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RESEARCH ARTICLE

Tracer distribution in legume roots and soluble rhizodeposits over a few weeks after a triple isotope (^{13}C, ^{15}N, ^{33}P) labeling

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Abstract

Background and aims Belowground carbon (C), nitrogen (N) and phosphorus (P) inputs by plants via roots and rhizodeposition are key drivers of these elements cycling in soils. Tracing and quantification of rhizodeposition using isotope enrichment techniques is based on assumptions that have not simultaneously been tested for C, N and P. Our objectives were: (i) to compare the elemental and isotopic composition (IC) of roots and soluble rhizodeposits for C, N and P; and (ii) to compare the IC of root segments of different ages to assess the homogeneity of root system labeling.

Methods The legume *Canavalia brasiliensis* was grown in sand and labeled with ^{13}C, ^{15}N and ^{33}P by using a novel tri-isotope method in two different experiments lasting two (percolate collection) and three weeks (root observation) after labeling.

Results Soluble rhizodeposits were less isotope enriched than roots at each time point, and each element showed a different course over time. The ^{13}C:^{15}N and ^{13}C:^{33}P ratios of rhizodeposits were higher shortly after labeling than at later samplings, highlighting faster transfer of ^{13}C than ^{15}N and ^{33}P into rhizodeposits. Phosphorus fertilizer increased the difference between IC of P in roots and rhizodeposits. Youngest root segments were more isotopically enriched than older segments, again with element specific time course, showing that root segments of different ages differ in IC.

Conclusion Assumptions underlying the quantification of rhizodeposition are not met. Temporal and spatial differences in IC of roots and soluble rhizodeposits are element specific, which needs to be considered in stoichiometric studies.

Keywords Legume · Tri-isotope plant labeling · Rhizodeposition · Root exudation · C:N:P stoichiometry

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Introduction

Belowground plant carbon (C), nitrogen (N) and phosphorus (P) inputs via roots and root-derived organic matter are a major source of soil organic matter and affect the availability of nutrients and their cycling in the soil (Cotrufo et al. 2019). Growing plants release a variety of organic materials through their root system such as lysates of sloughed-off cells and root debris and root exudates from intact root cells including diffusates, secretions and excretions (Neumann 2007; Obuger and Jones, 2018). The release of compounds from living plant roots, which includes ions and volatile compounds, has been termed rhizodeposition (Uren 2007). Belowground C input to soil largely affects soil C dynamics in agroecosystems (Keel et al. 2017) and may account for 30–90% of total organic C inputs to agricultural soils (Kätterer et al. 2011). Carbon from rhizodeposition may reside in soil considerably longer than C derived from aboveground crop residues and organic soil amendments (Rasse et al. 2005; Zhang et al. 2015). In the case of legumes, 15–30% of total assimilated plant N might end up as rhizodeposition (Mahieu et al. 2007; Hammelehle et al. 2018). While C and N rhizodeposition have been widely studied (Fustec et al. 2010; Kuzyakov and Schneckenberger 2004; Wichern et al. 2008), less is known about P release from roots (McLaughlin et al. 1987; Foyjunnessa et al. 2015).

Rhizodeposition is affected by abiotic and biotic factors, which influence the amount and elemental composition of compounds released by roots (Dakora and Phillips 2002; Jones et al. 2004; Nguyen 2009), and which in turn may affect the fate of rhizodeposition in the soil–plant system. Elemental relationships and their implications are the core of ecological stoichiometry, which has been defined by Sterner and Elser (2002) as "the balance of multiple chemical substances in ecological interactions and processes, or the study of this balance". The elements most often considered in ecological stoichiometry are C, N and P (Frossard et al. 2016). The C:N and C:P ratios of belowground inputs affect the rate of C incorporation in soils and change the levels of N and P available for the growth of plants and microorganisms in soils (Zechmeister-Boltenstern et al. 2015). For instance, P fertilization to a plant available P-limited tropical soil may affect root growth, root exudation and mycorrhizal association, directly influencing the quantity and composition of rhizodeposition (Rao et al. 1996; Lynch 2019; Chen et al. 2023). Therefore, a multi-element approach is needed for simultaneously studying C, N and P in rhizodeposition.

The fate of rhizodeposition in the soil–plant system can be studied with stable and radioisotope plant labeling techniques (Wichern et al. 2008; Fustec et al. 2010; Zang et al. 2015; Foyjunnessa et al. 2018; Kuzyakov and Domanski 2000). Labeling of carbon belowground inputs can be done by exposing plants to an isotope enriched CO_2 atmosphere in a labeling chamber (Meharg 1994; Soong et al. 2014). Pulse labeling with ^{13}C- or ^{14}C-CO_2 has been largely used to trace C compounds released from plant roots to the soil. While continuous labeling gives more homogeneous labeling of the plant C pools, allowing the study of long-term processes and fluxes, pulse labeling provides information on dynamics (Studer et al. 2014) and C distribution within the plant at a specific growth stage (Kuzyakov and Schneckenberger 2004).

In contrast to ^{13}C labeling, ^{15}N labeling techniques with mineral N forms usually imply plant manipulation by applying ("feeding") the ^{15}N labeled solutions to different plant organs (Wichern et al. 2008; Fustec et al. 2010). The leaf feeding and the cotton-wick stem feeding with a solution of ^{15}N-enriched urea have been mostly used to determine the fate of N within the plant-soil system (Ledgard et al. 1985; Palta et al. 1991; Russell and Fillery 1996; McNeill et al. 1997; Mayer et al. 2003a).

Although the P radioisotope plant labeling technique was developed in the 1960s to investigate the distribution and extent of living roots of annual crops (Racz et al. 1964), ^{32}P or ^{33}P plant labeling has rarely been used for studying rhizodeposition. Foyjunnessa et al. (2014) adapted the ^{33}P stem wick-feeding for labeling roots of canola and lupin and quantifying total P accumulation belowground and its transfer to wheat as a companion species (Foyjunnessa et al. 2015, 2016). Furthermore, Foyjunnessa et al. (2018) combined a stem feeding labeling with ^{15}N followed by ^{33}P to estimate the amount and the stoichiometry of belowground N and P. Finally, Stevenel et al. (2019) presented a novel tri-isotope (^{13}C, ^{15}N, ^{33}P) labeling approach to simultaneously study rhizodeposition of C, N and P by combining ^{15}N and ^{33}P cotton-wick stem feeding with $^{13}CO_2$ pulse labeling. However, this novel method has not yet been used to

study how the isotopic composition (IC) of C, N and P changes over time and space within the root system, and during rhizodeposition. This knowledge is essential for the application of multi-isotope labeling approach in stoichiometric studies.

Whilst rhizodeposition is conceptually defined as the release process from living roots (see above), it is operationally defined by researchers as the plant derived C, N and P remaining in the soil after separation of roots (Mayer et al. 2003a; Fustec et al. 2010). The rhizodeposited element in soil is then quantified by dividing the IC of the soil by the IC of the roots (Janzen and Bruinsma 1989). This calculation is based on two important assumptions: the IC of rhizodeposition is equal to the IC of roots (Janzen and Bruinsma 1989), and the IC of roots is constant over time and space (Sawatsky and Soper 1991; Jensen 1996). These assumptions have been tested for the IC of N: Sierra et al. (2007) showed that root exudates collected over five weeks from *Gliricidia sepium* ^{15}N-labeled with leaf feeding were less enriched than the roots. The ^{15}N enrichment of roots changed over time in a six-week lasting experiment after leaf labeling of *Trifolium pratense* L. (Gasser et al. 2015). Assumptions underlying C isotope labeling in rhizodeposition studies have not been systematically tested. Pausch and Kuzyakov (2011) reported the presence of "hotspots" with high ^{14}C activity within the root system of *Lolium perenne* by labeling plants with a single pulse of ^{14}C-CO_2 suggesting a heterogeneous root labeling. Similarly, Rasmussen et al. (2013) observed high variations in ^{15}N and ^{14}C tracer distributions within the root system of white clover and perennial ryegrass four days after labeling with the leaf feeding method. To the best of our knowledge, no study has investigated the homogeneity of root labeling with ^{33}P.

The overall aim of this study was to use a novel tri-isotope (^{13}C, ^{15}N, ^{33}P) labeling method (Stevenel et al. 2019) to quantify tracer distribution in the roots and rhizodeposits of the legume *Canavalia brasiliensis* Mart. Ex. Benth (Canavalia) over several weeks following isotope labeling. We used Canavalia, a tropical drought tolerant and fast growing multipurpose legume, that may derive about 70% of its shoot N uptake via symbiotic nitrogen fixation from the atmosphere (Douxchamps et al. 2010). We divided our study into two separate experiments: (i) a percolate collection experiment to compare the IC of roots and soluble rhizodeposits; and (ii) a root observation experiment to investigate tracer distributions within the root system over time and space. In the percolate experiment, we applied two P fertilizer doses in order to test the tri-isotope labeling and underlying method assumptions in the presence of a plant growth factor known to affect root growth and rhizodeposition. We also aimed to gain insight into root and rhizodeposition stoichiometry by comparing patterns of elemental and isotopic C:N and C:P ratios.

Material and methods

Both experiments (percolate collection, and root observation) were conducted in a C-free substrate under greenhouse conditions (mean temperatures 28.2/21.7 °C day/night, 12 h photoperiod). We grew the plants in a sand model system over a few weeks to study the rhizodeposition from plant roots without mycorrhizal inoculation. Seeds of canavalia were beforehand scarified and pre-germinated prior both experiments.

