C_P \). However the low residual variance left by fitting Eq. [2.11] to our data might be partially explained by the small range of \( n \) values opposed to the larger range of \( C_P \) values. The \( a, b, f \) and \( g \) coefficients of Eq. [2.10] and [2.11] were determined for each soil and treatment (Table 2.6). The values of \( a \) and \( f \) indicate, respectively, \( R/r_1 \) and \( n \) at a \( C_P \) of 1 mg P L\(^{-1}\), whereas \( b \) and \( g \) describe the extent of the effect of increasing \( C_P \) on the two kinetic parameters. As previously observed by Morel et al. (1994) for the \( n \) vs \( C_P \) relationship no consistent differences in \( f \) and \( g \) were observed between soils from the \( 0P \) and \( P \) treatments of Rümlang, Ellighausen, Oensingen and FAL. This suggests
Soil properties and P exchangeability

that whether conducted on the long term in the field or in the short term under laboratory conditions, P fertilization has the same effect on the kinetics of isotopic exchange confirming earlier observations made by Morel et al. (1994).

The soil dependency of the $R/r_1$ vs $C_p$ and $n$ vs $C_p$ relationships was studied by relating $a$, $b$, $f$ and $g$ with selected soil properties (Table 2.7). The parameter $a$ was positively correlated to soil pH ($p<0.01$) as the first independent variable and to $Fe_d$ ($p<0.05$) or SSA ($p<0.01$) as the second independent variable. Similarly, $f$ showed a positive correlation with soil pH as the first variable ($p<0.01$) and the sum of ammonium oxalate-extractable Fe and Al ($Fe+Al)_{ox}$ as the second ($p<0.01$). The exponents $b$ and $g$ of Eq. [2.10] and [2.11] showed weaker but significant correlations with both $Fe_d$ ($p<0.05$) and SSA ($p<0.01$).

Table 2.7. Relationships between the coefficients of Eq. [2.10] and [2.11] and soil properties

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Soil properties</th>
<th>Equation</th>
<th>$r^2$</th>
<th>S.e.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Fe$_d$, pH</td>
<td>$a = 1.11* \text{pH} + 0.09* \text{Fe}_d - 5.92$</td>
<td>0.90**</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>SSA, pH</td>
<td>$a = 1.25* \text{pH} + 0.06* \text{SSA} - 6.32$</td>
<td>0.92**</td>
<td>0.222</td>
</tr>
<tr>
<td>$b$</td>
<td>Fe$_d$</td>
<td>$b = 0.30* \ln(\text{Fe}_d) - 0.51$</td>
<td>0.55*</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>SSA</td>
<td>$b = 0.04* \text{SSA}^{0.74}$</td>
<td>0.64**</td>
<td>0.263</td>
</tr>
<tr>
<td>$f$</td>
<td>(Fe$<em>{ox}$+Al$</em>{ox}$), pH</td>
<td>$f = 0.011 + 0.0139* \text{pH} + 0.0115(\text{Fe}<em>{ox}+\text{Al}</em>{ox})$</td>
<td>0.86**</td>
<td>0.005</td>
</tr>
<tr>
<td>$g$</td>
<td>Fe$_d$</td>
<td>$g = 0.097 + (1.816/\text{Fe}_d)$</td>
<td>0.76**</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>SSA</td>
<td>$g = 0.41 - 0.0069* \ln(\text{SSA})$</td>
<td>0.59**</td>
<td>0.027</td>
</tr>
</tbody>
</table>

These findings confirm our preliminary hypothesis: $R/r_1$ and $n$ can be described as a function of $C_p$ and of soil properties controlling P sorption. Our results show that for a $C_p$ of 1 mg P L$^{-1}$, $R/r_1$ and therefore $E_{imin}$ increase with soil pH and the $Fe_d$ content while $n$ increases with pH and the sum (Fe+Al)$_{ox}$. These observations are coherent with a dilution, which increases with time, of the added radioactive P within the solution and within a fraction of P sorbed onto the solid phase. These fractions (P remaining in the solution and sorbed P) are controlled by the amount of added P, the concentration of metallic oxides, soil pH and, at higher pH values, by the presence of Ca.
compounds (Olila and Reddy, 1995; Holford, 1997; Rietra et al., 2001; Boruvka and Rechcigl, 2003).

**Isotopically exchangeable P and P Buffer Capacity in the incubated P-amended soils.**

E-values were calculated with Eq. [2.12], using the coefficients $a$, $b$, $f$ and $g$ listed in Table 2.7.

For $a$, $b$, and $g$ the relationship with $F_{d}$ was chosen over the one with SSA, since the former measurement is more commonly performed than the latter. The comparison between E-values obtained with Eq. [2.12] and the experimental E-values determined in a 60 minutes IEK experiment is shown in Figure 2.3a for $E_{\text{1min}}$ and in Figure 2.3b for $E_{\text{60min}}$. The slope and intercept of the regression lines between predicted and experimental E-values were (values ± standard error) $1.04 (±0.03)$ and $-0.17 (±1.52)$ for $E_{\text{1min}}$ and $1.00 (±0.03)$ and $1.68 (±2.65)$ for $E_{\text{60min}}$. Furthermore, the one-sample t-test showed that the average of all the ratios between predicted and measured values was not significantly ($p<0.01$) different from 1 (data not shown), confirming that our model could well predict short term measured E-values.
Since the $E_t$ vs $C_P$ relationship is a description of the P Buffer Capacity (PBC), we could calculate PBC in function of $C_P$, time and soil properties, as the first order derivative $dE_t/dC_P$ (PBC\text{iek}) as shown in Eq. [2.13] using the values of $a$, $b$, $f$ and $g$ listed in Table 2.7. The PBC\text{iek} values were compared to PBC values derived from chemical extractions (PBC\text{chem}) calculated either as the ratio between P-NaHCO$_3$ (as Q) and $C_P$ (as I) or as the ratio between P-AAEDTA (as Q) and $C_P$ (as I) as proposed by Célardin (2003). For the comparison, time in Eq. [2.13] was set to 60 minutes. The PBC\text{iek} after 60 min of isotopic exchange showed strong correlations with the PBC\text{chem} ($p<0.01$) (Figure 2.4). The best relationship was obtained with NaHCO$_3$-extractable P. This can be explained by the fact that the P extracted by this method is more rapidly isotopically exchangeable P than the P extracted by AAEDTA (see Chapter 3). Furthermore the $R/r_1$ values showed significant correlations with both PBC\text{iek} ($r^2 = 0.80^{**}$) and PBC\text{chem} ($r^2 = 0.88^{**}$, with $Q$ = NaHCO$_3$-extractable P), confirming that this kinetic parameter is as well related to the buffer capacity, as previously observed by Frossard et al. (1992b) and by Célardin (1991).
Table 2.8. The values of PBC determined by Eq. [2.13] for an exchange time of 1 and of 60 minutes

<table>
<thead>
<tr>
<th>Soil/Treatment</th>
<th>([\text{PBC}<em>\text{IEK}]</em>{\text{min}}) L kg(^{-1})</th>
<th>([\text{PBC}<em>\text{IEK}]</em>{60\text{min}}) L kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümlang P</td>
<td>41.1 aA</td>
<td>73.8 aB</td>
</tr>
<tr>
<td>Rümlang 0P</td>
<td>54.3 bA</td>
<td>112.1 bB</td>
</tr>
<tr>
<td>FAL P</td>
<td>30.4 aA</td>
<td>55.0 aB</td>
</tr>
<tr>
<td>FAL 0P</td>
<td>41.1 bA</td>
<td>86.4 bB</td>
</tr>
<tr>
<td>Oensingen P</td>
<td>45.4 aA</td>
<td>93.9 aB</td>
</tr>
<tr>
<td>Oensingen 0P</td>
<td>52.7 bA</td>
<td>118.3 bB</td>
</tr>
<tr>
<td>Ellighausen P</td>
<td>21.6 aA</td>
<td>37.5 aB</td>
</tr>
<tr>
<td>Ellighausen 0P</td>
<td>23.8 bA</td>
<td>45.2 bB</td>
</tr>
<tr>
<td>Changins 0P</td>
<td>43.7 A</td>
<td>90.3 B</td>
</tr>
</tbody>
</table>

* Different small letters indicate a statistically significant difference (p<0.05) between OP and P treatments; different capital letters indicate a statistically significant difference (p<0.05) between exchange times of 1 and 60 minutes

Table 2.9. The range of \(\text{PBC}_{\text{IEK}}\) (for 1 and 60 minutes) at three different P additions

<table>
<thead>
<tr>
<th>(\text{PBC}_{\text{IEK}})</th>
<th>P additions (mg P kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>(\text{[PBC}<em>{\text{IEK}]</em>{\text{min}}}_{\text{(L kg}^{-1})})</td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>16.4</td>
</tr>
<tr>
<td>Max</td>
<td>54.3</td>
</tr>
<tr>
<td>Average</td>
<td>37.1</td>
</tr>
<tr>
<td>St.Dev.</td>
<td>13.2</td>
</tr>
<tr>
<td>(\text{[PBC}<em>{\text{IEK}]</em>{\text{60min}}}_{\text{(L kg}^{-1})})</td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>30.0</td>
</tr>
<tr>
<td>Max</td>
<td>118.3</td>
</tr>
<tr>
<td>Average</td>
<td>74.2</td>
</tr>
<tr>
<td>St.Dev.</td>
<td>31.1</td>
</tr>
</tbody>
</table>

A significant increase (p<0.05) was observed when time in Eq. [2.13] increased from 1 to 60 minutes, confirming that pools of rapidly exchangeable P have a lower buffer capacity than pools containing slowly exchangeable P as observed by Morel et al., (2000) and Ehlert et al., (2003).
Soil properties and P exchangeability

Furthermore, the comparison of 0P and P treatments and of P additions in the amended soils (Table 2.8 and 2.9) showed that P fertilization decreased significantly PBCIEK (Holford and Mattingly, 1976).

Predicting \( R/r_1, n, E_{1\text{min}} \text{ and } E_{60\text{min}} \) for the soils of set B

The kinetic parameters \( R/r_1 \) and \( n \) and E-values for 1 and 60 minutes of the soils from the set B (Table 2.2) were calculated by estimating the \( a, b, f \) and \( g \) coefficients from their soil properties using the equations shown in Table 2.7 and then introducing the coefficients and the \( C_p \) values in Eq. [2.12].

The predicted values were then compared with the \( R/r_1 \) and \( n \) and E-values for 1 and 60 minutes that had been measured in these samples (Figures 2.5 a, b, c and d). Significant relationships (\( p<0.01 \)) between predicted and measured values were found for all parameters in both agricultural and grassland soils (Table 2.10). However, a 1:1 relationship was not consistently observed for all parameters. Predicted values of \( R/r_1 \) and \( E_{1\text{min}} \) were not different from the measured values in the cropped soils (one-sample t-test), whereas the predicted values of \( n \) underestimated the measured ones (Table 2.10). Consequently, the predicted \( E_{60\text{min}} \) values, which are calculated using \( n \) values, were underestimated as well. In the grassland soils, the prediction of all parameters was not statistically satisfactory: \( R/r_1 \) and \( E_{1\text{min}} \) were overestimated, whereas \( n \) and \( E_{60\text{min}} \) were underestimated, like in the agricultural soils.

