Quantifying element fluxes using radioisotopes

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Radioisotopes can be used to quantify element fluxes in ecosystems, such as plant phosphorus uptake from soil. On the occasion of a recent publication (Lekberg et al., 2024), this commentary briefly explains some challenges in the determination of element fluxes based on radioisotope labeling experiments along with strategies to avoid potential pitfalls. The intention of this contribution is to foster progress in the understanding of element fluxes in ecosystems based on the use of isotopes.

Radioisotopes can be used in quantitative and non-quantitative studies (for a review see Frossard et al. (2011)). In non-quantitative studies, radioisotopes are often used to demonstrate that specific elements or molecules move among different compartments, for instance among cells or organs. Using this approach it has been shown that mycorrhizal fungi transport elements from soil or a specific soil compartment to a plant. In contrast, other studies use radioisotopes to quantify the magnitude of an element flux. In these quantitative studies, the radioisotope is used as a tracer, i.e., a traceable proportion of the element in the studied system.

If an isotope is used as a tracer to quantify an element flux, rather than the flux of the tracer itself, it is essential to know the ratio of the amount of this isotope to the total amount of the element in the labelled pool (for a review see Di et al. (1997)). This is not a unique precondition in the use of radioisotopes. The same applies also when stable isotopes are used to trace fluxes. The difference is that radioisotopes are determined based on their radioactivity (for instance, ^{32}P activity) using scintillation counting, while stable isotopes are determined as the ratio of the added heavy isotope relative to the abundant light isotope of the element (for instance, the ^{15}N-to-^{14}N ratio) using isotope ratio mass spectrometry. Thus, when using radioisotopes to trace element fluxes, it is necessary to determine not only the amount of the radioisotope (based on its radioactivity), but also the amount of the non-labeled (or total) element in the system, in separate measurements.

If radioactive phosphorus, for instance ^{32}P, is added to a soil as phosphate, a large part of it will adsorb to soil minerals, while a second part will be taken up by microorganisms. The fraction of ^{32}P that remains plant-available in the soil (which can be as little as 1% of the added amount) will be strongly diluted by non-labeled phosphorus (for a review see Bünemann et al. (2015)). The plant will take up the radioisotope together with non-labeled phosphorus from the plant-available pool, and the ratio of radiophosphorus-to-non-labeled phosphorus (called specific activity) that is taken up can vary strongly among soils (Fig. 1). Hence, the amount of radioisotope in the plant by itself has only limited value for quantifying plant total phosphorus uptake during the labeling experiment (unless the soils are practically identical).

Soils differ strongly in their capacity to immobilize and release phosphorus due to differences in minerals, pH, texture, organic matter, microbial activity, and the extent to which binding places on minerals are saturated with phosphate. Hence, the proportion of the added radiophosphorus that remains plant-available after the first few minutes of isotope addition differs strongly among soils (Bünemann et al., 2015). In a phosphorus-poor soil, a smaller proportion of the added radiophosphorus will likely remain available for plant uptake than in a phosphorus-rich soil (assuming all other soil properties are the same). This is due to a lower saturation of minerals with phosphate (leading to a larger sorption) and a higher microbial need for phosphorus (leading to larger microbial phosphorus uptake). In addition, soils also differ in the concentration of plant-available phosphorus. Hence, radiophosphorus in the plant-available pool will be diluted to a different extent with non-labeled phosphorus in different soils (Fig. 1).

In order to calculate plant total phosphorus uptake in a labeling experiment with radiophosphorus, it is important to take into account the dilution of the radioisotope in the plant-available soil phosphorus pool by the non-labeled inorganic phosphorus. Total plant phosphorus uptake during the exposure time can be calculated by multiplying the amount of the radioisotope in the plant by the ratio of total inorganic phosphorus-to-radiophosphorus in the plant-available soil phosphorus pool. Organic phosphorus does not have to be considered in this context because plants only take up inorganic phosphorus (Lambers, 2022; Yang et al., 2024). In the two scenarios depicted in Figure 1, in which the soils received the same amount of radiophosphorus and the plants take up the same amount of radiophosphorus, plant phosphorus uptake is slightly larger in the phosphorus-poor system. Specifically, plant phosphorus uptake in the phosphorus-poor and the phosphorus-rich system is 18.8 and 16.1 arbitrary units of phosphorus during the exposure time, respectively (see equation in the figure and below). The difference results from the different ratios of non-labeled phosphorus-to-radiophosphorus in the plant-available soil pool of the two systems, which in turn has two reasons. First, the amounts of radiophosphorus in the plant-available soil pools differ because less radiophosphorus is immobilized (by adsorption and microbial uptake) in the soil of the phosphorus-rich system. Second, the radiophosphorus in the plant-available pool of the two systems is diluted to different extents with non-labelled phosphorus.