Percolate collection experiment

The percolate experiment consisted of 48 pots planted with one Canavalia plant each. The completely randomized experimental design comprised two P fertilizer doses (low, high), two labeling treatments (labeled, non-labeled reference), three harvest dates and four replicates. Seeds of Canavalia were sown into plastic pots (1 seed per pot) where an aluminum bag has been placed before filling with 1000 g of coarse quartz sand (0.7–1.2 mm, Carlo Bernasconi AG, Switzerland) previously washed with a 0.65 M HCl solution, rinsed with deionized water and oven dried at 60 °C for two days. Pots were daily weighed and watered with deionized water to 40–50% of the water holding capacity (WHC; 24 g water per 100 g dry sand determined by using gravimetric method) (Agroscope 2020) and received regularly 20 mL N- and P-free nutrient solution (Unkovich et al. 1994), with the following final concentrations for macronutrients (m*M*) $MgSO_4{\cdot}7H_2O$ (4), $CaCl_2{\cdot}2H_2O$ (4), K_2SO_4 (4), $FeSO_4{\cdot}7H_2O$ (0.1), and for micronutrients (µ*M*) H_3BO_3 (10), $MnSO_4{\cdot}H_2O$ (3.6), $ZnCl_2$ (2), $CuSO_4{\cdot}5H_2O$ (0.6), $Na_2MoO_4{\cdot}2H_2O$ (0.1) $NiSO_4{\cdot}6H_2O$ (0.1) and $CoSO_4{\cdot}7H_2O$ (0.1). Each plant

was inoculated twice over the first two weeks with *Bradyrhizobium* strain (CIAT 4969) freshly cultivated in liquid media (Somasegaran and Hoben 2012). Solution of 1 M $KH_2PO_4/K_2HPO_4 \cdot 3H_2O$ was added every week to reach two different P fertilizer levels until the start of the isotope labeling: 5 mg P (low) and 28 mg P (high) per pot. Additionally, a 0.05 M NH_4NO_3 solution was weekly applied to all pots to obtain a medium N level corresponding to 20 mg N per pot. Plants were grown for 36 days and were moved randomly each week within the greenhouse prior to labeling.

Plants were harvested at 1, 8 and 15 days after labeling (d.a.l). In order to determine the IC of soluble rhizodeposits, we needed to make sure that we worked without residual N or P from nutrient solution. To this end, two days before each harvest, eight plants (four plants from low P and four from high P supplies) were transplanted by carefully shaking off the sand, placing the plants into a new pot filled with sand and adding the N- and P-free nutrient solution. Thus, the measure of the IC at 1 d.a.l corresponded to the IC of the rhizodeposits collected between the labeling at day 0 and the excavation 1 d.a.l, while for 8 and 15 d.a.l the measured IC represented the IC of rhizodeposits collected from 6 to 8 d.a.l, and from 13 to 15 d.a.l, respectively.

At the beginning of all three harvests, deionized water was added to the pots, so that moisture content in the pot reached 100% WHC. Because moisture in pots had every day been readjusted to 50% WHC, the volume of water required was between 120 and 190 mL. The plastic pot containing the aluminum bag with the sand was removed, and the bag was placed on a square PVC platform where needles are jutting out above the surface and piercing the bag (Stevenel et al. 2019). The platform was linked to a peristaltic pump, and solution was pumped out between five and ten minutes after water addition and was collected in a 125 mL bottle. Percolation lasted approximately five minutes to collect between 80 and 100 mL of percolate. Thanks to this method, percolates containing soluble rhizodeposits could be collected without further disturbing the root system after the earlier sand replacement at transplanting (Stevenel et al. 2019). Percolates were filtered (Whatman no. 42 filter paper) to remove small particles and acidified to pH 3 using 9.8 M HCl to limit gaseous losses of ammonia and then stored at −18 °C until analysis. Shoots were removed by cutting the stem just above the sand surface and roots were carefully separated from the sand by shaking and soaking in deionized water for removing adhering sand particles.

Root observation experiment

The root observation experiment consisted of 32 rhizoboxes, each planted with one canavalia plant, and the randomized complete block design comprising one P level (medium), four harvest dates, two labeling treatments (labeled, non-labeled reference) and four replicates. Rhizoboxes (L x W x H: 15 cm × 1.5 cm × 45 cm) were filled with 1400 g of coarse sand. One canavalia seed was sown in each rhizobox and inoculated with freshly cultivated *Bradyrhizobium* (CIAT 4969). Similar to the percolate experiment, rhizoboxes were daily watered by weighing and received regularly 10 mL of N-free nutrient solution. In contrast to the pot experiment, only one P level was selected corresponding to a medium P fertilizer dose (11 mg P per rhizobox). Plants were grown for 41 days after germination prior to isotope labeling. During the entire plant growth, the root growth was regularly recorded by tracing the newly formed roots with permanent markers on the rhizobox observation window with a color code according to the age of root section (Fig. 1) (Lemming et al. 2016). Plants were harvested at 1, 8, 15 and 22 d.a.l. Shoots were cut at 1 cm above the sand surface and the rhizobox windows were carefully opened to get access to the root system. Root segments were collected according to the recorded age and classified as root segments grown from sowing to one week before labeling (r < 0d), within the last week before labeling (r1w < 0d) and roots developed for 1 to 8, 9 to 15, and 16 to 22 days after labeling (r1-8d, r9-15d and r16-22d). Additionally, roots not visible through the rhizobox window that could not precisely be categorized were collected, pooled and defined as bulk roots (rb). Results with ^{13}C enrichment (Fig. 6a) have already been reported before (Stevenel et al. 2019) but these are included again in this study for comparison.

Tri-isotope labeling

The same labeling procedure was applied to the percolate collection and root observation experiments.

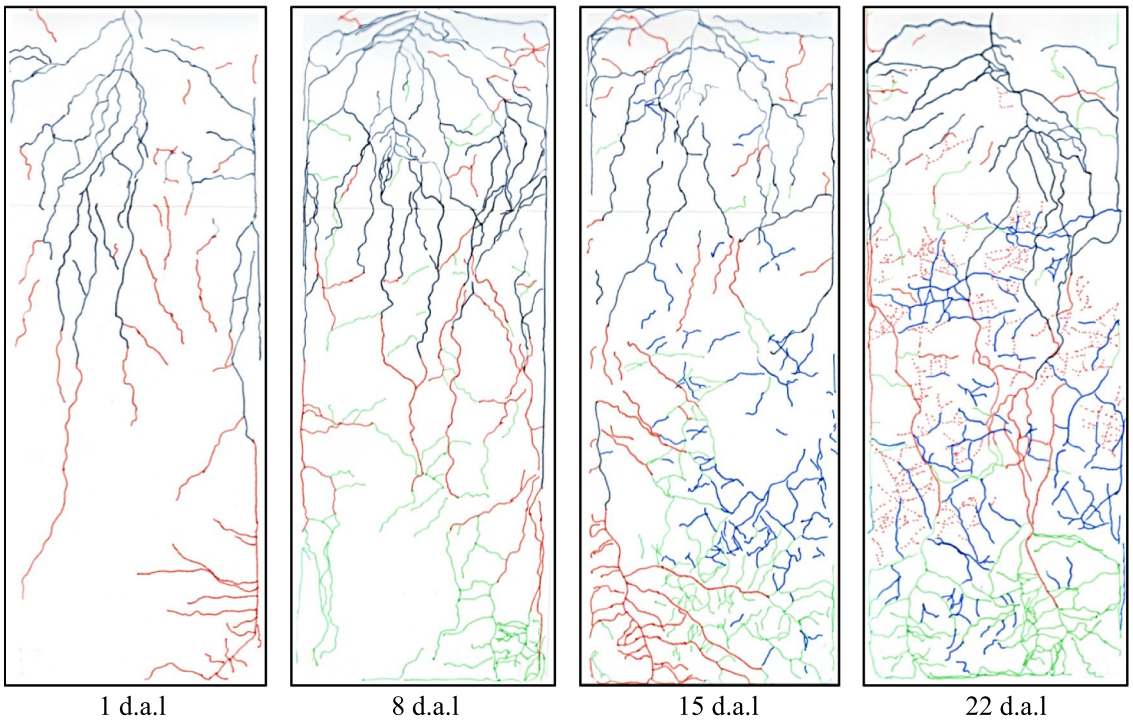


Fig. 1 Tracking of root development and distribution in sand during whole plant growth in the root observation experiment. Root system developed from sowing to one week before labeling (r < 0d, black line), within the last week before labeling (r1w < 0d, red line) and roots developed between 1 and 8 d.a.l (r1-8d, green line), 9 and 15 d.a.l (r9-15d, blue line) and 16 and 22 days after labeling (d.a.l) (r16-22d, dotted red line). Figure 1 22 d.a.l is adapted from Stevenel et al. (2019) and is licensed by Springer Nature Customer Service Center GmbH. Figure 1 22 d.a.l is not part of the open access content of this article

Firstly, a $^{13}CO_2$ single pulse labeling was applied to the canavalia shoots (Bromand et al. 2001). Therefore, plants were enclosed in a transparent airtight Plexiglas® chamber (120 cm × 100 cm × 90 cm) equipped with fans for air mixing and with ice packs for keeping temperature between 26 °C and 28 °C. The monitoring of total CO_2 and H_2O concentrations within the labeling chamber was done online with an infrared gas analyzer (LI-840A, LI-COR®, USA). The CO_2 concentration in the chamber was lowered to 300 µL L^{-1} with a CO_2 trap composed of soda lime enclosed in a cylinder. Then a pulse of 99 atom% $^{13}CO_2$ (Euriso-Top®, France) was injected up to a concentration of 750 µL L^{-1} and plants were kept approximately 3.5 h in the chamber until the CO_2 level fell to 300 µL L^{-1}. At the end of the labeling, the chamber was flushed by opening its door and plants were removed from it. To avoid any cross contamination of ^{13}C released by respiration from labeled plants, we grew labeled and non-labeled plants in separate greenhouses under the same growth conditions. In both experiments, non-labeled plants were used as reference samples in order to calculate atom% excess values.