Table 2.10. The coefficients of the linear regressions between the predicted and the measured kinetic parameters of the soils of set B.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Agricultural soils</th>
<th>Grassland soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope Intercept</td>
<td>( r^2 ) S.e.e.</td>
</tr>
<tr>
<td>( R/r_1 )</td>
<td>0.93 0.27</td>
<td>0.77** 0.676 0.979</td>
</tr>
<tr>
<td>( n )</td>
<td>0.74 0.12</td>
<td>0.71** 0.027 &lt;0.001</td>
</tr>
<tr>
<td>( E_{1\text{min}} )</td>
<td>0.75 2.13</td>
<td>0.92** 1.857 0.999</td>
</tr>
<tr>
<td>( E_{60\text{min}} )</td>
<td>0.85 9.43</td>
<td>0.76** 6.142 &lt;0.001</td>
</tr>
</tbody>
</table>

*The \( P \)-values of the one sample t-test indicate the significance of the hypothesis that the average of the predicted/measure ratio is statistically different from 1 and therefore that the prediction is not statistically significant.
The PBC_{IEK} was calculated for t = 60 minutes with Eq. [2.13] and related to the NaHCO_3-extractable P/C_P ratio (data not shown). For the cultivated soils the regression equation was [PBC_{IEK}]_{60\text{min}} = 0.33\cdot(P-\text{NaHCO}_3/C_P) + 4.67 (r^2 = 0.81**). The slope of the regression was close to the slope observed with the soils of set A, suggesting a good ability of our model in predicting the PBC of the cultivated soils of set B. On the other hand, the grassland soils showed a significant but weaker (r^2 = 0.55**) relationship between [PBC_{IEK}]_{60\text{min}} and P-\text{NaHCO}_3/C_P.
These positive correlations suggest that the relationships that were found between the parameters of isotopic exchange, the PBC and soil properties are of general value. These equations cannot, however, predict exactly the n and E_{60min} of cropped soils or the isotopic parameters of grassland soils. Higher levels of available P, of organic matter and of microbial biomass could explain the lack of ability of our model to predict the kinetic parameters of our grassland soils. Altogether these relations should be established on samples showing larger differences in soil properties e.g. by using soils from different geographical origins and under different land use.

Conclusions

In this study we evaluated the effects of soil properties controlling P sorption capacity and of solution P concentration (C_P) on P isotopic exchangeability and on P buffer capacity. We have shown that C_P and exchange time could not fully describe the changes in P exchangeability and on P buffer capacity if the effect of soil properties, such as the concentration of Fe oxy-hydroxides and soil pH, were not taken into account. Our findings allowed a good prediction of R/r_1 and of E_{1min} in a larger set of soils from a similar geographical and climatic context, whereas the kinetic parameter n and E_{60min} were consistently underestimated. This shows that relationships between the isotopic exchange kinetic parameters and soil properties should be studied in soils showing a wider range of properties and sampled under different land-use before being applied for modeling purposes.

Acknowledgements

We would like to thank Mr. Thomas Flura and Mr. Sven Bolomey (ETH Zurich, Institute of Plant Sciences) for providing the data of the soils of set B. This study was funded by the Swiss National Science Foundation (SNF), project n° 31-50528.97.
Exchangeability of phosphate extracted by four chemical methods


*J. Plant Nutr. Soil Sci.* 168: 89-93
Abstract

Isotopically exchangeable phosphate is a major source of phosphorus (P) for plants. In practice however, plant available P is assessed by chemical extractions solubilizing a mixture of P forms which availability is ill defined. We undertook an isotopic approach to assess the exchangeability of P extracted by (i) CO₂-saturated water (P-CO₂), (ii) ammonium acetate EDTA (P-AAEDTA) and (iii) sodium bicarbonate (P-NaHCO₃) compared to the exchangeability of P extracted by water. Five topsoils with similar P fertilization histories but different soil properties were studied. P was extracted from soils labelled with carrier free ³²P after a week of incubation, and the specific activity (SA = \(^{32}\)P / \(^{31}\)P) of the extracts was compared with the SA of P extracted by water to calculate the amount of P isotopically exchangeable that had been solubilized during the extraction. P-CO₂ extracted between 20 and 100 times less P than P-AAEDTA and P-NaHCO₃. The SA of P-CO₂ was not different from the SA of water extractable P, showing that P-CO₂ solubilized similar forms of P as water and that these forms can be considered as available. The SA of P extracted by the two other methods ranged between 25 and 63% for P-AAEDTA and 66 and 92% for P-NaHCO₃ of the SA of water extractable P. The fraction of exchangeable P extracted by AAEDTA decreased linearly with increasing soil pH, suggesting that this method dissolves slowly or non exchangeable P from calcium phosphates.
Exchangeability of extractable P

Introduction

Plant available phosphorus (P) is composed of phosphate ions that can move to the plant root during plant growth (Frossard et al., 2000). Isotopic techniques can be used to quantify the amount of P that can be transferred from the soil’s solid phase to the solution within a given time and accordingly, isotopically exchangeable P during a period of 3 months has been shown to be a major source of P for crops (Fardeau and Jappe, 1976; Frossard et al., 1994; Morel and Plenchette, 1994). However, because of the difficulties in manipulating radioactive P, isotopic approaches are not used in routine analyses for soil P availability. Instead, short term chemical extractions are preferred, although the amount of P extracted by these methods might reflect more the nature of the extractant than the real soil P availability for plants. In order to check this hypothesis, Fardeau et al. (1988) and Kato et al. (1995) studied the exchangeability of P solubilized by acidic or alkaline chemical extractions in soils previously suspended in water spiked with radioactive P. These authors showed that the specific activity (SA = \(^{33}\text{P} / {^{31}\text{P}}\)) of P in these extracts was often lower than the specific activity of P in the soil:water suspensions. This points to the fact that these extractants had solubilized proportionally more stable P than water, demonstrating that only a fraction of extracted P should be considered as available as isotopically exchangeable P. Barrow and Shaw (1976) and Fardeau et al. (1988) also calculated the gross flux of P being solubilized and the gross flux of P being re-adsorbed during chemical extractions, demonstrating the complexity of the processes occurring.

As non-point source pollution of P is becoming a wide problem in all intensively agriculturally used areas, it is necessary to adopt methods that describe accurately P availability and to base on these the appropriate management practices in order to limit P input and maximize its use by crops. This is why it is important to precisely assess the relationship between the amount and the nature of chemically extractable P and soil properties.

The objective of this work was to study the exchangeability and nature of P solubilized by three chemical extractions used in routine analyses: one operating at soil pH with a very low ionic strength (water saturated with CO\(_2\), Dirks and Scheffer, 1930, P-CO\(_2\)), another one operating at an acidic pH in the presence of ammonium acetate EDTA as a complexing agent (FAL et al., 1996, modified from Cottenie et al., 1982, P-AAEDTA) and a last one operating at an alkaline pH (Olsen et al., 1954, P-NaHCO\(_3\)) and to compare it with the exchangeability
of P extracted by water (P-w). P-CO₂ and P-AAEDTA are the two methods applied in Switzerland in routine soil P tests, whereas P-NaHCO₃ is considered adequate for assessing P availability in calcareous (Sen Tran and Giroux, 1985) and acid soils (Sims and Ellis, 1983). Five soils with similar P fertilization histories but different soil properties were selected for this study. The nature of extracted P was inferred by extracting soils that had been labelled with carrier free ³²P and by comparing the specific activity (SA) of P extracted by these three methods with the SA of water extractable P, considered as the SA of soil isotopically exchangeable P. Our hypothesis was that the nature of P extracted by P-CO₂ would be identical to that of P extracted by water (the SA should be similar), whereas the SA of P-AAEDTA and of P-NaHCO₃ would be lower than the SA of water extractable P as these methods would extract slowly or not exchangeable P from calcium phosphates (P-AAEDTA) and from metallic oxides and organic compounds (P-NaHCO₃), respectively. The effect of soil properties on the nature of P extracted by the tested methods was addressed as well.

Materials and methods

Soils

The soils used in this work come from five sites (Rümlang, FAL, Oensingen, Ellighausen and Cadenazzo) where field experiments have been established in 1989 by the Swiss Federal Research Station for Agroecology (Zurich), as described in detail in Gallet et al. (2003b). The study was carried out on samples taken in 1998 from plots where P was applied yearly as triple superphosphate in quantities equivalent to crops off-take (P treatment). Soils were sampled in the 0-20 cm layer (after harvest but prior to fresh fertilizer application), air dried and sieved at 2 mm. Some selected characteristics are presented in Chapter 2, Table 2.1. For Cadenazzo P (missing in Table 2.1) the soil properties were equal to the OP treatment as shown in Table 2.1 and the amount of total P was of 1235 mg P kg⁻¹.

Chemical extraction methods

P-w extraction was performed with a 1:10 soil water ratio for 16 hours. P-CO₂ extraction was performed with a 1:2.5 soil:extractant ratio for 1 hour using CO₂-saturated nanopure water at pH 3.5-4 and pCO₂ of 6 bars. P-AAEDTA was extracted in a 1:10 suspension for 1 hour by a mixture of 0.5M ammonium acetate, 0.5M acetic acid and 0.02M EDTA. P-NaHCO₃ was extracted by 0.5M NaHCO₃ at pH = 8.5 for 30 minutes with a soil:solution ratio of 1:20. P-w,
Exchangeability of extractable P

P-CO₂ and P-NaHCO₃ were extracted at room temperature (20 to 25°C) whereas P-AAEDTA was extracted at 23°C as recommended by FAL et al. (1996). Orthophosphate concentration in the extracts was determined by colorimetry with the malachite green method (Ohno and Zibilski, 1991) after filtration with a 0.2 µm acetate cellulose membrane filter (Sartorius). Total P in the NaHCO₃ extracts (Pₚ-NaHCO₃) was measured after digestion of the solution with potassium persulfate (Bowman, 1989) and the concentration of organic P in the extract (Pₒ-NaHCO₃) was calculated as the difference between total and inorganic NaHCO₃ extractable P.

Isotopic composition of P-NaHCO₃, P-CO₂ and P-AAEDTA.
Soils were incubated for one week in the dark at 21 ±1 °C after thorough mixing with 0.3 ml g⁻¹ soil (dry weight) of nanopure water containing carrier-free $^{33}$P (H₃$^{33}$PO₄ in HCl, Hartmann Analytic, GmbH with a specific activity at delivery >3.6 TBq $^{33}$P mg⁻¹ $^{31}$P) at the rate of 0.025 MBq g⁻¹ soil in order to label soil isotopically exchangeable P. P-w, P-NaHCO₃, P-CO₂ and P-AAEDTA were extracted from moist subsamples equivalent to 2 g of air-dried soil according to the methods described above. P and $^{33}$P concentrations were measured in each extract after filtration at 0.2 µm and used to calculate the specific activity of the extract ($S_{A_{ext}} = \frac{^{33}P \text{ extracted}}{P \text{ extracted}}$). The specific activity of P extracted by water is considered as a reference (as plants take up their P as orthophosphate from the soil solution) and is denominated as $S_{A_w}$ in the rest of the text. No significant differences were observed between $S_{A_w}$ determined in water extracts with soil:solution ratios and shaking time specific for each of the tested chemical methods (results not shown). $^{33}$P was measured by liquid scintillation counting. Measurements of radioactivity were corrected for quenching in the AAEDTA extracts. No quenching effect was observed for P-w, P-NaHCO₃ and P-CO₂ extractions. $^{33}$P associated with Pₒ-NaHCO₃ and P-NaHCO₃ was partitioned as proposed by Jayachandran et al. (1992).

