The nutrient cycle of soils is mainly determined by the concentration of macro and micro nutrients in the soil solution directed by the interactions between the solid phase of soil and the soil solution. These interactions are essential in case of nutrients, such as phosphate anion, strongly binding some soil components.

In this project, six types of soils were investigated (two arenosols (Calcaric (Humic) and Dystric (Humic)), Chernozem (Loamic), Calcic Vertisol (Gleyic), Rendzic Phaeozem (Hyperhumic) and Calcic Gleysol (Arenic, Humic)) by heterogeneous isotope exchange with a radioactive isotope of phosphorous ($^{32}P$). The phosphate transport between the soil and solution is studied and the rate of phosphate exchange is determined under a steady state condition. A model was proposed previously (Kónya and Nagy 2015) for the evaluation of the heterogeneous isotope exchange through which the quantity of phosphate dissolved in the soil solution ($m_1$) as well as the weakly sorbed/isotopically exchangeable phosphate on soil ($m_2$) can be studied under a steady state and equilibrium. These two quantities together are considered to be available for plants. In addition, the transport rate of phosphorus between the soil and solution, also under a steady state or equilibrium can be determined. The changes of phosphate fractions ($m_1$, $m_2$), tightly sorbed phosphorus ($m_{tightly}$), ammonium lactate soluble phosphorus ($P_{AL}$) and P taken up by plant ($P_{uptake}$) were determined as a function of P supply, incubation time and the plant culture.

At the typical pH range of soil solution (pH~7), the dominant phosphate species is $H_2PO_4^{2-}$. The sorption reactions of $H_2PO_4^{2-}$ on the soil components are expressed as below. A portion of phosphate is present as weakly or fast sorbed phosphate on soil (Barrow and Shaw 1975). The weakly sorbed and dissolved phosphate is in equilibrium or steady-state. The weak sorption of phosphate ions occurs on the surface sites of soil (S-OH) (Goldberg and Sposito 1984):

$$\mathrm{S-OH+H_2PO_4^- \rightleftarrows S-PO_4H^- + H_2O} \qquad (1)$$

where *S* means the surface of soil. The weakly sorbed phosphate can be transformed to tightly sorbed phosphate (Mansell et al. 1977; Sparks 1989) via a relatively slow process (Shuai et al. 2014), affecting the steady state between the dissolved and weakly sorbed phosphate. The significant portion of the tightly sorbed phosphate is produced by esterification:

$$\mathrm{S-OH+H_2PO_4^- \rightleftarrows S-O-PO_3H^- + H_2O} \qquad (2)$$

Phosphate ions can precipitate with some metal cations (aluminum, iron, and calcium ions), e.g.:

$$\mathrm{3Ca^{2+} + 2H_2PO_{4=}^- = Ca_3(PO_4)_2 + 4H^+} \qquad (3)$$

These processes are affected by many factors, e.g., soil type and composition, phosphate quantities of the original soil and phosphate fertilizer doses, the time elapsed since the fertilizer addition, and the agricultural use (plant culture), etc. Since the quantity of phosphate in the soil solution significantly controls the nutrient uptake by plants, the information gained on the phosphate species can be utilized to plan phosphate fertilization.

Many cultivated soils contain high amount of phosphate but mainly as tightly sorbed species. Thus, a large part of soil phosphate is not available directly for uptake by plants. For this reason, studies on phosphorous mobilization processes become more and more important, including the kinetics and equilibrium of the mobilization process from soil as well as the pathways of phosphorous in the complex systems of the soil, soil solution and plants.

During the heterogeneous isotope exchange, radioactive $H_2{}^{32}PO_4^-$ ions ($<10^{-8}$ μg/g soil) are added to the soil solution in equilibrium with the soil. In a short period time, the radioactive phosphate ions from the soil solution exchange with the weakly sorbed phosphate of soil:

$$\mathrm{S-PO_4H^- + H_2{}^{32}PO_4^- \rightleftarrows S-{}^{32}PO_4H^- + H_2PO_4^-} \qquad (4)$$

The kinetics of the exchange is:

$$x_{HIE} = \frac{m_2}{m_1+m_2}\left(1 - exp\left(-\frac{C}{m_1}\frac{m_1+m_2}{m_2}t\right)\right) \qquad (5)$$

$$y_{HIE} = \frac{m_1}{m_1+m_2} - \left(\frac{m_1}{m_1+m_2} - 1\right)\left(exp\left(-\frac{C}{m_1}\frac{m_1+m_2}{m_2}t\right)\right) \qquad (6)$$

where $t$ is time, $x_{HIE}$ and $y_{HIE}$ are the relative portions of radioactive phosphate in the soil and the soil solution, respectively, related to the total added radioactivity ($x_{HIE}+y_{HIE}=1$); $m_1$ is the mass of the phosphorus in the solution, $m_2$ is the mass of weakly sorbed phosphorus in the soil under a steady state; $C$ is the rate of the phosphate exchange in an equilibrium or steady-state. In equilibrium or steady state the transfer rates of phosphate from solution to the soil and vice versa are equal.