On the day after the ^{13}C pulse labeling, each ^{13}C -labeled plant was also labeled with a solution of ^{15}N-urea and carrier free $^{33}PO_4$ using the cotton-wick stem feeding technique described by Russell and Fillery (1996a). Briefly, a hole (Ø 0.5 mm) was drilled through the stem of the plant approximately 3 cm above ground and a thick cotton wick was passed through the hole using a fine needle. Then, the wick ends were passed through the perforated lid of a 1.5 mL vial that contained 0.6 mL of labeled solution composed of 0.1% (w/v) ^{15}N-urea (99 atom % ^{15}N, Sigma-Aldrich Chemie GmgH, Steinheim, Germany) and a total ^{33}P activity of 450 kBq with carrier-free ^{33}P-orthophosphate (Hartmann Analytic, Braunschweig, Germany). The wick parts in contact with air were protected

by flexible silicon tubes sealed against the stem with Terostat-IX® putty (Teroson, Henkel, Germany) to prevent evaporative losses of labeled solution and contamination. The total uptake of labeled solution by the plants was completed after 12–24 h and once the vial was empty, 1 mL of deionized water was added to rinse the vial and cotton wick to maximize the uptake of the ^{15}N and ^{33}P labeled solution. Then the vial and cotton wick were removed from all plants. To have representative controls, the same method was applied to the NL plants by replacing the 0.6 mL of labeled solution with deionized water.

Sampling and analytical methods

Plant samples (i.e. shoots and roots) of both experiments were dried at 60 °C for 48 h, weighed for dry matter (DM) determination and ground to a fine powder using a centrifugal mill (ZM 200, Retsch, Germany) and a ball mill (MM 200, Retsch, Germany) for shoots and roots, respectively. Root samples from the rhizobox experiment were manually powdered using a ceramic mortar to minimize losses. Total P was determined by ashing 0.2 g of DM at 550 °C for 4 h, dissolving the ash in 2 mL of 15 M HNO_3 and adjusting the volume to 50 mL with deionized water (Nanzer et al. 2014). The P concentration in the extract was then determined colorimetrically by using a malachite green method (Van Veldhoven and Mannaerts 1987). The ^{33}P beta-emission in the plant extracts and percolates were measured by liquid scintillation counting (TRI-CARB 2500 TR, Liquid scintillation analyzer, Packard Instruments, Meriden, CT, USA). The counts were corrected for radioactive decay and referred to the starting date of the labeling procedure. Plant N, C, $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were determined by EA-IRMS (Vario PYRO cube, Elementar, Germany and IsoPrime100 IRMS, Isoprime, United Kingdom). Percolates were analyzed for total organic C and total N concentrations using a TOC/TN analyzer (Shimadzu, Japan) (Supplementary material, Table S1). Total P was determined by a colorimetric method after digestion in 2.5 M H_2SO_4 and 0.2 M K-persulfate (Tiessen and Moir 1993). An aliquot of each percolate sample was also analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ determination using the EA-IRMS coupled with a liquid sampler.

Calculations and statistics

The ^{15}N and ^{13}C data were expressed as the atom% ^{15}N excess and atom% ^{13}C excess, respectively, which is the difference between the IC ($^{15}N/(^{15}N+^{14}N)$ and $^{13}C/(^{13}C+^{12}C)$, respectively) of the labeled plant and the non-labeled plant (control):

$$\text{Atom\% X excess} = \text{atom\% X}_{\text{labeled plant}} - \text{atom\% X}_{\text{control plant}} \quad (1)$$

where X corresponds to ^{15}N or ^{13}C. This formula also applies to the percolate calculations.

The radioactivity (^{33}P) was calculated back to the beginning of the experiment to correct for radioactive decay and expressed as kBq using the following equation:

$$R_0 = R_t / e^{\frac{-0.693t}{T_{1/2}}} \quad (2)$$

where R_0 is the initial radioactivity of ^{33}P, R_t is the radioactivity at counting day, t is time (days) between the counting day and the day of application and $T_{1/2}$ is the half-life time of ^{33}P (25.34 days). All activities are presented as the specific activities (SA, in kBq/mg P) and calculated by dividing the activity measured in a pool (i.e. shoot, roots or percolate, in kBq) with the total P content of this pool (mg P):

$$\text{SA (kBq/mg P)} = \frac{^{33}\text{P activity}}{\text{Total P content}} \quad (3)$$

In the root observation experiment, the IC of the total root system was calculated by the weighted arithmetic mean of all root segments using the following equation:

$$\text{atom\%}^{13}\text{C excess}_{\text{roots}} = \frac{\sum_{i=1}^{n} w_i X_i}{\sum_{i=1}^{n} W_i} \quad (4)$$

where w_i is the C content and x_i the atom% ^{13}C excess of a given root segment, with 1 denoting the root segment of r < 0d. The same relation was used for N by using root N and the atom% ^{15}N excess, and for P by using root P content and the specific activity.

The recoveries of ^{15}N and ^{33}P tracer in shoot and roots were calculated by relating the recovered amount of tracer and the total amount of tracer applied to the plant (Gasser et al. 2015). Despite the amount of ^{13}C supplied to the fumigation chamber was known, the exact ^{13}C amount assimilated by

the plant was unknown. Therefore, only relative ^{13}C recoveries were calculated by relating the amount of ^{13}C in a compartment and the total amount of ^{13}C recovered in the entire plant (i.e. in shoot and roots).

The C:N, C:P, ^{13}C:^{15}N and ^{13}C:^{33}P ratios of roots and percolates were obtained by relating the mass of C (or ^{13}C) to the mass of N (or ^{15}N) and P (or ^{33}P). Mass (m) of ^{33}P was calculated by using the following formula:

$$m\ ^{33}P\ (mg) = \frac{M * A * T_{1/2}}{N_A * 0.693} * 1000 \quad (5)$$

where M is the atomic weight (i.e. 33), A the activity in Bq, $T_{1/2}$ the half-life time of ^{33}P in s and N_A the Avogadro constant in mol^{-1}.

The effects of P level, plant part (i.e. shoot vs. root) and time after labeling on plant dry matter, C and P concentrations, C:N and C:P ratios, tracer recoveries and ^{13}C, ^{15}N and ^{33}P enrichments of plants and percolates were evaluated by factorial analysis of variance (ANOVA) followed by multi comparison post-hoc test using Tukey Honest Significant Differences test (HSD-test) using SigmaPlot ver.13.0 (Systat Software GmbH, Erkrath, Germany). The normal distribution test (Shapiro–Wilk) and homogeneity of variances test (Brown-Forsythe) were performed beforehand. Significant differences were determined at $\alpha = 0.05$.

Results

Percolate collection experiment

Plant dry matter and N and P concentrations Both root and shoot biomass significantly increased over the two weeks after labeling for both P levels, from 1.8 g to around 2.8 g for shoot and from 0.5 g up to 0.7 g for roots (Table 1). Phosphorus

Table 1 Dry matter (DM), nitrogen (N) and phosphorus (P) concentrations, C:N and C:P mass ratios of shoot and roots of canavalia grown at two different levels of P dose and from two different harvests at 1 and 15 days after labeling (d.a.l.) in the percolate collection experiment

Plant part	P level	Harvest (d.a.l)	Dry matter (g)	Concentration (g/kg DM)		C:N	C:P
				N	P		
shoot	low P	1	1.87[a]	17.5[a]	1.66[a]	23[a]	247[a]
		15	2.68[b]	24.9[b]	1.39[b]	17[b]	299[b]
	high P	1	1.84[a]	20.7[a]	4.75[a]	21[a]	87[a]
		15	2.93[b]	23.5[a]	4.30[a]	21[a]	98[a]
Significance level (p):							
P level			*ns*	*ns*	***	*ns*	***
harvest			***	*	*ns*	*	*ns*
P level x harvest			*ns*	*ns*	*ns*	*ns*	*
roots	low P	1	0.49[a]	22.0[a]	2.28[a]	17[a]	169[a]
		15	0.57[b]	30.6[b]	2.49[a]	14[b]	171[a]
	high P	1	0.55[a]	26.0[a]	8.67[a]	15[a]	46[a]
		15	0.74[b]	28.6[a]	6.10[a]	15[a]	73[b]
Significance level (p):							
P level			*	*ns*	***	*ns*	***
harvest			**	*	*ns*	*ns*	*ns*
P level x harvest			*ns*	*ns*	*ns*	*ns*	*ns*
plant part x harvest			*ns*	*ns*	*	*ns*	*ns*
plant part x P level			***	*ns*	*ns*	*ns*	***
plant part x harvest x P level			*ns*	*ns*	*ns*	*ns*	**

ns: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Different lower-case letters within columns denote significant differences at $p < 0.05$ across time points within P levels

fertilization did not affect shoot biomass but a higher P fertilizer dose increased by 30% 15 d.a.l root dry matter compared to lower P dose ($p < 0.05$). There was no difference between 1 and 15 d.a.l in N concentration of roots and shoots at high P dose, while both plant parts showed a significantly higher N concentration (+42% in shoot and +39% in roots) at 15 d.a.l compared to 1 d.a.l in the low P dose (Table 1). Consequently, N uptake increased over time in both shoot and roots for both low P (32.6 to 66.5 mg N in shoot, 10.8 to 17.5 mg N in roots) and high P dose (37.4 to 67.5 mg N in shoot, 14.1 to 21.8 mg N in roots) (data not shown). The C:N ratios ranged from 14 to 17 in roots and were slightly higher in shoots, 17 to 23, than roots. Significantly ($p < 0.001$) higher P concentrations were observed at high P dose in both shoot and roots. Total plant P uptake slightly increased over time at low P (4.2 to 5.1 mg P) and high P dose (13.6 to 17.0 mg P). Accordingly, C:P ratios in shoot and roots were lower under high P than under low P dose and increased with time in both, shoot and roots.