The ratio $S_{A_{ext}} / S_{A_w}$ indicates the fraction of extracted P that was isotopically exchangeable (Fardeau et al., 1988). This ratio can be used to calculate the amount of P (mg P kg⁻¹) extracted by a given method that was in an isotopically exchangeable form:

$\text{Ext P}_{\text{exc}} = \left(\frac{S_{A_{ext}}}{S_{A_w}}\right) \times \text{Extractable P} / 100 \quad [3.1].$

The amount of P isotopically exchangeable within a week ($E_{1wk}$, mg P kg⁻¹ soil) was calculated using P-w, the amount of radioactive P extracted by water after a week of
Exchangeability of extractable P incubation \( t_{\text{wk}} \), MBq) and the total amount of radioactivity introduced into the soil \( (R, \text{ MBq}) \):

\[
E_{\text{1wk}} = \frac{R}{t_{\text{1wk}}} \times P-w \quad [3.2].
\]

All radioactivity measurements were corrected for decay.
Isotopically exchangeable P within one minute \( (E_{\text{1min}}) \) was determined with an isotopic exchange kinetic (IEK) experiment as described by Frossard and Sinaj (1997) and in Chapter 2 of this dissertation.

Statistical analyses
All analyses were performed in triplicates. Means were compared with the Duncan’s Multiple Range Test of Statgraphics plus 3.1 for Windows (Statistical Graphics Corp.) and statistically significant differences are indicated at the 0.05 probability level. Linear regression analyses were as well performed with Statgraphics.

Results and Discussion
The amounts P and \( ^{33}\text{P} \) extracted by water and by the three other tested chemical methods and the \( \text{SA}_{\text{ext}}/\text{SA}_{\text{w}} \) ratios are shown in Table 3.1.

\( \text{CO}_2 \)-saturated water extracted the smallest amounts of P, whereas P-AAEDTA and inorganic P-NaHCO\(_3\) yielded amounts between 30 and 100 and 20 and 60 times larger than P-CO\(_2\), respectively. P-\( \text{NaHCO}_3\) accounted for 23 to 43% of total NaHCO\(_3\) extractable P (data not shown), but no radioactivity was measured in this fraction. In this discussion, only inorganic NaHCO\(_3\)-extractable P will be addressed.

\( \text{SA}_{\text{ext}} \) of P-CO\(_2\) was not significantly different \( (p < 0.05) \) from \( \text{SA}_{\text{w}} \) for all the tested soils, showing that P-CO\(_2\) and water solubilize similar forms of P. This is related to the fact that the initially low pH of the extractant is very rapidly buffered to reach soil pH (data not shown). As the P present in the soil solution is entirely and instantaneously plant available, this result demonstrates that the P extracted by water saturated with CO\(_2\) is also fully available.

\( \text{SA}_{\text{ext}} \) varied between 25 and 63% of \( \text{SA}_{\text{w}} \) for P-AAEDTA and between 66 and 92% of \( \text{SA}_{\text{w}} \) for P-NaHCO\(_3\). This results show that these reactants solubilized proportionally more stable P than \( ^{33}\text{P} \) compared to water, as already pointed out by Fardeau et al. (1988) for NaHCO\(_3\).
Table 3.1. P and $^{33}$P extracted by deionized water, CO$_2$-saturated water, AAEDTA and NaHCO$_3$ and the percentage of P extracted in an isotopically exchangeable form expressed as the ratio between the specific activities of extractable P (SA$_{ex}$) and SA of water soluble P (SA$_w$). Standard error of the mean is given below.

<table>
<thead>
<tr>
<th>Soils</th>
<th>P-w</th>
<th>P-CO$_2$</th>
<th>P-AAEDTA</th>
<th>P-NaHCO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>$^{33}$P</td>
<td>P</td>
<td>$^{33}$P</td>
</tr>
<tr>
<td></td>
<td>mg kg$^{-1}$</td>
<td>kBq kg$^{-1}$</td>
<td>mg kg$^{-1}$</td>
<td>kBq kg$^{-1}$</td>
</tr>
<tr>
<td>Rümlang P</td>
<td>1.8b(a)</td>
<td>531.6c</td>
<td>0.6b</td>
<td>181.9c</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>5.5</td>
<td>0.02</td>
<td>5.9</td>
</tr>
<tr>
<td>FAL P</td>
<td>2.8c</td>
<td>426.5b</td>
<td>1.0c</td>
<td>137.1b</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>5.0</td>
<td>0.01</td>
<td>4.2</td>
</tr>
<tr>
<td>Ellighausen P</td>
<td>3.1d</td>
<td>446.0b</td>
<td>0.9d</td>
<td>129.0ab</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>24.2</td>
<td>0.01</td>
<td>3.2</td>
</tr>
<tr>
<td>Oensingen P</td>
<td>1.2a</td>
<td>331.2a</td>
<td>0.4a</td>
<td>106.7a</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>8.0</td>
<td>0.00</td>
<td>0.3</td>
</tr>
<tr>
<td>Cadenazzo P</td>
<td>3.2d</td>
<td>709.3d</td>
<td>0.8c</td>
<td>152.8b</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>32.7</td>
<td>0.02</td>
<td>17.5</td>
</tr>
</tbody>
</table>

a Different letters indicate significant differences ($p < 0.05$) of extracted P, $^{33}$P and of the $SA_{ex}/SA_w$ ratio between soils within the same extraction method.

b For P-CO$_2$ $SA_{ex}$ is not significantly different from $SA_w$ at $p < 0.05$ for all soils.
The differences, for a given soil, in the proportion of exchangeable P extracted by NaHCO₃ and AAEDTA may be explained by looking at the action of each extractant. Sodium bicarbonate (at pH of 8.5) promotes the displacement of P, mostly from oxy(hydroxy)des and organic surfaces, by competition between P and bicarbonate ions and by increasing the negative charge on the surfaces; secondary reabsorption of desorbed P is limited by the bicarbonate present in the system (Barrow and Shaw, 1976).

On the other hand, AAEDTA extracts P by complexing metals and calcium from compounds to which P is bound and, because of its acidity, by dissolving Ca-P minerals (Sharpley et al., 1985; Woodard et al., 1994). The isotopic exchangeability of Ca-P minerals is reported to be lower than the one of P sorbed on the surface of oxy(hydroxy)des (Torrent et al., 1992; Castro and Torrent, 1998) and this can explain the better ability of P-NaHCO₃ in extracting isotopically exchangeable P.

![Figure 3.1](image)

Figure 3.1. The ratio of SA of P-AAEDTA to SA of water extractable P in function of soil pH. Dashed lines represent the 95% confidence intervals.

Soil properties affected the relative proportions of exchangeable and non-exchangeable fractions. For AAEDTA, the ratio between $SA_{\text{ext}}$ and $SA_{\text{w}}$ decreased linearly (i.e. the non-
Exchangeability of extractable P

exchangeable fraction increased) as soil pH increased (Figure 3.1). Although this data set is constituted of different soils and not of a soil that has been brought at different pH, this suggests that the dissolution of slowly or not exchangeable Ca-phosphates promoted by the AAEDTA extractant increases as pH increases. The quantities of exchangeable P extracted by AAEDTA and NaHCO₃ (P-AAEDTA<sub>exc</sub> and P-NaHCO₃<sub>exc</sub>) and the amounts of P isotopically exchangeable in one minute (E<sub>1min</sub>) and in one week (E<sub>1wk</sub>) are shown in Table 3.2.

Table 3.2. Amounts of P-AAEDTA and P-NaHCO₃ extracted in an isotopically exchangeable form as calculated from Eq. [3.1] and isotopically exchangeable P in one minute and in one week (the latter calculated from Eq. [3.2]). Standard error of the mean is given below.

<table>
<thead>
<tr>
<th>Site</th>
<th>P-AAEDTA&lt;sub&gt;exc&lt;/sub&gt;</th>
<th>P-NaHCO₃&lt;sub&gt;exc&lt;/sub&gt;</th>
<th>E&lt;sub&gt;1min&lt;/sub&gt;</th>
<th>E&lt;sub&gt;1wk&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümlang P</td>
<td>15.2&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9</td>
<td>64.7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.4</td>
<td>0.2</td>
<td>4.3</td>
</tr>
<tr>
<td>FAL P</td>
<td>17.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1</td>
<td>118.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.6</td>
<td>0.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Ellighausen P</td>
<td>13.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.1</td>
<td>121.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.9</td>
<td>0.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Oensingen P</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9</td>
<td>77.4</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>1.2</td>
<td>0.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Cadenazzo P</td>
<td>21.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>70.2</td>
</tr>
<tr>
<td></td>
<td>1.9</td>
<td>2.2</td>
<td></td>
<td>4.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Different letters indicate a statistically significant difference between P-AAEDTA<sub>exc</sub> and P-NaHCO₃<sub>exc</sub> within a given soil at p < 0.05  
<sup>b</sup> not determined

Despite the differences in extractable P, the amounts of exchangeable P extracted from a given soil by the two chemical methods were significantly different (p < 0.05) only in one soil. This result suggests that the amounts of exchangeable P extracted by two different chemical methods may reflect similar species of P, whereas the unexchangeable fractions may account for the difference in P extractability by different methods. Nevertheless a further investigation of the species of soil P dissolved by the two extractants would be necessary to confirm this hypothesis and perhaps to explain the significant difference between P-AAEDTA<sub>exc</sub> and P-NaHCO₃<sub>exc</sub> observed in one soil.
The amounts of exchangeable P extracted by the two methods were between 1.3 and 2.7 times higher than E₁in, which represents a pool of P that is immediately and completely available (Fardeau, 1996). P-AAEDTAexc and P-NaHCO₃exc accounted for only 11 to 31% of E₁wks, i.e. the pool of P that was exchanged during the incubation period. This shows that AAEDTA and NaHCO₃ had not extracted the total amount of P that had undergone isotopic exchange during the week of incubation. A similar conclusion was reached by Sinaj et al. (2004) for the exchangeability of chemically extractable zinc. The amounts of exchangeable P extracted by CO₂-saturated water (which correspond to P-CO₂ in Table 3.1, since all P-CO₂ is isotopically exchangeable) were one order of magnitude lower than the amount of P exchangeable within a minute.

**Conclusions**

The isotopic method employed in this study allowed the evaluation of the nature and the exchangeability of phosphate extracted by various chemical methods. Isotopically exchangeable P was the only source of P mobilized by CO₂-saturated water. This can be expected when such extractants are employed, i.e. solutions with low ionic strength (such as diluted CaCl₂) and operating at the pH of the tested soil. However, CO₂-saturated water strongly underestimates the amount of rapidly exchangeable P. The two other tested methods displaced non-exchangeable forms of P as well, depending on the interactions between the extractant and soil properties. Our results showed that AAEDTA is likely to dissolve significant amounts of P which may not be available, especially at higher soil pH. If this method is used in routine analysis, its interpretation must take into account the soil pH and probably the quantity of exchangeable cations. Furthermore such an analysis should not be carried after an addition of rock phosphate or lime as this would distort the meaning of the results. On the contrary, the NaHCO₃ extraction gave a better picture of P availability for the tested soils than AAEDTA, as the fraction of non-exchangeable P solubilized by this chemical was lower. However, a clear effect of soil properties on the exchangeability of P-NaHCO₃ was not observed in this study. Finally, both AAEDTA and NaHCO₃ extracted similar amounts of isotopically exchangeable P that were higher than the amount of rapidly exchangeable P, but lower than the amount of P exchangeable during the time course of incubation.
Acknowledgements