If plant phosphorus uptake is inferred only from the amount of radiophosphorus (^{32}P) transported from the soil into the plant, without accounting for immobilization of the tracer (on minerals and in microorganisms) and isotope dilution in the plant-available soil pool, the results can be highly misleading. In the study by Lekberg et al. (2024) the amount of ^{32}P was 7.8 times higher in plants growing in a phosphorus-rich soil than in plants growing in a phosphorus-poor soil, eight days after labeling. The authors reported the amount of ^{32}P per unit plant biomass and per unit biomass phosphorus, and concluded that phosphorus uptake into the plants was higher in the phosphorus-rich than in the phosphorus-poor soil during the labeling experiment. This might be the case. However, if ^{32}P dilution in the plant-available phosphorus pool was 7.8 times higher in the phosphorus-poor soil than in the phosphorus-rich soil (due to stronger adsorption and microbial uptake of the added ^{32}P in the P-poor system), total plant phosphorus uptake in both soils would have been the same. If ^{32}P dilution was more than 7.8 times higher in the phosphorus-poor soil than in the phosphorus-rich soil, plant total phosphorus uptake was larger in the phosphorus-poor system. Hence, without data on the ratio of radiophosphorus-to-non-labeled phosphorus in the plant-available soil phosphorus pool, it is impossible to quantify plant phosphorus uptake. Therefore, it is important to determine this ratio in the pool from where the transport occurs in studies that use isotopes as tracers to quantify element fluxes. This is particularly the case when fluxes in contrasting ecosystems are studied comparatively. Lekberg et al. (2004) briefly mentioned that the added radioisotope was likely diluted to different extents in the two soils, which decreased the accuracy of the estimate of plant phosphorus uptake. Yet, they do not consider that different adsorption and microbial uptake of radiophosphorus in the two soils has also a major impact on the ratio of non-labeled phosphorus-to-radiophosphorus in the plant-available phosphorus pool of the two soils, which might potentially even reverse the conclusion of their study.

In future studies that intend to determine plant phosphorus uptake based on radiophosphorus labeling (^{32}P or ^{33}P), it is important that the tracer is homogeneously applied in the soil or soil compartment (and not injected in one single spot). Second, the amount (i.e., the activity) of radiophosphorus and the concentration of inorganic phosphorus should be determined in the soil pool from where the plant acquires phosphorus using a mild extractant (as frequently as possible). Together with the determination of radiophosphorus in the plant, this allows the calculation of the total plant phosphorus uptake during the exposure time, following this equation (Di et al., 1997; Frossard et al., 2011):

$$\text{Plant P uptake (mg P plant}^{-1}) = \text{Plant total }^{32}\text{P (Bq plant}^{-1}) \times \text{Inorganic P in plant-available pool (mg P kg}^{-1}\text{ soil)}/\ ^{32}\text{P in plant-available pool (Bq kg}^{-1}\text{ soil)}$$

This calculation assumes (i) that the radiophosphorus (^{32}P) is uniformly distributed in the plant-available soil phosphorus pool, (ii) that it has the same probability to be taken up by the plant as the non-labeled phosphorus (i.e., no discrimination of phosphorus isotopes), and (iii) that no phosphorus is released by the roots into the soil (unidirectional transport). The concentration of non-labeled phosphorus (^{31}P) in the plant-available soil phosphorus pool is determined as the concentration of dissolved inorganic phosphorus since radiophosphorus is typically added to soils in extremely small (trace) amounts that have negligible effects on the soil phosphorus concentration (and can only be detected due to their radioactivity). One uncertainty in this approach is the definition and quantification of the pool from where the plant takes up phosphorus. This pool is typically called the plant-available soil phosphorus pool (or the isotopically exchangeable pool), and it is often operationally defined as a phosphorus pool that can be extracted with a specific extractant, for instance Bray-1, from soil. Another option is to determine total inorganic phosphorus and radiophosphorus in the plant-available pool based on diffusive gradients in thin films (DGT; Six et al., 2012).