From the relative radioactivities of P-32 in the soil ($x_{HIE}$) or the soil solution ($y_{HIE}$) vs time plot, $m_2/(m_1+m_2)$ and $C/m_1$ can be calculated; and when $m_1$ is measured by an independent analytical method, $m_2$ (weakly sorbed or water soluble/exchangeable phosphorus in the soil) and $C$ (the exchange rate of phosphorus between the soil and solution under equilibrium or steady state) can be calculated. The $m_1+m_2$ give the total quantity of dissolved + weakly sorbed phosphorus in the whole system (soil + solution). This means that the tightly sorbed phosphate is not involved in the heterogeneous isotope exchange process. Thus:

$$x_{HIE} = \frac{m_2}{m_1+m_2} \tag{7}$$

and

$$y_{HIE} = \frac{m_1}{m_1+m_2} \tag{8}$$

The quantity of the tightly sorbed (immobilized/organic) phosphorus ($m_{tightly}$) can be obtained by subtracting the sum ($m_1$+$m_2$) from the total phosphorus quantity of the system ($P_{total}$, the sum of the phosphorus content of the original soil and the added phosphorus):

$$m_{tightly} = P_{total} - (m_1 + m_2) \tag{9}$$

If plant is grown in the soil before heterogeneous isotope exchange studies, the phosphorous taken up by plant ($m_{uptake}$) has also to be considered:

$$m_{tightly} = P_{total} - (m_1 + m_2 + m_{uptake}) \tag{10}$$

## Experimental

1. The basic properties of soils were determined.
2. Soils were incubated with inactive and radioactive phosphate ions for 1, 3, 13 weeks; with 0, 40, 80, 160, and 320 mg/kg P/soil P rates. (P was added as $KH_2PO_4$ solution but the quantities are expressed for P.) The water content during incubation was 60 % of the total water capacity of soil. The results of additional incubation of non-radioactive soils as long as 22 weeks (see later) were also taken into consideration.
3. The non-radioactively incubated soils were studied in heterogeneous isotope exchange batch experiments: after the formation of a steady state between the soil and the soil solution, radioactive $^{32}P$ isotope was added to the solution, the isotope exchange was studied as a function of time (kinetic studies).
4. The dissolution kinetics of phosphate of the radioactively incubated soils was studied in batch experiments. From the kinetic curves, the exchangeable phosphate quantity of soil and the transport rate of phosphate between soil and soil solution at a steady state were determined.
5. When reaching equilibrium, the phosphate concentration of soil solution was determined both in radioactive and non-radioactive incubated soils.
6. The non-radioactive soils incubated at different times (1, 3, 13 weeks) and with different phosphorous quantities (0, 40, 80, 160 and 320 mg/kg phosphorous/soil) were divided into

three parts. Two parts of them were used to plant ryegrass (*Lolium perenne, L.*) for two month in two parallel experiments. Harvesting was done twice. The third parts of each soil samples were incubated on, and this served as control soil when comparing to the soils with plant.

7. After harvesting, the heterogeneous isotope exchange studies were done (Section 3). The exchangeable phosphate quantity was determined using radioactive $^{32}PO_4^{3-}$ ion; at the same time, the exchangeable phosphate quantities of control soils were determined also. When reaching equilibrium, the phosphate concentration of soil solution was determined (Section 5).
8. The wet and dry mass and phosphorous content of the cut ryegrass was measured. These studies gave information on the phosphorous uptake of plants, and the effect of plant on the quantity of water-soluble/exchangeable phosphate as well as the ratio of water-soluble/exchangeable to mineralized/organic phosphorous.

Main conclusions

1. The total P content of the original soils increases as the humus content increases. The ratio of the phosphorus species are mainly determined by humus content of soil. The $m_1$ and $m_2$ values are the smallest in the soils with the highest humus content. The effect of clay content is less pronounced. Clay plays a role in the formation of soil types because of swelling and nutrient content.
2. The distribution of $^{32}P$ strongly depends on the source of that; i.e. whether the $^{32}P$ was originally in the soil or in the solution phase. After the incubation of the soils, a part of the phosphate is present as tightly sorbed phosphate. However, the heterogeneous isotope exchange studies show that only the weakly sorbed (water-soluble/exchangeable) species exchange with the P-32 isotope of the solution.
3. The rate of weakly to tightly sorbed P transformation (the half-lives are about 22, 20-30, 11-15, 14, 10 and 7-8 weeks for Calcic Gleysol (Arenic, Humic), Calcic Vertisol (Gleyic), Chernozem (Loamic), Rendzic Phaeozem (Hyperhumic), Dystric Arenosol (humic), and Calcaric Arenosoil (Humic), respectively) is inversely proportional to the humus content, except for Rendzic Phaeozem (Hyperhumic).
4. The phosphorous taken up by plant increases as the P rate increases. The highest increase (4-5x compared to the untreated soils) is observed for the soils with high humus content (Rendzic Phaeozem and Calcic Gleysol, while 1.5-2x increase is for Chernozem and

Arenosols. However, a significant portion of added phosphate strongly sorbs on the soil, becomes tightly sorbed as fast as one week incubation before sowing; thus cannot be directly utilized by plant. The transformation is mainly determined by the soil type: its degree is rather high for the soils with great humus content (Rendzic Phaeozem and Calcic Gleysol) and Chernozem, and the smallest for Arenosols (lowest humus content). The incubation time and P rate have smaller effects. This transformation also happens during plant growing: plant slightly influences the transformation of P into tightly sorbed form.