We would like to thank Dr. F. Mächler and Mrs. T. Rösch (ETH Zürich, Institute of Plant Sciences) for measuring P concentration in solution. This research was funded by the Swiss National Science Foundation (SNF), project n° 31-50528.97.
Assessment of phosphate release from agricultural soils using a flow-through reactor system.
Abstract
A large amount of information exists on the relations between phosphorus (P) availability in soil and plant uptake whereas less information exists on the relations between P availability and the amount of P that can be lost to the environment. The potential of phosphorus release to subsurface drainage waters was evaluated with 3 unfertilized (0P) and 3 fertilized (P) agricultural soils using a flow-through reactor made of a column containing repacked soil leached with 2 mM CaCl₂ for 28 days. Two sets of experiments were carried out. First, samples that had been incubated for 28 days in the presence of carrier-free ³³P were introduced in the columns and the pH and the concentrations of ³³P and P were measured in the eluates. In the second series, soils that had been incubated for 28 days without ³³P were introduced in the columns and were removed after specific leaching times to assess P exchangeability with the isotopic exchange kinetics method. Between 1.3 and 32.6 mg P kg⁻¹ and up to 39% of the added ³³P were released from the columns after 28 days. 0P soils released smaller amounts of P and of ³³P than the P soils. In two of the unfertilized soils, the release of ³³P became non detectable after 48 hours of leaching. Two phases could be distinguished during the release of P from the columns. The first phase was marked by a significant pH decrease in the eluates and by high concentrations of P and ³³P and lasted about 4 days. P release during this phase was probably resulting from a complex series of processes involving desorption/sorption and dissolution reactions. The second phase was characterized by stable pH values and by low rates of ³³P and P release. P release during this phase resulted probably from desorption and diffusion processes. Results allowed measuring a D-value (mg P kg⁻¹) which was the amount of isotopically exchangeable P having the same specific activity as the P released from the column. This amount was interpreted as the total amount of P that could be potentially released from the soil columns and varied between 172 and 284 mg P kg⁻¹ in the soils where it could be calculated. Results obtained with the isotopic exchange kinetic experiment conducted on the soils that had been incubated without ³³P and then leached with CaCl₂ confirmed the complex processes that took place during the early days of the leaching experiment and showed that leaching strongly affected soil P exchangeability. Highly significant correlations were found between the amounts of CaCl₂ extractable P measured in the soils before the leaching experiment and both the amounts of P released within 28 days from the columns and the amounts of P present in the rapidly leached pool. The interest and limits of this flow through reactor to assess P losses from soils were then discussed.
Introduction

The continuous inputs of phosphorus (P) in agroecosystems in amounts exceeding crop requirements have led to the accumulation of available P in the surface horizon of many agricultural soils of developed countries. This has accelerated phosphorus losses through overland flow or leaching from agricultural land to surface and/or ground waters, causing adverse effects on water quality (Foy and Withers, 1995). In order to reduce the risks of phosphorus losses from arable land while maintaining the desired level of crop production, it is important to optimize the utilization of soil and fertilizer phosphorus by plants (Frossard et al., 2000). In Switzerland, this has been evaluated by studying the effects of various fertilization schemes on plant production and P availability (Gallet et al., 2003a, b). However, there is no information on the effect of these fertilization schemes on the potential phosphorus losses from agricultural land to water bodies.

Soil P tests have been proposed to estimate the concentrations of dissolved P in run-off or drainage waters (Heckrath et al, 1995; McDowell and Sharpley, 2001; Maguire and Sims, 2002; Sims et al., 2002). McDowell et al. (2001) showed that isotopically exchangeable P (Eᵢ-values) measured with low soil:solution ratios in deionized water were strongly correlated to the concentrations of P observed in overland flow, whereas Eᵢ-values measured at higher soil:solution ratios in 0.01M CaCl₂ were better correlated to the concentrations of P found in drainage waters. The use of a low soil:solution ratio in batch experiments probably represents natural conditions of run-off, in which a large amount of water mixes rapidly with a very limited amount of soil from which P is then removed (Schärer, 2003). However, a batch experiment probably does not properly reflect the processes that can occur during the leaching of P, as the destruction of soil microstructure due to shaking affects the release of P to the solution (Wang et al., 2001). Undisturbed and disturbed soil column and flow-through experiments have been used to study reactions, mobility and leaching of P from soils (e.g. Van der Zee et al., 1992; Coale et al, 1994; Chen et al., 1996, Chardon et al., 1997; Wang et al, 2001, Maguire and Sims, 2002). These experiments gave valuable information on the processes controlling phosphorus losses by leaching and gave actual amounts of P leached from the columns but were not able to yield the total amount of P that could be released by leaching. To achieve this aim, Van der Zee et al. (1987), Freese et al. (1995), Lookman et al. (1995) and Koopmans et al. (2001) extracted soil P with an iron oxide impregnated filter paper or with an amorphous iron oxide in a dialysis tube.
These approaches allowed these authors to hypothesize that the total amount of P bound to amorphous Al and Fe oxides could be released from the soil provided that the desorption time would be long enough. However, these approaches also have their limits. First these are also batch experiments during which soil microstructure might be altered. Then the amounts of desorbable P can be confounded by soil particles remaining on the iron oxide impregnated paper (Freese et al., 1995); and when P release is promoted by a dialysis tube filled with an amorphous Fe oxide, the build up of relatively high P concentration in solution prevents a proper modeling of the potential amount of P that can be desorbed (Koopmans et al., 2001). These problems could be circumvented by leaching columns containing soils that had been pre-incubated with radioactive $^{33}$P. In analogy with the measurements of soil phosphate availability obtained by measuring the specific activity of P ($^{33}$P/$^{31}$P) in plants grown on labeled soils (the L-values; Sibbesen, 1984; Frossard et al., 1994; Bühler et al., 2003) we can assume that the isotopic composition of P released from a $^{33}$P labeled soil column will be a characteristic of the pool of potentially releasable soil P.

In this work, we used a flow-through reactor to study the potential P release from three agricultural soils that have been either annually fertilized with P since 1989 (P) or that have not received any P fertilizer since 1989 (OP). Our objectives were (i) to evaluate the effects of different P inputs on the kinetics of P release; (ii) to assess the size of the pool of soil P that could potentially be released; and (iii) to observe the changes in soil P exchangeability after defined leaching times. To do so, we performed two sets of experiments: first we studied the kinetics and the isotopic composition of P released from soils which had been previously incubated with carrier-free $^{33}$P and then we followed the changes in soil P exchangeability after various leaching times by carrying out isotopic exchange kinetics experiments (Fardeau, 1996) on depleted soils that had been incubated without $^{33}$P.

**Material and Methods**

**Soils**

The soils used for this study came from three long-term field experiments on P fertilization established in 1989 by the Swiss Federal Research Station for Agroecology (FAL), Zurich. One field trial is located at the FAL research station itself, while the other two are in Rümlang.
P release from soils with a flow-through system

(Canton of Zurich) and in Cadenazzo (Canton of Ticino). In all sites, soils were under crop rotations. Samples were taken in 2003 in the OP (no P application since the beginning of the experiment) and in the P (P applied as triple superphosphate in quantities equal to P uptake by the crops) treatments. Further details on the experimental design, the crop rotations and on sampling are given in Gallet et al. (2003b). Samples were taken from the ploughed horizon (0-20 cm), air dried, sieved at 2 mm and stored in the dark at room temperature. The main soil properties (pH, total organic carbon, texture, cation exchange capacity) were determined according to FAL et al. (1996) and are shown in Table 2.1 of Chapter 2 of this dissertation.

**Phosphorus analyses**

Ammonium oxalate-extractable P (POx) was determined after 4 hours of extraction and with a soil:extractant ratio of 1:40 (Loeppert and Inskeep, 1996). The extracts were filtered at 0.2 μm with a cellulose-acetate membrane filter (Sartorius). P concentration in the oxalate extracts was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Isotopically exchangeable P (Et-values, mg P kg⁻¹) and the kinetic parameters of isotopic exchange were determined with isotopic exchange kinetic experiments (IEK) as described in Chapter 2.

**Soil incubation and labeling**

Samples of the six studied soils (three soils and two P treatments) were incubated with 0.3 ml g⁻¹ of deionized water for 28 days in a growth chamber at 22°C, 50% of atmospheric humidity and in absence of light. A first set of samples was labeled with carrier-free ³³P ions (H₃³³PO₄ in HCl, specific activity at delivery day >3.6 TBq ³³P mg⁻¹ ³¹P, Hartmann Analytic GmbH) diluted in the deionized water added for the incubation. The amounts of radioactivity were the same for both OP and P treatments of a given soil and were (at the time of labeling: May 11th 2004 for the OP soils and June 14th 2004 for the P soils) 29.6 MBq g⁻¹ for the Cadenazzo soils and 40.7 MBq g⁻¹ for the FAL and Rümlang soils. Radioactivity was added in three separate steps after pre-wetting of the soils with two thirds of the total added water. Thorough mixing of the wet soils followed each step of radioactivity addition, in order to obtain a homogeneous labeling. For each soil, 100 g (dry weight) of sample was used. These soils will be referred to, throughout the text, as “labeled soils”. Another set of samples (300 g dry weight) was incubated under the same
conditions but without radioactivity and will be called "unlabeled soils" hereafter. The pots containing the soils were covered to prevent evaporation during the incubation.

Measurement of P release with a flow-through reactor

The flow-through system employed in this study is derived from the system proposed by Freese et al. (1999). The reactor column (Figure 4.1) was a polymethylmetacrylate cylinder with an internal diameter of 25 mm and contained, from bottom to top: a ring-shaped silicon gasket; one 1.6 μm Whatman GF/A glass microfibre filter; about 4 g of acid washed silica sand (Siegfried
AG, Switzerland); 10 g (dry weight) of soil sample; other 6 g of silica sand; one 0.7 μm Whatman GF/F glass microfibre filter. After placement in the column, the soil sample was packed by gently pressing with a cork stopper in order to minimize the volume of air pockets and obtain a similar soil volume (about 10 cm³) for all the columns. The columns were tightly closed at each end with polycarbonate in-line filter holders (Fischer Scientific AG, Switzerland) and contacts between the column and the caps were sealed with Teflon tape. The columns were connected with Tygon tubes (Fischer Scientific AG, Switzerland, internal diameter of 2.06 mm) to a 24 channels peristaltic pump (Ismatec, IPC-N - ISM 939). The outflows were led by Tygon tubes into 250 ml conical flasks, where leachates were collected. An upward flow was chosen in order to reduce the effects of filters clogging by fine soil particles. The eluent was 2mM CaCl₂ and it was fed to the pump from glass bottles which were replenished daily with fresh solution. The flow rate was set to 0.2 ml min⁻¹, which corresponded to an average of 2.2 pore volumes per hour.

Two sets of experiments were carried out in a growth chamber at 22°C and in absence of light. On a first set, the system was run with ³²P labeled samples (see above) for 28 days, corresponding to about 1460 pore volumes. Eluates were collected twice in the first day and then every 24 hours, weighed and an aliquot of 20 ml was filtered at 0.2 μm with a cellulose-acetate membrane filter (Sartorius). ³²P was measured in a 2ml aliquot by scintillation counting. ³¹P was measured colorimetrically (Ohno and Zibilski, 1991). For the OP soils, after the first 3 days of experiment, the ³¹P concentration in the eluates of two soils (FAL and Rümlang) was too low to be measured directly. Therefore 10 ml of leachate was centrifuged overnight under vacuum until all the solution was evaporated. The residue was dissolved in 2 ml of deionized water in which the colorimetric essay was carried out. The specific activity of released P (SA, Bq μg⁻¹) was calculated as the ratio between released ³²P (Bq g⁻¹) and released ³¹P (μg g⁻¹). All radioactivity measurements were corrected for decay back to the day of labeling. The pH of the eluates was measured with a glass electrode every day during the first week of experiments and then once a week.