Isotopic tracer recoveries within the plant Total recoveries in the plant (i.e. in roots and shoot) were 97–108% of applied ^{33}P and 72–88% of applied ^{15}N (Fig. 2). Both tracers were mostly recovered in shoots

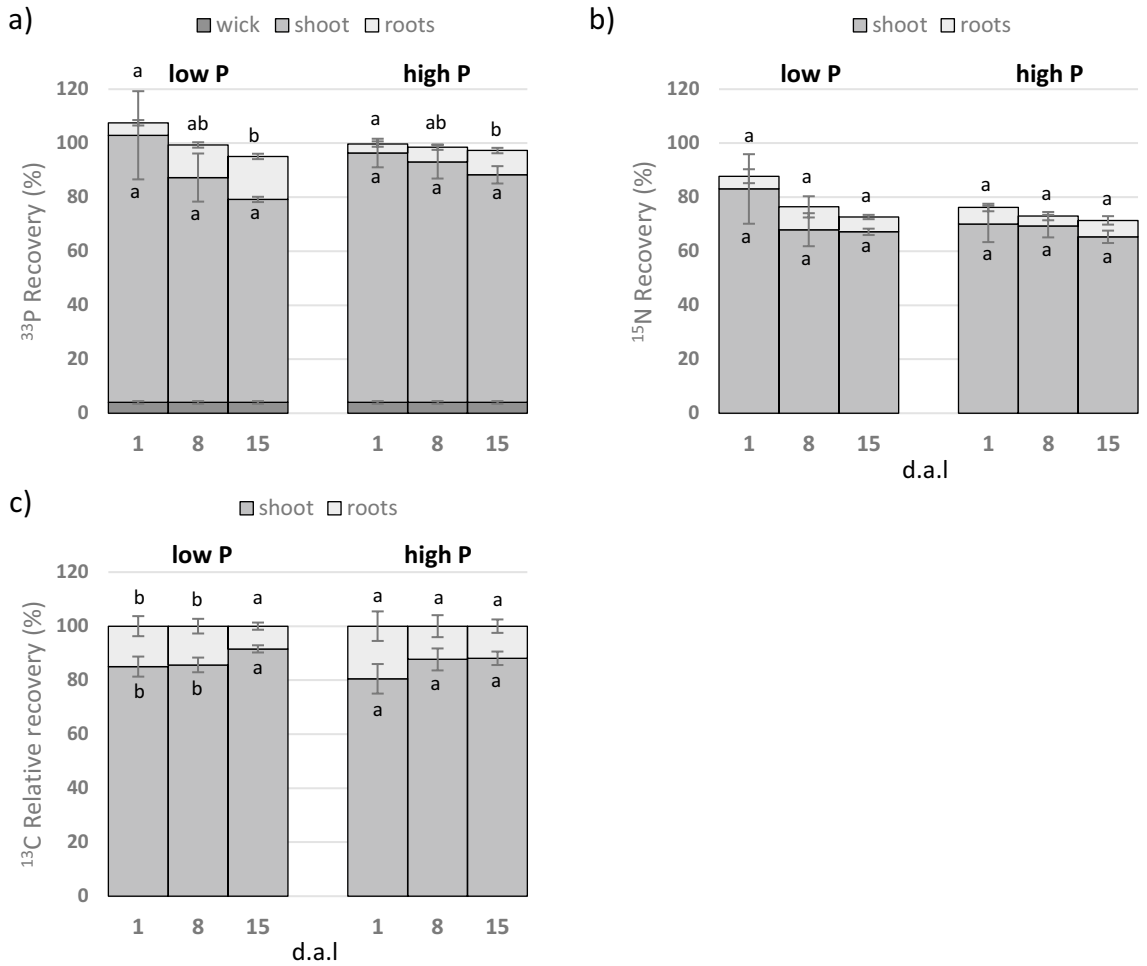


Fig. 2 (**a**) ^{33}P and (**b**) ^{15}N tracer recoveries within the plants related to the total amount of tracer applied to the plant, and (**c**) ^{13}C tracer recoveries as relative distribution recovered in the plant parts (recoveries in shoot plus root = 100%) in the percolate collection experiment 1, 8 and 15 days after labeling (d.a.l). The ^{33}P radioactivity recovered in "wick" represents the average of the activity measured in six labeling devices. Different letters denote significant differences at $p < 0.05$ across time points within a same plant part. Error bars represent the standard deviation ($n = 4$)

with more than 75% for ^{33}P and 65% for ^{15}N, while recoveries in roots did not exceed 16% for ^{33}P and 9% for ^{15}N. The P fertilizer levels did not affect ^{33}P and ^{15}N distributions within the plant. Whereas no significant difference was observed in ^{15}N distributions over time in both roots and shoot, ^{33}P tracer recovered in roots was significantly higher at 15 d.a.l than at 1 d.a.l for both P levels. Of the total ^{13}C tracer amount recovered in the plant, between 81–85% was in shoot and 15–20% was in roots at 1 d.a.l. Two weeks after labeling, relative ^{13}C recoveries (in % of total recovered in the plant) in shoot increased up to 88–92%, while they decreased accordingly in roots (down to 8–12%), with a significant evolution in low P treatment. The P fertilizer dose did not significantly affect ^{13}C partitioning in plant although there was a trend

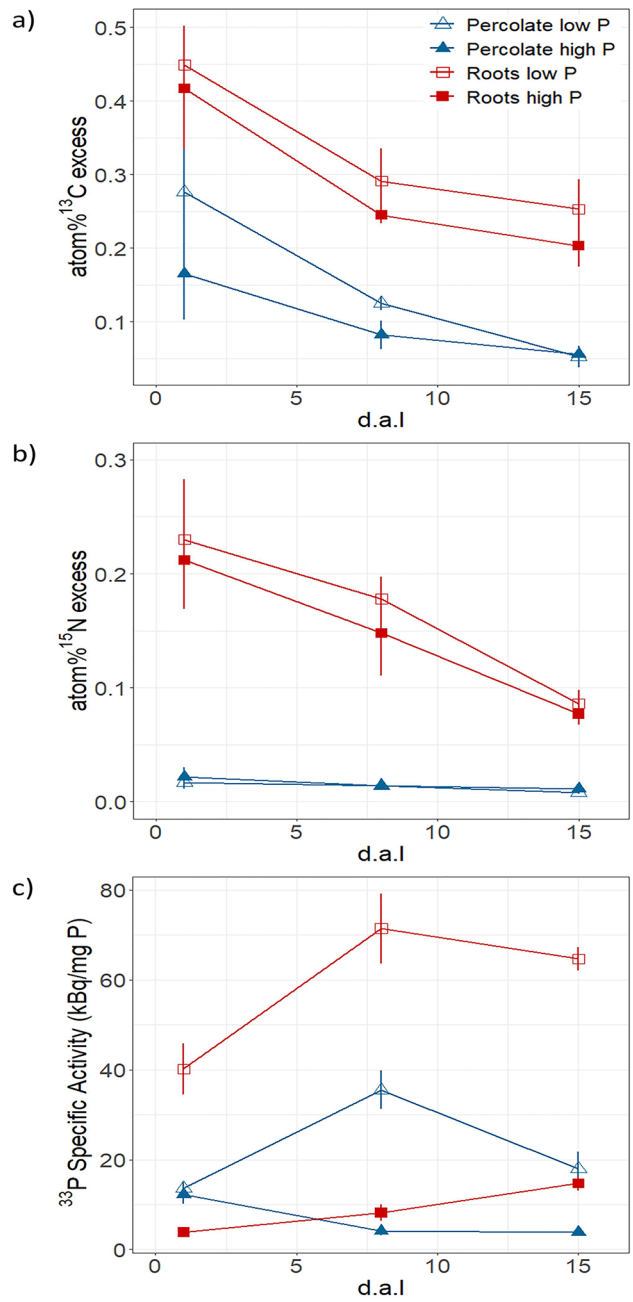


Fig. 3 Atom% ^{13}C excess (**a**), atom% ^{15}N excess (**b**) and specific activity (**c**) in roots and percolate from plants grown at two different P treatment levels (low, high) in the percolate collection experiment. Error bars represent the standard error of the mean ($n = 4$)

to a greater proportion recovered in roots at high P treatment.

Isotopic composition of roots and percolates Percolates were less enriched in labelled isotopes than roots for all three elements at all time points and with both P levels (Fig. 3). The IC of roots and percolates showed similar patterns over time and in response to P level. The ^{13}C enrichments of roots were about double those of percolates for both low and high P levels. The ^{13}C excess of roots and percolates tended to be lower at high P than low P fertilizer dose. The ^{13}C excess values decreased by half over the first two weeks after labeling in both roots and percolates. The ^{13}C enrichment of percolates showed a significant decrease from 62 to 23% of the ^{13}C enriched roots along the experimental period at low P dose (Figure S1). At high P treatment, ^{13}C percolate enrichment was stable, at around 34 and 41% of the root enrichment.

The ^{15}N enrichments in roots were 10 times higher than in percolates and were not significantly affected by P levels. The ^{15}N excess in roots decreased from around 0.2 atom% excess at 1 d.a.l to 0.1 atom% excess at 15 d.a.l. Percolate enrichments ranged from 6–11% at low P and from 11–15% of root enrichment at high P fertilizer dose.

The SA of ^{33}P in roots and percolates were affected by the P fertilizer levels. The SA of both roots and percolates were significantly higher at low P than high P dose. Similar temporal patterns were observed for SA in roots and percolates at low P dose with a maximum SA at 8 d.a.l. Percolates had constantly about half the SA of the roots (35–51%) at low P dose (Figure S1). In contrast, percolates had three times higher SA than roots (282%) at 1 d.a.l with high P treatment followed by a significant ($p < 0.05$) decrease to finally reach only 29% of the root enrichment at 15 d.a.l.

Elemental and isotopic stoichiometry of roots and percolates Elemental and isotopic stoichiometry often differed between roots and percolates (Fig. 4). Overall, C:N ratios ranged between 10 and 20 and tended to decrease over time. The only significant difference ($p < 0.05$) in C:N ratio was observed in percolate at high P dose between 1 and 15 d.a.l. The P treatment level did not affect the C:N ratio measured in roots and percolates (Fig. 4). In contrast, the C:P ratio of roots and percolates were significantly lower ($p < 0.01$) under high P than under low P level and did not change over time. There was no significant difference between C:P ratio in roots and C:P ratio in percolates at high P dose whereas C:P ratios were significantly lower ($p < 0.01$) in percolates (90–104) than in roots (156–182) at low P level.

The isotopic ratios differed much from elemental ratios, as they directly depend on the level of labeling (Fig. 4). They also differed often between roots and percolates and were affected by the P level. The ^{13}C:^{15}N ratios in roots were not affected by the P fertilizer level and had an average of 34, which was about twice higher than the C:N ratios of roots (14–17). The main differences between C:N and ^{13}C:^{15}N ratios were observed in percolates. Already at 1 d.a.l, the ^{13}C:^{15}N ratios were 312 and 112 at low and high P treatments, respectively, and these values significantly decreased to reach a similar ^{13}C:^{15}N ratio as in roots at 15 d.a.l. The P fertilizer level only affected the ^{13}C:^{15}N ratios in percolates shortly after the plant labeling.