Taken together, when using isotopes as a tracer to quantify element fluxes it is necessary to determine the isotope dilution in the studied system, and specifically in the labeled pool. In contrast to experiments with stable isotopes in which tracers are detected as isotope ratios, this requires additional measurements in radioisotope studies.

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Competing interests

The authors declare that they have no competing interest.

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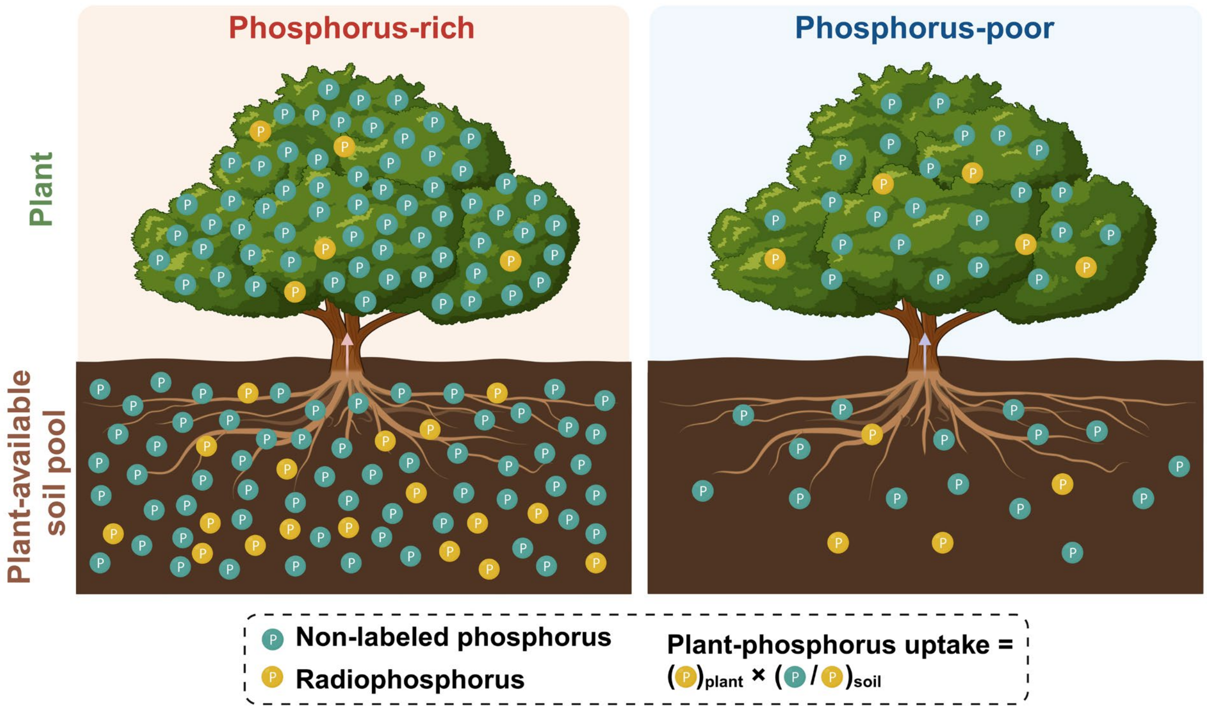

Figure 1: Schematic drawing of isotope dilution in a radiophosphorus labeling experiment involving a phosphorus-rich and a phosphorus-poor system. For each system two pools are shown (the plant and the plant-available soil phosphorus pool). To calculate the total plant phosphorus uptake during the labeling experiment, it is necessary to account for the ratio of non-labeled phosphorus-to-radiophosphorus in the plant-available soil pool. The same amount of radiophosphorus taken up by the two plants in the phosphorus-rich and in the phosphorus-poor system (5 arbitrary units) indicates a different total plant phosphorus uptake due to differences in the ratio of non-labeled phosphorus-to-radiophosphorus in the plant-available soil pool. The soils received the same amount of radiophosphorus and the difference in the plant-available soil pool is caused by differences between the two systems in radiophosphorus immobilization in the soil by adsorption and microbial uptake (not shown in the figure) and dilution of radiophosphorus with non-labeled phosphorus in the plant-available soil pool. The figure was created with BioRender.com.