5. As a result of plant growing the $m_1$ and $m_2$ values decrease, plant takes up P from the soil solution and weakly sorbed phosphate. The addition of P increases these values in the highest degree in the soils with high humus content, enhancing the phosphorous quantity available for plant.
6. A significant correlation is found between $m_1$ and $P_{uptake}$ as well as the sum of $m_1+m_2$ and $P_{uptake}$ of plant, except for Calcic Vertisol (the mass of plant was extremely low). $m_1$ and $m_2$ are in equilibrium and both are utilized by plant. $m_1+m_2$ is a better indicator of plant available P fraction than $m_1$ or $m_2$ and can be quantitatively measured by P-32.
7. There is a significant correlation between the $m_1$ and $P_{AL}$ as well as $m_1+m_2$ and $P_{AL}$ values. The traditionally applied $P_{AL}$ for the characterization of P supply is proportional to the values obtained by heterogeneous isotope exchange. The relations between PAL and $m_1$ or $m_1+m_2$ can be characterized by a straight line; however, the slope strongly depends on the soil type. For the different soil types, the slope of $P_{AL}$ vs $m_1$ ranges from 0.863 to 1.967. The $m_1+m_2$ values is fairly close to the $P_{AL}$ fraction, that is the slope on Arenosols and Chernozem, while for the other soils the $m_1+m_2$ (Calcic Vertisol, Rendzic Phaeozem and Calcic Gleysol) is significantly lower than the $P_{AL}$. This shows that the $P_{AL}$ can well be applied to characterize the P supply only some soil types. The heterogeneous isotope exchange, however, can provide more general, quantitative information on different soil types.

Our studies clearly reveal that the soils are very unique; each soil type is a complex system. The type of soil is the basic factor determining the P uptake by plants. The effect of phosphate fertilization is rather small. Instead, we have to transform the tightly sorbed phosphate of original soils (if its quantity is suitable) to weakly sorbed phosphate before or during the vegetation period, e.g., by microbiological treatments as intended recently in Hungary by Moment Consulting Ltd. Together with them, we have already made some field experiments with different soils types and plants. Our research group made heterogeneous isotope exchange studies to determine the quantity of P forms. The evaluation of the results is in progress.

We have already published the method to differentiate the short- and long-time processes using one short-live radioactive isotope (Nagy and Kónya, 2018) when the changes in soil happen before the addition of radionuclide. The results on the effect of P dose and incubation time on the phosphorous species in Chernozem and Arenosol (Calcaric (Humic)) (Nagy et al. 2019). The effect of plant growing was analyzed on the phosphorous species in the case of the same soils at different P doses and preincubation times. Soil preincubation and greenhouse pot experiment was conducted to follow up the sorption processes of added P-fertilizer with using radioisotope tracer technique. These results were also published (Balla Kovács et al. 2021). The results of the other four soil types (Dystric Arenosols (Humic), Calcic Vertisols (Gleyic), Rendzic Phaeozem (Hyperhumic), Calcic Gleysols (Arenic, Humic)) are summarized in a manuscript (Buzetzky et al. 2021, submitted to Soil and Tiilage Research (Q1, impact factor: 5.374)). In addition, the general conclusions were published in a book chapter (Nagy and Kónya, 2021). (Only the international journal papers are mentioned here, the other publications are uploaded to the NKFIH website.)

We made some new evaluations of the isotope exchange kinetic plots which were not aimed the original working. The previous results show that the heterogeneous isotope exchange cannot be described well enough by an exponential kinetic law. This suggests that more than one weakly sorbed/isotopically exchangeable phosphate species is present in the soil. Using the separation of the weakly sorbed P species, their quantities can be quantitatively determined separately. A manuscript is during preparation. A PhD student deals with this topic (Zoltán János Vörös) who presented the results in the Radiochemical Scientific Committee of HAS and gain the Attila Vértes Performer Award.

In the framework of the project, a young researcher (Dóra Buzetzky) was applied in 15 months; this assisted the finalization of her PhD work. Eight students did BSc and MSc theses within the project. We could purchase some valuable instruments such as X-ray fluorescence spectrometer, balances, alpha/beta/gamma sandwich-type radiation detector, etc. In addition, we could take part at scientific conferences, and can present the results of other research fields. For this reason, the publications not strongly attached to the P species of soils are also involved in the publication list.

Unfortunately, due to the COVID-19, the participants had much extra work this year; the consultation between the researchers was prohibited. There were no conferences in 2020.

For these reasons, I requested the prolongation of the project with an additional year (approved by N. modification: KFF-24-15/PALY).

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