Although the ^{13}C:^{33}P ratios were significantly higher ($p < 0.001$) than C:P ratios, ^{13}C:^{33}P ratios showed a similar temporal pattern as ^{13}C:^{15}N ratios, with a significant decrease between 1 and 8 d.a.l.

Root observation experiment

Tracer distributions and recoveries within the plant The ^{15}N and ^{33}P recoveries ranged from 80 to 88% for ^{15}N and from 84 to 93% for ^{33}P of the isotope contained in the labeling solution (Table 2). For ^{33}P, 1.3% ($n = 6$) was recovered in the labeling device, including wick, tubing and vial. The major proportion of ^{15}N and ^{33}P tracers remained in shoot with 70–75% for ^{15}N and 74–80% for ^{33}P. The isotope recovery in the entire root system of canavalia was greater for ^{15}N than ^{33}P, with an allocation of 5–18% of the plant recovered ^{15}N and 2–11% for ^{33}P recovered in the roots. The recoveries of both tracers in roots were significantly higher at 22 d.a.l than at 1 d.a.l showing a continuous allocation of tracers to the root system. During that time span, root dry matter increased by 50% whereas shoot dry matter doubled (Table S2). The P and N concentrations in the roots tended to increase with time while they decreased in the shoots

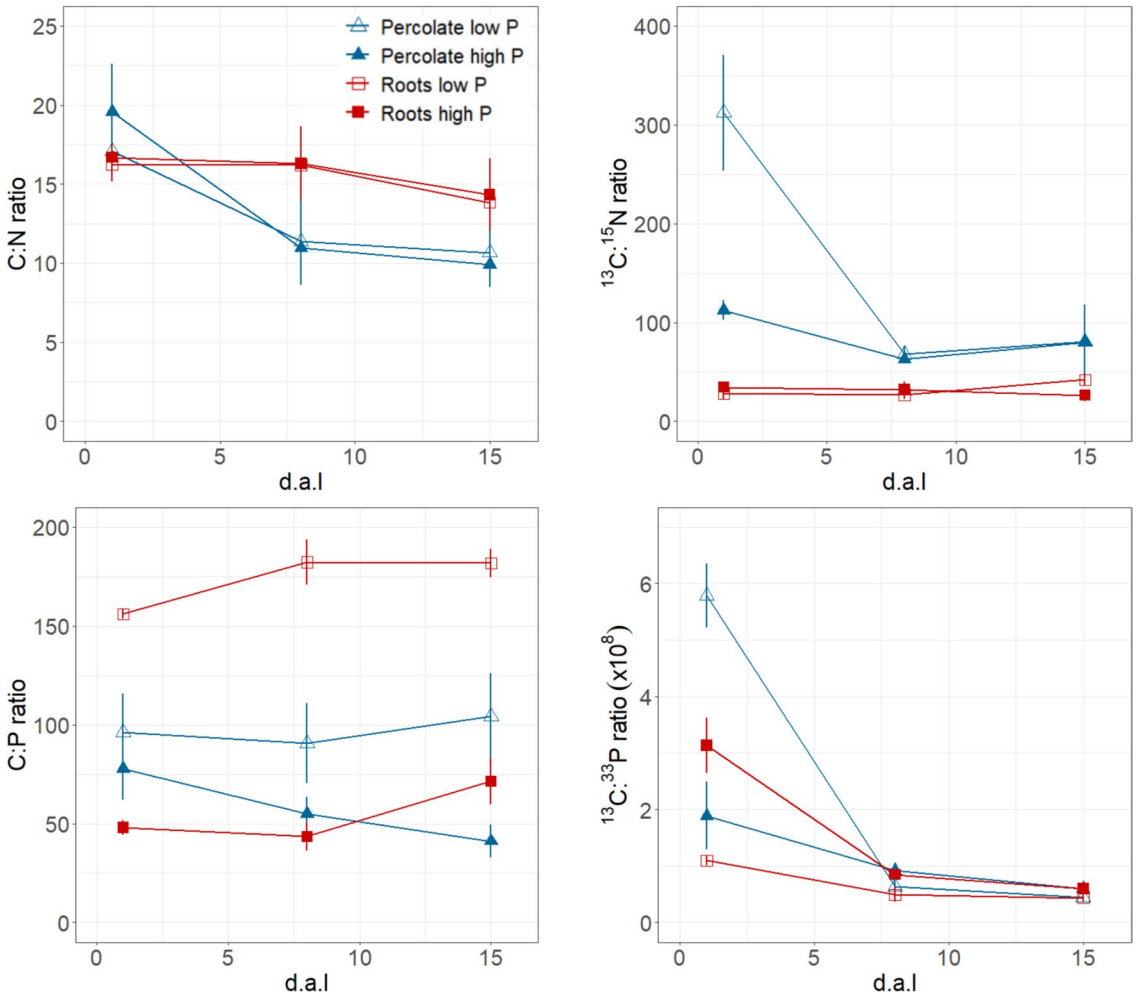


Fig. 4 C:N, ^{13}C:^{15}N, C:P and ^{13}C:^{33}P mass ratios in roots and percolates of transplanted plants grown at two different P treatment levels (low, high) in the percolate collection experiment. Error bars represent the standard error of the mean ($n = 4$)

(Table S2). However, C:N and C:P ratios increased over time in both, shoots and roots.

The recoveries of ^{15}N and ^{33}P in the different root segments grown after labeling (r2-8d, r9-15d and r16-22d) tended to decrease with time due to a lower dry matter of younger root segments (Table 3). Within roots, 79% of the ^{15}N and 83% of ^{33}P recovered in roots remained in roots grown before labeling ($r < 0d$ and $r1w < 0d$). This proportions significantly decreased ($p < 0.001$) to 33% for ^{15}N and 57% for ^{33}P at 22 d.a.l. A similar decrease, from 35 to 20% of ^{15}N and from 18 to 10% of ^{33}P, was observed in roots grown after the labeling during the whole duration of the experiment. In contrast, the proportion of tracer recovered in the non-categorized roots (rb) increased and represented 46% of ^{15}N and 33% of ^{33}P contained in roots, at the end of the experiment due to the increasing biomass of this segment.

Isotopic composition of root weighted mean and shoot The ^{13}C enrichment of both shoots and roots significantly fell by 50% over 21 days due to growth-induced dilution with non-^{13}C-enriched C assimilation, respiration and root exudation following the ^{13}C-CO_2 pulse labeling (Fig. 5a). A constant atom% ^{15}N excess (0.8%) was observed in the shoot while the enrichment tended to increase ($p > 0.05$) from 0.2 to 0.4 atom% in the weighted average for the entire root system (Fig. 5b). Similar to ^{13}C, the ^{33}P SA values

Table 2 ^{15}N and ^{33}P tracer distribution and recovery within the plant at 1, 8, 15 and 22 d.a.l in the root observation experiment

		% tracer recoveries									
		Total biomass		Root segments						Device	Total
tracer	d.a.l	shoot	roots	r < 0d	r1w < 0d	r2-8d	r9-15d	r16-22d	rb		
^{15}N	1	75 ± 4^{a}	5.0 ± 0.5^{a}	2.2 ± 0.4^{a}	1.7 ± 0.3^{a}	*na*[1]	*na*	*na*	1.0 ± 0.7^{a}	-[2]	80 ± 4^{a}
	8	74 ± 4^{a}	8.8 ± 4.0^{ab}	2.5 ± 1.6^{a}	1.0 ± 0.3^{a}	2.8 ± 0.7a	*na*	*na*	2.5 ± 1.9^{a}	-	83 ± 3^{ab}
	15	73 ± 5^{a}	15 ± 5^{ab}	3.9 ± 1.4^{a}	1.2 ± 0.4^{a}	1.8 ± 0.6a	1.3 ± 0.3^{a}	*na*	6.9 ± 2.3^{ab}	-	88 ± 2^{b}
	22	70 ± 9^{a}	18 ± 7^{b}	4.6 ± 0.7^{a}	0.92 ± 0.42^{a}	1.9 ± 1.1a	1.1 ± 0.4^{a}	0.66 ± 0.51	8.5 ± 4.4^{b}	-	87 ± 3^{b}
^{33}P	1	80 ± 3^{ab}	2.2 ± 0.6^{a}	1.2 ± 0.3^{a}	0.52 ± 0.12^{a}	*na*	*na*	*na*	0.40 ± 0.37^{a}	1.3 ± 0.9	84 ± 3^{a}
	8	80 ± 4^{ab}	5.0 ± 3.3^{ab}	2.9 ± 2.5^{ab}	0.36 ± 0.13^{a}	0.75 ± 0.29^{a}	*na*	*na*	0.97 ± 0.69^{a}	1.3 ± 0.9	86 ± 3^{a}
	15	84 ± 6^{a}	7.5 ± 3.0^{ab}	3.9 ± 2.0^{ab}	0.60 ± 0.25^{a}	0.60 ± 0.07^{a}	0.35 ± 0.05^{a}	*na*	2.3 ± 0.9^{ab}	1.3 ± 0.9	93 ± 4^{b}
	22	74 ± 1^{b}	11 ± 3^{b}	5.6 ± 1.3^{b}	0.48 ± 0.18^{a}	0.71 ± 0.34^{a}	0.37 ± 0.06^{a}	0.40 ± 0.01	3.6 ± 1.2^{b}	1.3 ± 0.9	86 ± 1^{a}
Significance level (p):											
tracer		**	*	*ns*	***	***	***	*ns*	*		*ns*
d.a.l		*ns*	***	***	*	*ns*	*ns*	*na*	***		***
tracer x d.a.l		*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*na*	*ns*		*ns*

[1] *na*: not available due to experimental design, [2] -: not available due to the limit of quantification, *ns*: not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Different lower case letters within columns denote significant differences at $p < 0.05$ across time points. Data are given as the recovery in percent of applied ^{15}N and ^{33}P. Values presented are mean ± standard deviation ($n = 4$)

Table 3 Distribution of ^{15}N and ^{33}P tracer recovered within the roots at 1, 8, 15 and 22 days after labeling (d.a.l.) in the root observation experiment

	% tracer recoveries within the roots [1]			
	d.a.l	Roots grown before labeling	Roots grown after labeling	Roots not categorized
^{15}N	*1*	79.4^{a}	*na*[2]	20.6^{a}
	8	39.0^{b}	34.6^{a}	26.5^{a}
	15	34.0^{b}	20.7^{b}	45.4^{b}
	22	33.2^{b}	20.3^{b}	46.4^{b}
^{33}P	*1*	83.4^{a}	*na*[1]	16.7^{a}
	8	63.7^{ab}	17.6^{a}	18.8^{ab}
	15	58.7^{b}	9.9^{a}	31.3^{b}
	22	57.3^{b}	9.6^{a}	33.1^{b}

[1] entire root system being 100%, [2] *na*: not available due to experimental design since no root segment has been identified only 1 d.a.l, ns: not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$. Different lower case letters within columns denote significant differences at $p<0.05$ across time points

in shoot were significantly lower at 22 d.a.l than at 1 d.a.l ($p=0.02$). In contrast, the SA values in roots increased significantly over the experimental period, from about 4 to 11 kBq/mg P (Fig. 5c).