If we consider that ³¹P and ³²P have a similar behavior in a soil/solution system, then the entire population of P ions present within the column that possess a specific activity identical to that of the released P (measured at any nᵗʰ sampling step) can be potentially released from the column. The total amount of P that can be potentially released from a soil (D-value, mg P kg⁻¹), can be
P release from soils with a flow-through system

calculated using Eq. [4.1] by analogy with the measurements of soil phosphate availability obtained by measuring the specific activity of P ($^{33}$P/$^{31}$P) in plants grown on soils that had been labeled with $^{33}$P (the L-values; Larsen, 1952; Sibbesen, 1984; Frossard et al., 1994; Buhler et al., 2003):

$$D_n = \left( R - \sum_{i=1}^{n-1} r_{Di} \right) / P_{Di} + \sum_{i=1}^{n-1} P_{Di} \quad [4.1]$$

where: $R$ is the total amount of radioactivity added to the soil (Bq $^{33}$P g$^{-1}$) and $r_{Di}$ (Bq $^{33}$P g$^{-1}$) and $P_{Di}$ (μg P g$^{-1}$) are, respectively, the amounts of $^{33}$P and $^{31}$P released from the soil at the $n$th sampling step. The sums $\sum_{i=1}^{n-1} r_{Di}$ and $\sum_{i=1}^{n-1} P_{Di}$ are the cumulative amounts of $^{33}$P and $^{31}$P released between the first and the $(n-1)^{th}$ step.

In the second set of experiments, the system was run with unlabeled soil samples for 1, 3, 14 and 28 days. The soils were then removed from the columns and IEK and ammonium-oxalate extractions were performed on subsamples of the extruded moist soils. The initial conditions were defined by performing IEK on moist incubated unlabeled samples which had not undergone leaching. IEK experiments were carried out with 2mM CaCl$_2$ as background solution for consistency with the eluent solution.

**Statistical analyses**

The leaching experiments were carried out on four replications for the labeled soils and three for the unlabeled soils. Means of all the experimental measurements were compared with a one-way ANOVA procedure of StatGraphics Plus 3.1 (Statistical Graphics Corp.) and statistically significant differences between means were determined with Duncan's multiple range test at the 95% ($p<0.05$) or 99% ($p<0.01$) confidence level. The experimental curves of P release were modeled using the linear and non-linear regression procedures of StatGraphics. Goodness of the fits was evaluated with the coefficients of determinations ($r^2$) and the standard error of the estimate. Significance of the regressions is given at the 95% (*) or 99% (**) confidence level.
Figure 4.2. The changes with time in the concentration of $^{31}$P ($^{31}$C$_P$) and of $^{33}$P ($^{33}$C$_P$) in the leachates. The dashed lines in the 0P soils show the quantification limit of $^{33}$P. Values below the limit are not shown.
Results and Discussion

\(^{31}P\) and \(^{33}P\) release during the column experiment

The changes with time in the concentration of \(^{31}P\) and \(^{33}P\) in the eluates are shown in Figure 4.2. In the OP soils, the concentration of \(^{31}P\) decreased within the first 96 hours and then leveled off to 0.014, and 0.001 mg P L\(^{-1}\) for Cadenazzo and Rümlang, respectively. In FAL OP, a slight but significant (p<0.01) increase was observed from the minimum value observed at 96 hours (0.002 mg P L\(^{-1}\)) to 0.005 mg P L\(^{-1}\), reached after about 240 hours. In the P soils, after an initial rapid decrease observed within the first 48 hours of the experiment, the \(^{31}P\) concentration continued to decrease at a very slow rate until the end of the experiment in the Rümlang and FAL soils. The Cadenazzo P soil showed a continuous non-linear decrease. After 28 days, \(^{31}P\) concentration in the eluates had reached 0.027, 0.021 and 0.017 mg P L\(^{-1}\) in the Cadenazzo, FAL and Rümlang P soils, respectively. The final P concentration in the eluates of FAL and Rümlang OP was below 0.01 mg P L\(^{-1}\), which is, according to Vollenweider (1968), the limit above which P can begin to trigger eutrophication of surface water bodies, whereas Cadenazzo OP and all the P soils showed higher values.

The concentrations of \(^{33}P\) in the eluates of the OP and of the P soils followed patterns similar to the concentrations of the stable isotope. Radioactivity measurements dropped under the quantification limit (ten times the average background noise of CaCl\(_2\)) for both FAL and Rümlang OP after 48 hours and for Cadenazzo OP after 600 hours of leaching. Radioactivity measurements taken after these times are therefore not further discussed. The \(^{33}P\) concentrations of the P soils remained always above the quantification limit.

The cumulative releases of \(^{31}P\) and \(^{33}P\) are shown in Figure 4.3. A maximum of release was not reached within the duration of our experiments. For a given soil, the trends of \(^{31}P\) and of \(^{33}P\) release were generally comparable and there was a consistent difference in the patterns of release between the OP and the P soils. For the OP soils, two distinct steps of P release were observed: a non-linear phase until 96 hours and a linear phase afterwards. In the P soils, P release was non-linear and continuous (Figure 4.3). The discussion of the kinetics of P release is presented further on in the text.
Figure 4.3. Cumulative curves of $^{31}\text{P}$ ($^{31}\text{P}_R$) and $^{33}\text{P}$ ($^{33}\text{P}_R$) release from the $^{33}\text{P}$ labelled soils.
P release from soils with a flow-through system

The total amounts of $^{31}$P and $^{33}$P leached after 28 days are shown in Table 4.1. Our flow-through system mobilized relatively small amounts of both isotopes, compared to the amount of oxalate extractable P considered by Lookman et al. (1995) to be the total amount of desorbable P and to the total amount of radioactivity added to the soils. We note nevertheless that the experiment removed higher percentages of $^{33}$P than of $^{31}$P. This is consistent with the influence of previous time of contact between P and soil on P sorption/desorption reactions (Barrow and Shaw, 1975). The time of contact of $^{33}$P with the soil was shorter (28 days) than the time elapsed since the last $^{31}$P additions (14 years for OP soils and several months for the P soils).

Table 4.1. Released $^{31}$P and $^{33}$P after 28 days expressed as total amounts (with standard errors) and as fractions of oxalate-extractable P ($P_{ox}$) and of total added radioactivity (R).

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>$^{31}$PR mg kg$^{-1}$</th>
<th>$^{33}$PR MBq kg$^{-1}$</th>
<th>$^{31}$PR / $P_{ox}$ %</th>
<th>$^{33}$PR / R %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadenazzo</td>
<td>0P</td>
<td>10.9</td>
<td>0.13</td>
<td>1.8</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>32.6</td>
<td>0.98</td>
<td>4.8</td>
<td>14.0</td>
</tr>
<tr>
<td>FAL</td>
<td>0P</td>
<td>3.3</td>
<td>0.08</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>19.4</td>
<td>0.32</td>
<td>3.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Rümlang</td>
<td>0P</td>
<td>1.3</td>
<td>0.08</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>17.0</td>
<td>0.28</td>
<td>3.8</td>
<td>9.6</td>
</tr>
</tbody>
</table>

*a nd = not determined

pH values in the eluates of the 0P and P soils

The pH values of the eluates were similar in the 0P and P soils, therefore the average values of both treatments are shown in Figure 4.4. The pH values decreased strongly in the first 48 hours of the experiment to reach values which were 1 (Cadenazzo), 0.6 (FAL) and 0.5 (Rümlang) pH units lower than the value measured after three hours. After 48 hours, pH values remained constant until the end of the experiment. This decrease in pH can be explained by an initial sorption of Ca$^{2+}$ followed by the release of protons to the eluent.

Altogether these results suggest that the release of P during the first 100 hours can be explained by the flush of P that had equilibrated with the solution during the preliminary incubation, by the dissolution of P compounds caused by the decrease in pH and probably by the desorption of P
P release from soils with a flow-through system

reversibly sorbed onto the surfaces of soil particles and aggregates. In the meantime P sorption probably also occurred as Ca sorption increases P sorption through a decrease in soil negative charges (Rietra et al., 2001). After the first 100 hours of leaching, P release was then probably mostly controlled by desorption from the surfaces and its diffusion through particles and aggregates (Barrow, 1983a; Van Riemsdijk et al., 1984).

The isotopic composition and the exchangeability of released P

As the $^{33}$P measurements for FAL and Rümlang OP were too low since the very early stages of the experiment, we discuss only the isotopic composition of the P released from Cadenazzo OP (until 600 hours) and from all the P soils (until the end of the experiments, 672 hours). The curves describing the changes in the specific activity ($SA = ^{33}P/^{31}P$) of P release can be divided in two regions (Figure 4.5): SA values increased until about 100 hours and then slowly decreased.
until the end of the experiment. For all soils, SA after 96 hours was significantly higher ($p<0.05$) than SA measured at the first sampling step (3 hours for Cadenazzo OP and 7 hours for the P soils). The SA at the last sampling was significantly lower ($p<0.05$) than the SA after 96 hours. The lower SA at the beginning of the experiment might be related with the initial dissolution of unlabelled P compounds due to the pH decrease. The subsequent decrease in SA can be explained by the continuing exchange between P ions located in the soil solution and P ions located on the solid phase (Fardeau, 1996), which results in decreased $^{33}$P concentrations in the eluate. The low rate of this decrease is due to the slow kinetics of exchange, since the fast exchange reactions are considered to have taken place during the incubation of $^{33}$P with the soil. The rate of decrease of SA in Cadenazzo was higher in the OP than in the P treatment, suggesting that the rate of the exchange reactions occurring after the incubation was higher in a soil relatively poorer in available P. This is consistent with the increase of the kinetic parameter of isotopic exchange $n$ observed with decreasing concentrations of P in the soil solution (Chapter 2; Morel et al., 1994).

Table 4.2. The amounts (with standard error) of ammonium-oxalate extractable P ($P_{ox}$), of the pool of isotopically exchangeable P potentially releasable (D-values) calculated for the time of incubation plus the time of the column experiment from Eq. [4.1] and the fraction (%) of $D_{8wks}$ that was recovered in the leachates.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>$P_{ox}$ mg P kg$^{-1}$</th>
<th>$D_{8wks}$ mg P kg$^{-1}$</th>
<th>$P_{r}/D_{8wks}$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadenazzo</td>
<td>OP</td>
<td>601.1</td>
<td>172.2</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>683.1</td>
<td>247.0</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>636.9</td>
<td>284.6</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>442.5</td>
<td>194.7</td>
<td>8.7</td>
</tr>
</tbody>
</table>

The values of D decreased during the first 100 hours and then increased steadily to reach 172, 247, 285 and 195 mg P kg$^{-1}$ in Cadenazzo OP, Cadenazzo P, FAL P and Rümlang P, respectively at the end of the experiment (Figure 4.6). As for the SA values, the differences of D between the first sampling and 100 hours and between 100 hours and the end of the experiment were statistically significant ($p<0.05$). The changes in D values until 100 hours are difficult to explain given the complexity of the processes that are taking place in the early phases of the experiment.
The subsequent increase in D can be explained by the slow ongoing isotopic exchange between P ions located in the solution and P ions located on the solid phase of the soil. Since the exchange reactions continued during the leaching experiment, the final D-values are considered for a time equal to the incubation plus the leaching time (i.e. 8 weeks). The final amounts of released P (P_f)
after 28 days (Table 4.2) were only 6% (Cadenazzo 0P), 13% (Cadenazzo P), 7% (FAL P) and 9% (Rümlang P) of the D_{weeks}-values, showing that only a limited fraction of the potentially releasable pool assessed by isotopic exchange was released from our P soils. Altogether our results show that the major source of P released from the P soils over 8 weeks of experiment was isotopically exchangeable, i.e. it belonged to the pool of soil P in which the added $^{33}$P had diluted itself since the beginning of the incubation.