Isotopic composition of root segments of different age over time In the oldest root segment (r < 0d), corresponding to roots growing from planting to one week before labeling, the ^{13}C excess showed a significant decrease ($p<0.01$) between 1 and 22 d.a.l (different harvests of that segment in Fig. 6a). Also, in all other segments, the ^{13}C excess decreased from one harvest to another (significantly for r2-8d), showing that the ^{13}C excess was not constant over time. At a given harvest date, the ^{13}C excess was higher in younger than in older root segments for segments up to r2-8d, while segments grown later than 9 days after labeling (i.e. r9-15d and r16-22d) had lower ^{13}C excess ($p<0.05$). Considering that younger root segments had explored different space (Fig. 1), this shows that the ^{13}C excess was not constant over space.

In contrast to ^{13}C enrichment, ^{15}N enrichments of any given root segment did not significantly differ between harvests, i.e., the ^{15}N excess was constant over the three weeks of study (Fig. 6b). However, the ^{15}N IC varied between root segments of different ages, i.e. spatially: it was greater in root segments grown until 8 d.a.l, similarly to ^{13}C, while segments grown during the last two weeks tended to lower ^{15}N enrichment.

The SA values in root segments grown from planting to 1 week before labeling (r < 0d) were significantly increasing with time (different harvests of that segment in Fig. 6c). However, the following root segment (i.e. r1w < 0d, the second oldest one) showed similar SA values among all harvests. For younger root segments grown later than 2 days since labeling (from r2-8d to r16-22d), the SA values increased significantly ($p<0.05$) over time of harvest and were higher in younger segments, suggesting a translocation of the ^{33}P tracer from aerial plant part to newly formed roots from labeling until the end of the experiment. Thus, the SA was not constant in either space or time.

Discussion

The simultaneous tracking and quantification of belowground C, N and P inputs and their fate in soil–plant systems require the combination of different isotope plant labeling approaches. In this study, we successfully combined a $^{13}CO_2$ single-pulse labeling with the simultaneous ^{15}N and ^{33}P cotton-wick stem feeding and for the first time quantified the distribution of these three tracers in the plant-sand system. The use of these tracers to track belowground C, N and P is based on assumptions presented in the introduction. The percolate collection experiment revealed lower IC in soluble root rhizodeposits than in the roots during 15 days since labeling (Fig. 3). Results from the root observation experiment that lasted for 22 days showed that the IC of a given root segment changed over time (Fig. 6). In addition, root segments of different ages usually had different ICs, indicating spatially inhomogeneous root labeling. These temporal and spatial differences in IC were isotope, i.e., element specific. These results are discussed below, including their implications for stoichiometric studies of plant derived belowground inputs.

Soluble rhizodeposits and roots differ in isotopic composition for C, N and P

The IC of C, N and P contained in percolates were always lower than the IC of roots for all three tracers

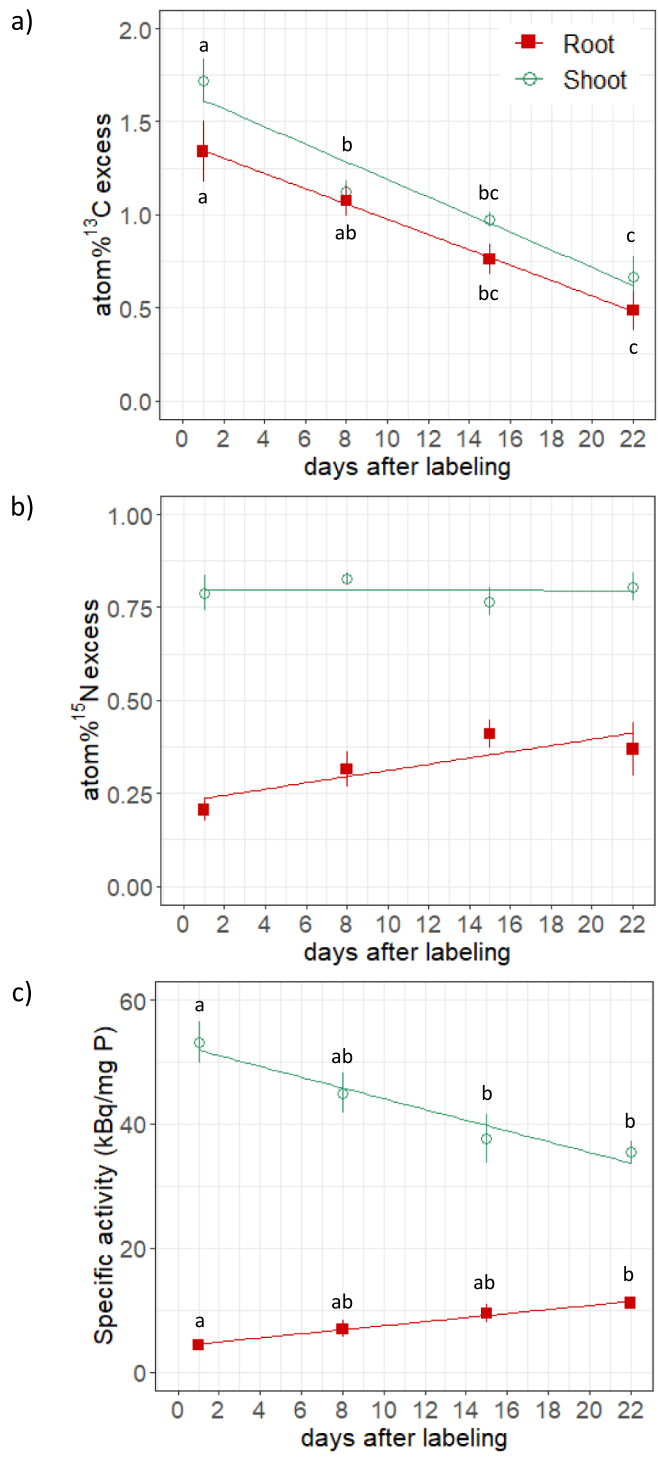


Fig. 5 Atom% ^{13}C excess (**a**), atom% ^{15}N excess (**b**) and specific activity (**c**) in shoot and roots at 1, 8, 15 and 22 days after labeling in the root observation experiment. For the root part, each dot corresponds to the weighted mean of all root segments. Different letters denote significant differences at $p < 0.05$ across time points. Error bars represent the standard error of the mean ($n = 4$)

but the magnitude varied in function of isotopes and time (Fig. 3). Similarly to the atom% ^{13}C excess of roots, the atom% ^{13}C excess in percolates was also decreasing over time suggesting a comparable dilution effect. Transplantation enabled to measure the IC of exudate produced over only two days, which