**Modeling the kinetics of P release from the 0P and P soils**

For the 0P soils, the non-linear increase of released P within the first 96 hours ($P_{R66}$) was significantly ($p<0.01$) described by the following equation (Lookman et al., 1995; Koopmans et al., 2001):

$$P_{R96} = P_{R0} \cdot (1-e^{-k_0 t})$$  \[4.2\]

where $P_{R0}$ (mg P kg$^{-1}$) is the size of the pool of $P_{R96}$ and $k_0$ (hours$^{-1}$) is a constant. The increase of released P with time after 96 hours ($P_{R>96}$) was fitted to a linear function ($p<0.01$).

$$P_{R>96} = v \cdot t + w$$  \[4.3\]

where $v$ (mg P kg$^{-1}$ h$^{-1}$) is the constant rate of increase of released P with time. The estimates of $k_0$, $P_{R0}$, $v$ and $w$ are shown in Table 4.3. Eq. [4.2] and [4.3] describe the release of P from two pools, one with fast and one with slow, kinetics, as proposed by Lookman et al. (1995). In their work conducted on P-rich soils, Lookman et al. (1995) observed that the release of P from the fast and the slow pools were both described by a first order kinetic equations like Eq. [4.2], whereas in our 0P soils only the fast pool was described by this type of equation. This difference might be explained by the P status of the soils and by differences in the applied methodologies. Our 0P soils have a low P availability (E_{min} values in deionized water of 3.9, 4.7 and 1.9 mg P kg$^{-1}$ for Cadenazzo, FAL and Rümlang, respectively) (Bolomey, personal communication), and contain P that is probably strongly bound to soil particles that can only be desorbed at a slow rate. Besides, in our experiment P was released in the CaCl$_2$ flowing through the soil reactor, whereas in Lookman et al. (1995) P was continuously trapped on an iron oxide inserted in a dialysis bag allowing for the continuous depletion of P from the solid phase.
Table 4.3. Estimates of the parameters $P_{R_0}$ and $k_0$ of Eq. [4.2] and $v$ and $w$ of Eq. [4.3]. The 95% confidence intervals are given in parenthesis below the estimates.

<table>
<thead>
<tr>
<th>Soil</th>
<th>$P_{R_0}$</th>
<th>$k_0$</th>
<th>$r^2$</th>
<th>$v$</th>
<th>$w$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg P kg$^{-1}$</td>
<td>hours$^{-1}$</td>
<td></td>
<td>mg P kg$^{-1}$ hour$^{-1}$</td>
<td>mg P kg$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Cadenazzo $OP$</td>
<td>2.96</td>
<td>0.119</td>
<td>0.013</td>
<td>0.001</td>
<td>0.999**</td>
<td>0.0155</td>
</tr>
<tr>
<td></td>
<td>(2.65-3.27)</td>
<td>(0.011-0.015)</td>
<td></td>
<td>(0.00154-0.0165)</td>
<td>(0.495-0.552)</td>
<td></td>
</tr>
<tr>
<td>FAL $OP$</td>
<td>0.62</td>
<td>0.012</td>
<td>0.039</td>
<td>0.002</td>
<td>0.997**</td>
<td>0.0048</td>
</tr>
<tr>
<td></td>
<td>(0.59-0.65)</td>
<td>(0.034-0.045)</td>
<td></td>
<td>(0.0047-0.0049)</td>
<td>(-0.045-0.059)</td>
<td></td>
</tr>
<tr>
<td>Rümlang $OP$</td>
<td>0.41</td>
<td>0.013</td>
<td>0.036</td>
<td>0.003</td>
<td>0.992**</td>
<td>0.00167</td>
</tr>
<tr>
<td></td>
<td>(0.38-0.44)</td>
<td>(0.028-0.044)</td>
<td></td>
<td>(0.00161-0.00173)</td>
<td>(0.170-0.219)</td>
<td></td>
</tr>
</tbody>
</table>

*a A.s.e. = Asymptotic standard error
In the P soils, P release could be described by the two pools model proposed by Lookman et al. (1995) where each pool is characterized by first-order kinetics:

\[ P(t) = P_{1i} \cdot (1-e^{-kt}) + P_{2i} \cdot (1-e^{-kt}) \]

where each pool is characterized by first-order kinetics:

\[ P(t) = P_{1i} \cdot (1-e^{-kt}) + P_{2i} \cdot (1-e^{-kt}) \]

Table 4.4. Estimates of the parameters \( P_{R1i}, k_i \) and \( k_2 \) of Eq. [4.4]. \( P_{R2} \) is calculated as the difference between \( D_{\text{weeks}} \) (Table 4.2) and the estimate of \( P_{R1} \). 95% confidence intervals are given in parenthesis below the estimates.

<table>
<thead>
<tr>
<th>Soil</th>
<th>( P_{R1i} ) (mg P kg(^{-1}))</th>
<th>( k_i ) (hours(^{-1}))</th>
<th>( P_{R2} ) (mg P kg(^{-1}))</th>
<th>( k_2 ) (hours(^{-1}))</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimate</td>
<td>A.e.</td>
<td>Estimate</td>
<td>A.e.</td>
<td>Estimate</td>
<td>A.e.</td>
</tr>
<tr>
<td>Cadenazzo P</td>
<td>10.8  0.2</td>
<td>0.0089  0.0003</td>
<td>236</td>
<td>1.5\times10^{-4}</td>
<td>2.0\times10^{-6}</td>
</tr>
<tr>
<td></td>
<td>(10.3-11.2)</td>
<td>(0.0084-0.0094)</td>
<td></td>
<td>(1.42\times10^{-4}-1.49\times10^{-4})</td>
<td>1.000**</td>
</tr>
<tr>
<td>FAL P</td>
<td>1.9  0.1</td>
<td>0.0155  0.0027</td>
<td>283</td>
<td>9.6\times10^{-5}</td>
<td>1.0\times10^{-6}</td>
</tr>
<tr>
<td></td>
<td>(1.6-2.2)</td>
<td>(0.0100-0.0210)</td>
<td></td>
<td>(9.4\times10^{-5}-9.6\times10^{-5})</td>
<td>0.999**</td>
</tr>
<tr>
<td>Rümlang P</td>
<td>3.3  0.5</td>
<td>0.0066  0.0011</td>
<td>191</td>
<td>1.1\times10^{-4}</td>
<td>4.0\times10^{-5}</td>
</tr>
<tr>
<td></td>
<td>(2.3-4.2)</td>
<td>(0.0043-0.0090)</td>
<td></td>
<td>(1.04\times10^{-4}-1.20\times10^{-4})</td>
<td>0.999**</td>
</tr>
</tbody>
</table>
P release from soils with a flow-through system

show that most of the released P was originally in an isotopically exchangeable form, $P_{R2}$ can be written as the difference between the $D_{weeks}$ value and $P_{R1}$. The estimates of $P_{R1}$, $P_{R2}$, $k_1$ and $k_2$ are given in Table 4.4. The size of the fast releasable pool ($P_{R1}$) estimated from Eq. [4.4] was 10.8, 1.9 and 3.3 mg P kg$^{-1}$, and was reached after about 100, 48 and 72 hours in Cadenazzo, FAL and Rümlang, respectively. For FAL and Rümlang this corresponds approximately to the time when the change in the rate of decrease of P concentration in the eluates occurred and when the pH values started to stabilize (Figure 4.2).

Although the experimental conditions used in our work are different from the desorption studies of Lookman et al. (1995) and Koopmans et al. (2001) and from the assessment of isotopically exchangeable P (in soil solution systems at steady state for P, Fardeau, (1996)), we can discuss our results in relation with those obtained in the afore mentioned works. Lookman et al. (1995) considered the amount of P present in the slow pool ($P_{R2}$) to be equal to the difference between the amount of oxalate extractable ($P_{ox}$) and the amount of P located in the fast pool ($P_{R1}$) whereas our results suggest the presence of an intermediate pool, $D_{weeks}$, which is smaller than $P_{ox}$ in all our studied soils (Table 4.2). We suggest that in our experimental conditions released P came first from the soil solution, then from $P_{R0}$ (for the OP soils) or $P_{R1}$ (P soils) (through the combination of processes that occurred during the first 100 hours of experiment), then from $D_{weeks}$ (through desorption and diffusion) and then, if enough time is given, P can be released from the last pool ($P_{ox} - D_{weeks}$), provided that all $P_{ox}$ is really desorbable as assumed by Lookman et al. (1995). As D-values increased with time after 100 hours of leaching (Figure 4.6) at a rate of 1 and 2 mg P kg$^{-1}$ day$^{-1}$, it was not possible, in the time-frame of our experiments, to test experimentally whether $D_{\infty}$ was similar to $P_{ox}$. However, extrapolating the observed rates of increase of D with time and considering them as constant, D-values would reach the amounts of $P_{ox}$ for these soils in 200-320 days.

Our results confirm, as suggested by Fardeau (1996) for isotopically exchangeable P, that P potentially releasable from these soils can be described by a compartmental analysis in which P mobility is expressed as a function of the quantity of P present in different compartments and as a function of time needed for the P present in these compartments to reach the soil solution. However, whether exchangeable P can be described as a catenary structure (where P flows from a low mobility to high mobility compartments until it reaches the soil solution, or on the contrary from the solution to compartments containing less and less mobile P) or as a mamillary structure
(where independent compartments containing P of different mobility are directly linked to a central compartment containing the rapidly available P) can not be assessed with our data.

**Effect of P depletion on isotopically exchangeable P and on the kinetics of P isotopic exchange**

IEK experiments were carried out using 2mM CaCl₂ as background electrolyte on subsamples of the soils removed from the columns after 24, 72, 336 and 672 hours of leaching of unlabelled soils and the results were compared with the results obtained on the non-leached moist soil sampled directly after incubation.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Cadenazzo</th>
<th>FAL</th>
<th>Rümlang</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.042 d</td>
<td>0.025 c</td>
<td>0.019 d</td>
</tr>
<tr>
<td>24</td>
<td>0.036 c</td>
<td>0.022 c</td>
<td>0.016 c</td>
</tr>
<tr>
<td>72</td>
<td>0.028 b</td>
<td>0.013 b</td>
<td>0.013 b</td>
</tr>
<tr>
<td>336</td>
<td>0.017 a</td>
<td>0.010 a</td>
<td>0.007 a</td>
</tr>
<tr>
<td>672</td>
<td>0.017 a</td>
<td>0.010 a</td>
<td>0.006 a</td>
</tr>
</tbody>
</table>

The values of P concentration in the solution of the soil suspension where IEK were performed (Cₚ) at the end of the column experiments were significantly lower than those measured at the beginning in all soils (Table 4.5). In the 0P soils, however, there was no significant further decrease already after 336 hours. In the P soils, only FAL showed a similar behavior, whereas in Cadenazzo and Rümlang, Cₚ continued to decrease significantly between 336 and 772 hours. The decrease of CaCl₂ extractable P (ΔP-CaCl₂, mg P kg⁻¹) after a given time was generally one order of magnitude smaller than the amounts of P released within the same time.
Figure 4.7. The amounts of released P at defined leaching times in function of the decrease of CaCl₂-extractable P at the same leaching time.

Figure 4.8. Plots of P in the fast releasable pool (as determined by P_{fo} for the OP soils or P_{f1} for the P soils) (a) and of P_{R} after 28 days of leaching (b) in function of the initial amount of CaCl₂-extractable P. The regression equations are: (a) \( y = 10.78x - 2.38; r^2=0.90^{**} \); (b) \( y = 33.60x - 3.72, r^2=0.97^{**} \).
The plots of ΔP-CaCl₂ vs Pₚ for any chosen leaching step (Figure 4.7) show that at the beginning of the leaching experiment CaCl₂-extractable-P was a net source of P as it strongly decreased with time, whereas after 2 weeks in all 0P soils and in FAL P, P-CaCl₂ remained constant. Finally, CaCl₂ extractable P measured on the moist samples before the leaching experiment was highly correlated with the amount of ³¹P released after 28 days and to the amount of P calculated to be in the fast releasable P pool (Pᵢ₀ for 0P and Pᵢ₁ for P soils, Figure 4.8)

![Figure 4.9](image-url)  
*Figure 4.9. Plots of the kinetic parameter Rᵢ₁ of P isotopic exchange in function of Cₚ at different phases of leaching. Regression equations are: Cadenazzo: 0.94x⁻⁰.₆₄, r²=0.98**; FAL: 4.51x⁻⁰.₄₀, r²=0.86**; Rümlang: 2.99x⁻⁰.₄₆, r²=0.85**. For each soil, 0P and P are considered together.*

No clear trend was observed with the n kinetic parameter (data not shown), whereas Rᵢ₁ increased with decreasing Cₚ or increasing P depletion (Figure 4.9). For all sampling steps of the 0P and P treatments of each soil it was possible to find a highly significant relationship between
P release from soils with a flow-through system

R/\gamma_1 \text{ and } C_P \text{ of the type } R/\gamma_1 = a \cdot C_P^{-b} \text{ described in Chapter 2 of this dissertation. This observation confirms, for increasing } P \text{ depletion, what we previously observed following the addition of increasing quantities of } P \text{ to soils, i.e. that } R/\gamma_1 \text{ is determined both by the concentration of } P \text{ in the soil solution and by soil properties.}

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>E_{imin} (mg P kg(^{-1}))</th>
<th>E_{60min} (mg P kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cadranazzo</td>
<td>FAL</td>
</tr>
<tr>
<td>0</td>
<td>3.0 b 0.1</td>
<td>4.6 c 0.1</td>
</tr>
<tr>
<td>24</td>
<td>3.0 b 0.1</td>
<td>4.9 c 0.2</td>
</tr>
<tr>
<td>72</td>
<td>2.2 a 0.1</td>
<td>3.3 b 0.1</td>
</tr>
<tr>
<td>36</td>
<td>2.1 a 0.1</td>
<td>2.8 a 0.1</td>
</tr>
<tr>
<td>672</td>
<td>2.2 a 0.3</td>
<td>2.5 a 0.1</td>
</tr>
</tbody>
</table>

Isotopically exchangeable P within one minute (E_{imin}) and 1 hour (E_{60min}) after 772 hours of experiment were significantly lower than in the non-depleted samples (Table 4.6) except for Rümlang P (E_{imin}) and FAL and Rümlang P (E_{60min}). In all the P soils, except E_{imin} in Cadranazzo P, E_{imin} and E_{60min} increased significantly after 72 hours, before decreasing when the leaching experiment progressed. These transient increases are probably the result of the complex processes.
that occurred during the first 100 hours: P dissolution due to the decrease in pH and increased P sorption related to the sorption of Ca on soil particles and P desorption. This probably explains the lack of correlation between the decreases of $E_{\text{imin}}$ and $E_{\text{50min}}$ at any given sampling time and the corresponding amounts of released P.

The amount of P isotopically exchangeable within 8 weeks ($E_{\text{weeks}}$, mg P kg$^{-1}$, equivalent to the total period of incubation and column experiments) was calculated from short term IEK data for Cadenazzo $0P$ and the three $P$ soils as proposed by Fardeau et al. (1985) and compared to the $D_{\text{weeks}}$ values. If the process of isotopic exchange was the major process explaining the isotopic composition of released P than $D_{\text{weeks}}$ and $E_{\text{weeks}}$ should be similar.

![Figure 4.10. Plot of the relationship between $E_{\text{weeks}}$ values extrapolated from short term isotopic exchange kinetics and the $D_{\text{weeks}}$ values determined from the specific activity of released P. The line represents the 1:1 relationship](image-url)

Figure 4.10. Plot of the relationship between $E_{\text{weeks}}$ values extrapolated from short term isotopic exchange kinetics and the $D_{\text{weeks}}$ values determined from the specific activity of released P. The line represents the 1:1 relationship.
Data presented in Figure 4.10 show that $D_{\text{weeks}}$ was higher than $E_{\text{weeks}}$ for FAL and Rümlang and lower for Cadenazzo 0P, but was not significantly different ($p<0.05$) for Cadenazzo P. $D_{\text{weeks}}$ overestimated $E_{\text{wk}}$ by 41 and 46% for Rümlang and FAL respectively, while it underestimated $E_{\text{weeks}}$ by 23% for Cadenazzo 0P. Given the complexity of the processes that occurred during the early phases of the experiments and the sometimes low reliability of $E$-values extrapolation (Frossard et al., 1996; Bühler et al., 2003), it would be illusory to look for equality between these results. It is enough to state that they are of similar order of magnitude and that both approaches give a similar picture of the exchangeability of soil P.

**Interests and limits of the proposed approach to assess the potential amounts of P to be leached from agricultural soils**

**Interests**

The proposed flow-through reactor system allowed to measure the amount of P released from soils during a period of 28 days. Furthermore it was possible, in P-rich soils, to measure the amount of radioactive P released from the soil columns and to calculate the D-values, i.e. the amount of P that has the same specific activity as the released P and therefore that can be potentially released from the column. The use of a weak electrolyte (2 mM CaCl$_2$) and of soil samples labeled with radioactive P allowed measuring the amount of P that can be potentially released from a soil without having to use an infinite sink for P therefore allowed to circumvent problems linked to it described by Freese et al. (1995) and by Koopmans et al. (2001). Furthermore the use of a weak electrolyte without an infinite sink is probably closer to a leaching situation e.g. in winter when plants do not grow and P released from the soil can be transported by subsurface flow. The observations of data obtained from the leachates were congruent with those obtained from the isotopic exchange kinetics method conducted in an independent experiment with leached soils. Finally the results reflected the expected differences between fertilized and non-fertilized soils, and highly significant correlations were observed between the amounts of CaCl$_2$ extractable P measured before the leaching experiment and the amount of P released within 28 days and with the amounts of P present in the fast releasable pool.
Limits
We measured only the release of inorganic phosphate whereas organic P can be an important component of leaching, especially when organic fertilizers are applied (Chardon et al., 1997). The use of CaCl₂ resulted in significant pH decreases during the first 100 hours of the leaching experiment causing probably dissolution and sorption to occur. Such effects could probably be avoided by using deionized water. Schärer (2003) compared the effects of Ca-rich waters and of rainwater on P losses by run-off and showed that Ca strongly affects P mobility through its effect on P sorption. Therefore, water with properties close to that of rainwater should be used to study P losses by run off. The use of deionized water in our flow through system was however not possible as it rapidly resulted in particle dispersion and in filter clogging (personal observation). Whereas our system probably conserves the soil microstructure of a given sample, it ignores the soil macrostructure and the effect it can have on P losses through preferential flow through the entire profile as studied by Stamm et al. (1998) and Sinaj et al. (2002). Furthermore, the upward flow that we employed may affect the gravity-driven, downward transport and distribution of fine and colloidal particles within the soil sample which occurs in natural leaching conditions. This may, in turn, affect the processes of P release.
Finally our method is not adapted to low-P soils in which it is not possible to calculate the D-values without using large amounts of radioactivity. However, this is not a major problem, since potential P losses must only be characterized in P rich soils.
In conclusion the amount of P released from soils using our flow-through reactor within a given time (Pₖ) and the amount of P that could be potentially released from soils (D-values) can only be considered as indicators of the potential for P losses by leaching. These results must be confirmed with a higher number of soils having a wider range of properties and sampled under different land-uses and by comparing the Pₖ and D results to actual leaching data obtained e.g. from lysimeters.

Acknowledgements
We are grateful to Mr. René Flisch (Swiss Federal Research Station for Agroecology, Zurich) for providing the soil samples (already dried and sieved) used for this study and to Mr. Thomas Flura for the ICP-MS measurements of oxalate-P. In a moment of need, Mr. Mathimaran Natarajan took excellent care of the flow-through reactor, by assuring the daily supply of fresh leaching
solution. Mr. Sven Bolomey kindly provided the $E_{1\text{min}}$ values in water. This work was funded by the Swiss National Science Foundation (SNF), project n° 31-50528.97.
P release from soils with a flow-through system
General Discussion and Conclusions

Introduction

The main objective of this thesis was to study the factors influencing P isotopic exchangeability and the potential of P release in agricultural soils.

P exchangeability was determined with isotopic exchange kinetics (IEK) experiments. In order to separate the effects of soil properties and solution P concentration ($C_P$) on P exchangeability, IEK experiments were carried out on soils where a range of $C_P$ was obtained by incubating samples with P, while keeping the soil properties constant (Chapter 2).

The exchangeability of P extracted by routine chemical methods was assessed by extracting samples that were previously labelled with $^{33}$P and comparing the specific activity ($SA = ^{33}P/^{31}P$) of chemically extracted P with the one of water-extractable P (Chapter 3).

The release of P from fertilized and unfertilized soils was studied in a flow-through system where P was continuously depleted for 28 days from $^{33}$P-labelled and unlabelled soils (Chapter 4). The SA of P released from labelled soils allowed to calculate the exchangeability of released P, whereas the changes in P exchangeability at increasing stages of depletion were studied with the unlabelled soils.

A summary and a discussion of the main results are given hereafter.

Phosphate isotopic exchangeability

Isotopically exchangeable P and kinetic parameters of exchange

The results presented in Chapter 2 showed that isotopically exchangeable P ($E_t$) is not only a function of concentration of P in solution ($C_P$) and of exchange time, as previously reported by Morel et al., (1994), but also of soil properties. In our studied soils, the pH and the content in Fe and Al oxides controlled the relationships between $C_P$ and the kinetic parameters of isotopic exchange ($R/t_1$ and $n$). Consequently, E-values could be calculated as a function of pH, citrate-dithionite-bicarbonate extractable Fe, the sum of ammonium oxalate-extractable Fe and Al and $C_P$ (Table 2.6, Table 2.7 and Eq. [2.12]). E-values thus calculated for 1 and 60 minutes of exchange were not significantly different from the ones determined experimentally with IEK experiments (Figure 2.3a and 2.3b).

The P buffer capacity (PBC), calculated as $dE_t/dC_P$ and with the soil properties taken into account in the determination of $E_t$ (Eq. [2.13], Table 2.8, Table 2.9), was well correlated with the $R/t_1$ kinetic parameter. Results from Chapter 2 and Chapter 4 show that the changes of $R/t_1$ with $C_P$
could be observed either when soil samples were enriched in P (Chapter 2: Figure 2.2a and Table 2.6) or when they were depleted (Chapter 4, Figure 4.8). This is consistent with a decrease in PBC expected when $C_p$ is increased or vice versa, as suggested by the non-linearity of P sorption isotherms. The effect of $C_p$ on the kinetic parameter $n$ (which describes the rate of increase of $E_t$ with time) was observed when $C_p$ was increased by P additions (Chapter 2), but not when $C_p$ was decreased by depletion (Chapter 4).

**Exchangeability of extractable P**

The results presented in Chapter 3 showed that an extraction method operating at low ionic strength and rapidly buffered to soil pH (CO$_2$-saturated water) solubilized only isotopically exchangeable P. However, the amounts of P extracted by CO$_2$-saturated water were lower than isotopically exchangeable P within 1 minute, suggesting that this extraction, although removing only forms of very labile P, might underestimate P availability. With more aggressive extradants such as NaHCO$_3$ or ammonium acetate EDTA (AAEDTA), higher amounts of P were extracted, but variable and significant fractions of slowly or non exchangeable P were mobilized. P-NaHCO$_3$ contained a larger proportion of exchangeable P than P-AAEDTA, for which the pH of the soil clearly affected the fraction of non exchangeable P in the extract (Figure 3.1). Due to the acidic pH of the AAEDTA extractant we believe that mainly Ca-P compounds accounted for the amount of P extracted in a non exchangeable form. A clear effect of soil properties on the exchangeability of NaHCO$_3$-extractable P was not observed.