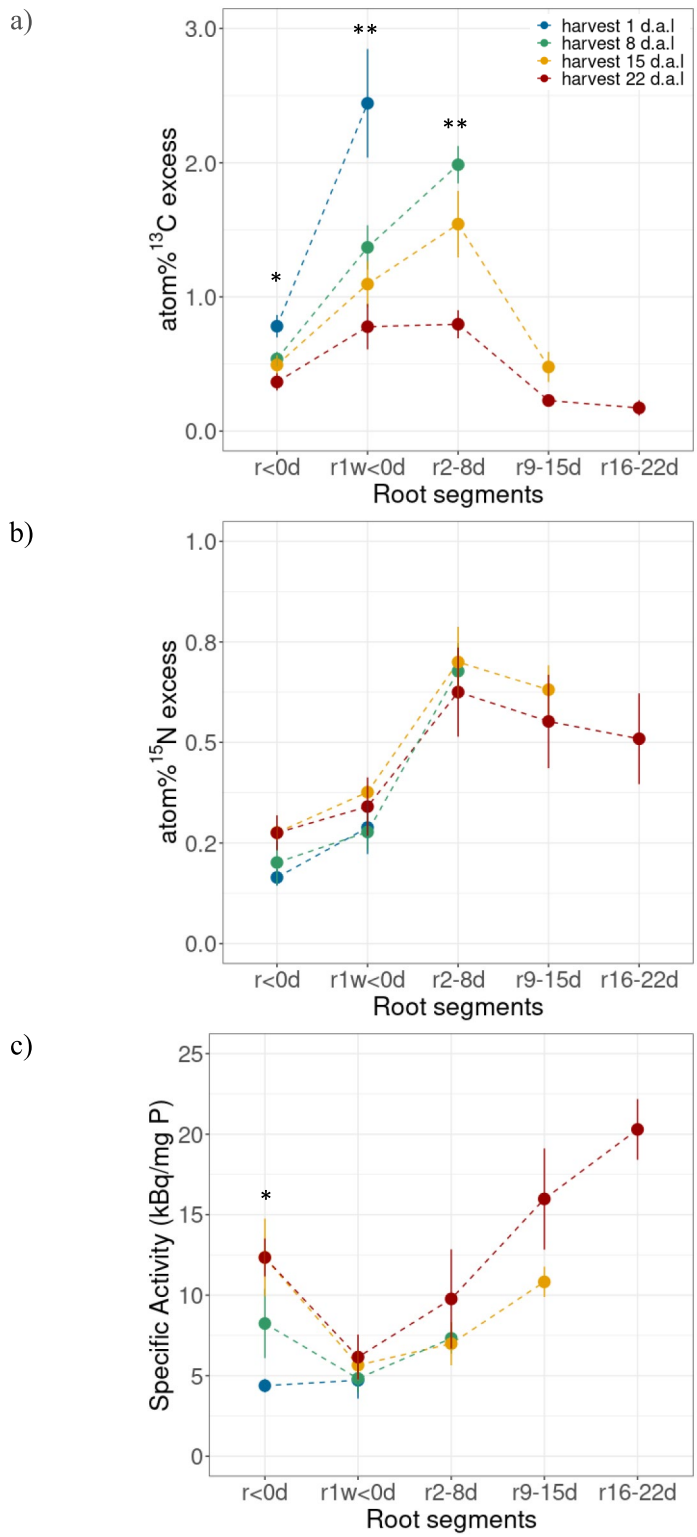


Fig. 6 Atom% ^{13}C excess (**a**), atom% ^{15}N excess (**b**) and specific activity (**c**) in sampled root segments at 1, 8, 15 and 22 days after labeling (d.a.l) in the root observation experiment. Root segments were divided in roots growing from planting to 1 week before labeling (r < 0d), 1 week before labeling (r1w < 0d), 2 to 8 d.a.l (r2-8d), 9 to 15 d.a.l (r9-15d) and 16 to 22 d.a.l (r16-22d). Error bars represent the standard error of the mean ($n = 4$) and stars indicate significance for unequal enrichment within a same root segment. Figure 6a is adapted from Stevenel et al. (2019) and is licensed by Springer Nature Customer Service Center GmbH. Figure 6a is not part of the open access content of this article

was long enough to collect sufficient volume of exudates with C, N and P concentrations above the detection and quantification level (Table S1). In the time frame of our experiment and using filtered percolates, rhizodeposition is probably largely composed of diffusates and secreted compounds such as sugars, organic and amino acids, and mucilage (Uren 2007; Farrar et al. 2003). However, transplantation provokes a stress to the plant which might affect rhizodeposition (Kerbiriou et al. 2013). While maximum care was applied to avoid root damage, we cannot fully exclude the existence of small root debris (smaller than the 2.5 µm pore size of the filters), root secretion or exudation in response to the change in the root environment. However, we assume that root turnover was a minor component of the soluble rhizodeposits collected two days after transplanting.

Damage of delicate root cells, particularly from root hairs, could have been another impact of transplanting of young plants. It could have caused some leakage of cell metabolites. As root hairs grow behind the root tip, in the maturation zone, they mostly develop in very young root segments. The IC of ^{13}C and ^{33}P of younger root segments often were higher than those from older segments (Fig. 6). Damaging them would have resulted in overestimated values of IC of soluble rhizodeposits in the percolate collection experiment, even though these values were always lower than those of the IC of roots. Similarly, damage of mycorrhizal hyphae could have also caused some bias in our estimates because these hyphae are an important sink for C assimilates of plants (Zhou et al. 2020). However, we point out that mycorrhizal networks were not considered as a component of this study. Firstly, the duration of our experiments was short for mycorrhizal networks to develop without inoculation (Thonar et al. 2011). Secondly, the sand pre-treatment condition described in Material and Methods section would have reduced the possibility of developing an effective mycorrhizal network without inoculation (Liao et al. 2003; Maitra et al. 2021).

Carbon in exudation derives mainly from C assimilated a few hours to days before (Högberg et al. 2001; Kuzyakov and Cheng 2001). This fast C transfer from shoot to soil (Domanski et al. 2001; Studer et al. 2014) may explain the relatively high ^{13}C enrichment observed in percolate C at 1 d.a.l, followed by a decrease (Fig. 3a). However, percolates were 40% less enriched than roots at 1 d.a.l and this difference significantly increased up to 80% at 15 d.a.l at low P fertilizer dose while they were about 60% constantly less enriched at high P dose (Figs. 3a and S1). This would result in underestimated C rhizodeposition, if the IC of the root would be used for calculating it. In addition to the allocation of C in metabolites, plants invest a substantial proportion of C for structural compounds which show longer residence time in the roots (Studer et al. 2014). Thus, the enrichment of assimilates in exudates declined more rapidly than in roots.

The N rhizodeposition had a lower enrichment than root N over two weeks after labeling (Fig. 3b). This is in agreement with Sierra et al. (2007), who labeled the legume *Gliricidia sepium* with a ^{15}N-leaf feeding. They also observed that root exudates were always depleted in ^{15}N relative to the roots, in spite of the longer time scale, and another root exudate collection method used by Sierra et al. (2007). Considering the slow translocation of the ^{15}N tracer from shoot to roots over time, we might expect an increase in the rhizodeposition enrichment after a longer period when more root turnover is involved. Thus, a repeated collection of rhizodeposits over the experimental period like our or Sierra et al. (2007) can be used to determine the IC of percolates and correct the bias on the rhizodeposition quantification induced by such a difference in values of IC between root and rhizodeposition.

The atom% ^{15}N excess in the percolates changed little over time. Studies using ^{15}N labelled urea for leaf-feeding of clover (Gasser et al. 2015) and soybean (Araujo et al. 2023) suggested leakage of ^{15}N through the root system, indicated by a rapid appearance in the soil of ^{15}N applied to the legume. Gasser et al. (2015) suggested that ^{15}N labelled ammonium, produced from the hydrolysis of urea, was a main carrier of leaked ^{15}N. In our experiment, we did not see a faster appearance of ^{15}N in percolates than for ^{13}C or ^{33}P, when compared to the enrichment of root system (Fig. 3). Thus, ^{15}N tracer leakage seems not to have taken place.

The ^{33}P SA values of percolates and roots differed, with lower SA of percolate P especially at low P level (Fig. 3). Because rhizodeposition in soils is quantified using the IC of the roots, its quantity would be underestimated at low P dose in short-term experiments where root debris deposition would be negligible. In contrast, under the high P fertilizer level at 1 d.a.l., the

SA of P in percolates was higher than in roots, which also contrasts with the observations following ^{15}N and ^{13}C labelling, where the enrichments were always higher in roots than percolates. This suggests a significant release of ^{33}P, agreeing with the ^{32}P root "loss" (term by Emmert 1959) observed in beans growing in hydroponic systems (Emmert 1959). This might result in an overestimation of short-term P exudation. This confirms the observations of Emmert (1959) who showed that ^{32}P root release increased exponentially with available P concentration. Loss of radioactive P from roots might, according to McLaughlin et al. (1987) and Weigl (1968), be attributed to an isotopic exchange on the cell membranes instead of exudation per se. We could also assume that the ^{33}P re-uptake by plants was limited under higher P treatment. Still, our experiment has been carried out under low to moderate P fertilizer dose, and with small N addition in order to stimulate symbiotic fixation.

Elemental isotope ratios differ more than elemental mass ratios between roots and soluble rhizodeposits

The isotopic ratios directly depend on the amounts of tracer accumulated within the plant, which in turn depend on the amounts of tracer used for labeling. Therefore, the isotope ratios do not allow quantifying rhizodeposition under the given labeling method used, but facilitate investigating the evolution of isotopic ratios over time compared to elemental ratios. This is relevant, because multi-isotope labeling is used for multi-element (stoichiometric) studies. The C:N ratios observed in roots and percolates in our study changed over time and revealed that elemental composition of soluble rhizodeposits was similar to root composition and were not affected by P levels whereas C:P ratios were impacted (Fig. 4). In contrast, ^{13}C, ^{15}N and ^{33}P stoichiometry in percolates showed different patterns. While the temporal evolution of ^{13}C:^{15}N ratios in roots was in line with the C:N ratios, plants allocated a relatively greater proportion of ^{13}C to exudates than into roots shortly after the $^{13}CO_2$ pulse labeling, resulting in high ^{13}C:^{15}N ratios in percolates, especially at low P dose. The resulting high ^{13}C:^{15}N and ^{13}C:^{33}P ratios indicated a faster incorporation of ^{13}C in soluble rhizodeposits than of ^{15}N and ^{33}P, confirming the fast C transfer from shoot to soil (Högberg et al. 2001; Kuzyakov and Cheng 2001; Studer et al. 2014). We do not know the molecular nature of the released compounds. Mucilage would be a compound with high C:N ratio, as it is mainly composed of polysaccharides and of up to 6% w/w proteins (Nguyen 2009). While the C:N ratio of amino acids ranges from 1.3 to 7.7 (mean value = 3.6), mucilage has been reported to have a C:N ratio of 64 (Nguyen 2009). However, given its low solubility, we assume that only a very low part of root mucilage was collected in our study (Grimal et al. 2001). In addition to mucilage, sugars and organic acids contain between 34 and 42% w/w of C and no N and P, greatly increasing the C:N and C:P ratios of the total rhizodeposition. Thus, the high ^{13}C proportion recovered in percolate at low P treatment suggests that canavalia plants enhanced exudation of C-compounds such as carboxylates when P fertilizer dose was severely limiting (Vance et al. 2003; Neumann and Römheld 2007). The ^{13}C pulse labeling enabled the labeling of C-rich exudates that have been released shortly after the pulse while ^{15}N and ^{33}P stem feeding highlighted a longer release process of more complex mixture of compounds, including the proportion of N-rich compounds such as amino acids and border cells and P-rich compounds such as DNA. Thus, ^{13}C:^{15}N and ^{13}C:^{33}P ratio would induce a bias on C:N and C:P ratio of rhizodeposition in short-term experiments or by harvesting plants shortly after the labeling.