When either NaHCO$_3$- or AAEDTA-extractable P was used as Quantity factor for the calculation of the PBC (as Quantity/Intensity ratio, with $C_p$ as intensity), the best correlation with either the PBC determined from IEK ($dE_t/dC_p$ for $t = 60$ minutes) or with $R/t_1$ was observed when Q was expressed as NaHCO$_3$-extractable P (Figure 2.4).

**Factors controlling P exchangeability**

Summarizing, the factors that control P exchangeability in our soils are the concentration of P in the solution, the soil properties controlling P sorption on the solid phase and the speciation of P on the solid phase.

In Chapter 3 we had indirect evidence that forms of P that are sorbed on the surface of soil constituents, most likely Fe and Al oxy-hydroxydes, are more rapidly exchangeable than Ca-P
General Discussion and Conclusions

mineral compounds. This was shown by the larger fractions of exchangeable P extracted by NaHCO₃, which most likely displaces P from adsorption surfaces, rather than dissolving large amounts of P precipitates like the AAEDTA extractant is expected to do, especially in calcareous soils. Similar conclusions were drawn by Bühler et al. (2002) with sequential extractions on ³²P labeled, low-P tropical soils. However, due to the very high P fixing capacity of their soils, the exchangeability of P extracted in a given extraction step may have been confounded by the specific sorption of the labeled phosphate. In our relatively P-rich soils, this is less likely to occur.

Finally, different fertilization rates did not show significant effects on P exchangeability besides the influence that fertilization has on increasing and decreasing C_P, presumably because they did not significantly affect soil pH and the content and reactivity of Fe and Al compounds. It is expected that other treatments such as liming would have a significant effect on the kinetics of P exchange.

**Generalization and implications of the results**

In Chapter 2, we have shown that, at first approximation, our findings on the factors controlling P exchangeability could be applied to other cropped soils for predicting R/r₁ and E₁min, that is, (i) a parameter related to PBC and to P sorption capacity (R/r₁); and (ii) the quantity of instantaneously plant available P (E₁min), which has been shown to be an excellent indicator of P bioavailability for plants (see e.g. Fardeau, 1996).

Provided that such relationships can be improved by being established on a larger number of soils having a wider range of properties and sampled under different land-use (e.g. grassland), they may have a relevant implication for the study of P cycling in soils, either from an agronomical or an environmental point of view. A large body of published results has shown that the IEK approach is a valuable tool to study the fate and the availability of nutrients and of pollutants in soils and soil-plant systems. However, such a method cannot be proposed as a routine test, either for agronomical or environmental purposes, because of the high cost and the safety issues derived from working with radioactive compounds. If the factors controlling P exchangeability are well established, the prediction of isotopically exchangeable P and of its kinetic parameters will be possible using readily accessible parameters, such as soil pH, Fe and Al oxides content and C_P.
Potential P release

Laboratory methods alternative to batch experiments should be evaluated to estimate the potential of P losses from agricultural soils to subsurface waters. During periods without plant growth (e.g. in winter or during fallow) a fraction of the P released from a soil can be potentially transferred to water bodies. However this release might be smaller than that observed in the presence of a biological sink, like the root system of growing plants. Therefore, results from laboratory batch methods employing sinks for P (e.g. Freese et al., 1995; Lookman et al., 1995) may overestimate the potential of a soil to loose P to the environment in such conditions, whereas they give a good estimation of the total amount of P that can be taken up by plants.

We developed a flow-through reactor system (Figure 4.1) and we measured the amounts and the kinetics of P release in unfertilized (OP) and fertilized (P) soils (Chapter 4). We evaluated the results in order to verify whether such an approach can give valuable information and its improvement should be pursued.

Amounts and forms of released P and processes of release

The amounts of P released ($P_r$) from the $0P$ soils were significantly smaller than from the $P$ soils (Table 4.1), confirming that omitting fertilization and decreasing P availability have an effect on the ability of the solid phase to release P to a leaching solution. By measuring the specific activity of P released from $^{33}$P-labelled soils (in one $0P$ and in the 3 $P$ soils), we could quantify the amount of soil P that could potentially be released (D-values). In the $P$ soils, $P_r$ was only a small fraction (7 to 13%) of D (Table 4.2). This confirms that the release process, in absence of growing plants, is relatively slow. The amounts of isotopically exchangeable P determined from short-term batch IEK experiments (E-values) and extrapolated to the timeframe of the column experiments, were in the same order of magnitude as the D-values (Figure 4.10). This suggests that both E- and D-values give a similar picture of P exchangeability. All together our results from the labeled soils point to the fact that isotopically exchangeable P was a major source of P release under our experimental conditions.

The initial amounts of CaCl$_2$-extractable P were significantly positively correlated with the amounts of $P_r$ after 28 days (Figure 4.7), suggesting that P-CaCl$_2$ could be used as an indicator to predict P losses.
The kinetics of P release that we observed in the first 100 hours of experiments were determined by a rather complex combination of processes, partially due to experimental conditions. Among them we can consider (i) flush of P equilibrated with the soil solution during the incubation; (ii) dissolution of P due to the initial decrease of pH of the eluent; (iii) desorption of reversibly sorbed P from soil surfaces; and (iv) partial readsorption of P caused by the decrease of the negative charge on the soil surfaces due to the adsorption of Ca²⁺ added with the eluent. After the first 100 hours, desorption from the surfaces in contact with the solution and a slow outward intra-particle or intra-aggregate diffusion were probably the processes controlling the release. In the OP soils, the rate of removal by the flowing solution was close to equilibrium with the rate of P supply from the solid phase, since the concentration of ³¹P in the leachates were constant (Figure 4.2) and thus the amounts of cumulative P in increased linearly (Figure 4.3a, c and e).

The previous time of contact between the soil and the last addition of P (14 years or several months for ³¹P in the OP and P soils, respectively, and 28 days for ³³P) affected the ratio between released ³¹P and oxalate extractable P (Pox) or between released ³³P and the amount of ³³P incubated with the soil (R) (Table 4.1).

All these observations suggest that the results yielded by our system are coherent with the processes governing the release of P from the soil to the solution in field conditions.

**Outlook**

*Improvement of the study of P exchangeability*

We believe that enough information has been gathered during the years on the IEK method and on the results it produces. It is probably not worthwhile to invest further research in understanding the chemico-physical meaning of R/r₁ or of n.

However, relationships of the kind we have found between soil properties, Cₚ and isotopically exchangeable P should be further explored and improved. Extending the range of the tested parameters and finding relationships specific for a given land use may help to refine the models and increase the accuracy of their predictions. Provided this knowledge is acquired, P exchangeability could be predicted from simple laboratory measurements. When the IEK method is applied on a regular basis to study the exchangeability of other elements (either nutrients or pollutants) and/or in other systems (e.g. sewage sludge, manure, soil amendments) the factors
affecting the exchange processes should be evaluated initially, in order to develop, if possible, reliable models for the specific element/system.

The exchangeability of the various forms of soil P can be further investigated with the approach of chemical extractions (single and/or sequential) targeting specific forms of soil P and performed on $^{32}$P-labeled samples. Beauchemin et al. (2003) were able to successfully identify, at a molecular level, the forms of P in P-rich soils by X-ray Absorption Near-Edge Structure Spectroscopy (XANES). By combining these spectroscopy techniques with isotopic exchange methods and sequential chemical extractions, we might magnify the detail of our knowledge on the speciation of isotopically exchangeable P. With such an approach, Diesing (2004) was able to identify the species of isotopically exchangeable zinc and relate them to plant uptake.

**Improvement of the estimation of potential P release**

Since we obtained promising results from the flow-through system with regards to the potential P release, it will be probably worthwhile to overcome some of its methodological limits, which were addressed at the end of Chapter 4.

The possibility of using water instead of CaCl$_2$ should be properly evaluated, since we hypothesized that the presence of Ca$^{2+}$ in the eluent solution caused complexities in the initial phase of the experiments. Furthermore, a downward flow would probably cause changes in the movement of fine soil particles and colloids through the column and the possible effect on the amounts and the kinetics of P release should be tested. Dissolved organic P can be a relevant fraction of P released from soils, especially when organic fertilizers are used (Chardon et al., 1997). The measurement of total P in the leachates would give better information on the speciation of released P.

A flow-through system probably does not disturb significantly the soil micro-structure, but it surely does not take the macro-structure into account and therefore preferential flow paths. These are factors that have a relevant influence on P losses (Stamm et al., 1998; Sinaj et al., 2002). This is why the results obtained from a laboratory flow-through system should be compared to undisturbed soil columns. Finally the number of studied soils and the range of their properties should be extended and the results compared with leaching data, obtained, for instance, from lysimeter studies. This might provide conversion factors between the measurements of P released from laboratory soil columns and the actual P losses under field conditions and to quantify the
effects of specific soil properties on P release. With more research in this direction, we believe that flow-through systems could be a useful laboratory tool for the environmental issues of P research.

Towards an environmentally sound evaluation of P availability

The current Swiss regulations for P fertilization schemes take into account classes of soil P fertility based on P extractability and calibrated in function of plant needs. However in order to achieve the desired reduction of P losses from agricultural land, the amounts of P present in agricultural topsoils must be evaluated not only from an agronomical point of view but also with regard to the potential P losses. Soils considered as low in available P (and therefore allowed to be fertilized) may indeed have a significant potential for P losses. Furthermore, fertilization does not only affect the amount of available P, but also the P buffer capacity of the soil (Chapter 2). These aspects should be taken into account when devising fertilization strategies: soils should be classified based on their fertility and vulnerability to P losses.

To do so, the results presented in this dissertation allow us to suggest that two chemical extractions (one less and one more aggressive) should be routinely performed in order to define the need of fertilization of a soil. Deionized water should be preferred, as the less aggressive extraction, to the CO₂-saturated water method, because the latter, although extracting only exchangeable P, underestimates the amounts of readily available P described by E_{\text{min}} (Chapter 3). On the other hand, a more aggressive extraction can better estimate the Quantity factor and is therefore more meaningful from an agronomic point of view. However, the a priori choice of the more aggressive method should be avoided. The AAEDTA extraction has the advantage that not only P but also other elements can be measured with a single extraction. Nevertheless, if only P availability is investigated, we suggest that the NaHCO₃ method should be considered as an alternative option, especially in neutral and calcareous soils.

The ratio between P extracted by the aggressive method (Quantity) and the concentration of P (C_p) in the extract of the less aggressive method (Intensity) would give an indication of the soil P buffer capacity (PBC). This parameter should be taken into account when assigning a soil to a P availability class. For a given level of available P determined by the aggressive extraction, a soil with a higher PBC would present a lower risk of P losses since its capacity to retain added P on the solid phase would be higher. However, in Chapter 2 we have shown that P additions cause a
decrease in soil PBC and therefore this parameter should be monitored with time in soils where fertilizers are applied. Although the current Swiss interpretation schemes propose to correct the extraction data with the soil clay content as an indicator of PBC, our results suggest that the extent of the effect of fertilization on PBC depends on the content of Fe oxides and on soil pH (Chapter 2). Provided that these relationships are confirmed for larger sets of soils, we suggest that such soil properties should be taken into account. This would allow assessing soil-specific limits of C_P above which the PBC is low and any further P addition is distributed in the soil solution, increasing the risk of losses.

Finally, a water extraction, combined with the above mentioned soil properties, can give access to P exchangeability without having to perform costly experiments with radioactive isotopes.
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