Non-homogeneous labeling of the root system for C, N and P

The IC of entire root system and segments changed with time, and different temporal patterns were observed for the three isotopes (Figs. 5, 6). Data from both experiments showed a decrease with time of the atom% ^{13}C excess in shoot and roots (Figs. 3a, 5a). This is typical of the ^{13}C tracer dilution effect due to growth with new non-labeled C assimilated following an enriched-^{13}C pulse labeling (Swinnen 1994; Swinnen et al. 1994; Studer et al. 2014). As previously discussed in Stevenel et al. (2019) and in agreement with Pausch and Kuzyakov (2011), the actively growing new roots were more ^{13}C-enriched than the older ones, suggesting that ^{13}C-pulse labeling preferentially labels newly formed roots to meet the demand for C assimilates (Meharg and Killham 1988; Meharg 1994).

Entire root system enrichment variations over time as well as dissimilarities within the root system

were also observed for ^{33}P and ^{15}N tracers. We recovered less ^{33}P in the root system than Foyjunnessa et al. (2015). Indeed, we recovered 2–16% of ^{33}P tracer in roots while recoveries obtained by Foyjunnessa et al. (2015) were 17–50% and even up to 64–71% (Foyjunnessa et al. 2018). The ^{33}P distribution within the plant is probably driven by plant species, growth stage and P availability for the plants. In our study, the ^{33}P labeling has been done during the vegetative stage of Canavalia while Foyjunnessa et al. (2015, 2016, 2018) applied the tracer at early flowering stage of lupin and canola, when plants have an extensive root system with a high root-hair density (Misra et al. 1988). Additionally, we harvested plants at different time points after labeling and observed a significant increase of ^{33}P recoveries and SA in roots from 1 to 22 d.a.l with no difference in root P concentrations at all P supplies (Table 1, Fig. 2). By assuming that this increase stays steady over time, a greater recovery (> 20%) might be detected at 60 d.a.l, the time point when Foyjunnessa et al. (2018) harvested the plants. In both our experiments, the ^{33}P recoveries and the SA values increased in roots with time indicating a progressive ^{33}P translocation from shoot to roots (Figs. 2 and 5c) with a higher ^{33}P root recovery at 15 d.a.l with low P than high P fertilizer dose. Moore (1949) also observed a greater downward movement of P to the roots when the roots were growing in a P-deficient medium indicating that plants re-allocated shoot P to the root system for its development for a better soil P acquisition. The ^{33}P translocation mainly occurred to roots recently formed (i.e. until 22 d.a.l, Fig. 6c), suggesting that the cotton-wick stem feeding method provides a continuous labeling over a specific time period. This contrasts with the pulse labeling obtained with ^{13}C which also labels the root system but only for a few hours to days after labeling (Studer et al. 2014).

The translocation pattern showed different time lapse between ^{33}P and ^{15}N. Although no difference over time was detected in ^{15}N recovery in both shoot and roots in the percolate collection experiment (Fig. 2), we observed a significant increase of ^{15}N recovery in roots in the root observation experiment suggesting a continuous translocation of the tracer from shoot to roots, similarly to the ^{33}P tracer (Fig. 5). However, the shoot–root partitioning of ^{15}N (70–80% in shoots, 5–18% in roots) was consistent with previous studies (Russell and Fillery 1996; Mayer et al. 2003b; Mahieu et al. 2009) and the ^{15}N enrichment of roots did not vary significantly over time in both experiments as already observed in previous studies (Gardner et al. 2012; Gasser et al. 2015). This ^{15}N redistribution toward the root system kept the root segment specific enrichments constant to compensate for growth dilution, as also reported by Palta et al. (1991). The observed ^{15}N distribution within roots (Fig. 6b) was in agreement with the expected pattern for single pulse labeling, which generally results in a higher enrichment in roots newly formed following the labeling than older ones (Wichern et al. 2008). Our data showed an increase of the enrichment in the youngest roots over the first week after labeling (r2-8d) and a decrease until the end of the experiment. Gasser et al. (2015) observed similar pattern and attributed this time shift to slow release of ^{15}N compounds (i.e. urea and ammonium) from vacuoles of leaves and roots. Interestingly, the ^{15}N tracer seemed to be less mobile in roots than the other tracers since at a given root age, no significant change in the enrichment was detected. Results from this study indicate that the youngest root segments were more isotope enriched than older segments over the first week after labeling and even longer for ^{33}P, showing that root segments of different ages do not have the same IC.

Consequences on rhizodeposition estimates and studies using isotope labelled roots

To limit the over- or underestimation of the rhizodeposition or the root decomposition rate induced by the change in IC of the root system over time for all the tracers, temporal variation needs to be taken into account by sampling roots several times during the experimental period (not only at the end) and derive a time integrated IC of the root (Gasser et al. 2015). The rhizodeposition can then be quantified using the mean root enrichment over time, calculated as weighted mean (Chalk et al. 2014) or weighted geometric mean (Hammelehle et al. 2018). Since changes in IC in the root system are different for each element, an element-specific function is needed. Thus, a time repeated sampling of the roots is essential to integrate these changes in studies of longer duration. Still, this correction does not account for the differences between the IC of roots

and soluble percolates found in our study. Assuming that soil-based experiments often last longer than in sand, and thus root turnover may become increasingly important, this bias may lose importance with time. Nevertheless, the question on whether the IC of the roots properly reflects the IC of the source of rhizodeposition needs further testing.

The root observation experiment clearly showed that additionally to the changes in IC of root segments over time, we observed for the three tracers a marked heterogeneity in the spatial distribution of the root segments. This might also affect the rhizodeposition quantification, for instance if not the entire rooted soil is sampled, or if rhizodeposition mainly coming from a specific root segment of specific age would be collected. Root segments of different age were not located in the same areas suggesting a space-dependent change in IC (Fig. 1). Although there was space limitation in the rhizobox, the root system of canavalia has been described as a deep pivoting root system with long lateral roots (Alvarenga et al. 1995; Douxchamps et al. 2012; Kahle et al. 2012). Regarding the lower SA in older root segments, we can assume that deeper, younger root segments might be more ^{33}P-enriched than older root segments that are located in the topsoil. The same would apply for the ^{15}N enrichment, whilst the pattern for the ^{13}C enrichment would be even more dynamic across root segments of different ages and space.

Continuous labeling of plants with all three isotopes throughout their life (e.g., a split root system for ^{15}N and ^{33}P, and a constantly enriched atmosphere for ^{13}C) could alleviate the problem of non-homogenous labeling (Mahieu et al. 2007; Studer et al. 2014). For N, the use of the ^{15}N natural abundance should further be explored, since it was shown to be robust for tracking and quantifying the transfer of (mostly belowground) legume N to associated grass (Hammelehle et al. 2024).

Conclusions

The triple isotope labeling approach allowed to obtain labeled roots and simultaneously track the belowground C, N and P of a tropical legume within the plant-sand system. The translocation of tracers from the shoot to the roots resulted in a temporal and spatial variation of IC within the root system, mainly driven by the age of the root segments, with the youngest root segments being more enriched than the older ones. The IC values of soluble rhizodeposits were always lower than those of roots and varied during two weeks after labeling. The lower IC of soluble rhizodeposits from roots would lead to an underestimation of the amount of rhizodeposition, if the IC of the root were considered as the source of rhizodeposition. The ^{13}C:^{15}N and ^{13}C:^{33}P ratios differed from the C:N and C:P ratios in percolates, which was to be expected since the isotope ratios depend on the amount of isotope used for labeling. However, the change over time of the ^{13}C:^{15}N and ^{13}C:^{33}P, and the difference of these ratios between roots and rhizodeposits differed markedly from that of C:N and C:P, implying that the tracers did not reflect the stoichiometry of soluble rhizodeposits, nor of roots. This would result in an overestimation of C rhizodeposition relative to N and P rhizodeposition. The results of this study on the labeling of all three isotopes did not validate the assumptions underlying the quantification of rhizodeposition proposed by Janzen and Bruinsma (1989). To reduce the bias caused by the violation of the assumptions, repeated sampling of the root system during the experimental period and collection of rhizodeposition data to integrate these changes would be necessary, also for studies of longer duration. We recommend also to further explore the use of continuous labeling methods that follow the plants' uptake and assimilation pathways of these elements. More studies are needed to evaluate how tracer distribution in roots and rhizodeposition would be affected by growing plants in soil.

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Data availability Data will be made available on request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Agroscope, 2020. Determination of the maximun water holding capacity. Ed. Agroscope, Reckenholz. Reference Methods, version 1.2, 2020, 3 pp.

Alvarenga, R.C., Costa, L.M. da, Moura Filho, W., Regazzi, A.J., 1995. Potential of some green manure cover crops for conservation and recuperation of tropical soils. Pesqui. Agropecu. Bras. Braz.

Araujo KEC, Vergara C, dos Santos RC, Santos, W.d.M., de Freitas Souza, R., de Farias Silva, C., Guimarães, A.P., Jantalia, C.P., Urquiaga, S., Araujo, E.S., Alves, B.J.R., Boddey, R.M., (2023) Can 15N leaf-labelling reliably quantify rhizodeposited nitrogen remaining after a nodulated legume crop? Nutr Cycl Agroecosys 125:235–260. https://doi.org/10.1007/s10705-022-10238-w

Bromand S, Whalen JK, Janzen HH, Schjoerring JK, Ellert BH (2001) A pulse-labelling method to generate 13C-enriched plant materials. Plant Soil 235:253–257. https://doi.org/10.1023/A:1011922103323

Chalk PM, Peoples MB, McNeill AM, Boddey RM, Unkovich MJ, Gardener MJ, Silva CF, Chen D (2014) Methodologies for estimating nitrogen transfer between legumes and companion species in agro-ecosystems: A review of 15N-enriched techniques. Soil Biol Biochem 73:10–21. https://doi.org/10.1016/j.soilbio.2014.02.005

Chen Z, Wang L, Cardoso JA, Zhu S, Liu G, Rao I, Lin, y., (2023) Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. Front Plant Sci 14:1094157. https://doi.org/10.3389/fpls.2023.1094157

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Spectrom 25:2538–2560. https://doi.org/10.1002/rcm.5129

Cotrufo MF, Ranalli MG, Haddix ML, Six J, Lugato E (2019) Soil carbon storage informed by particulate and mineral-associated organic matter. Nat Geosci 12:989–994

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47. https://doi.org/10.1023/A:1020809400075

Domanski G, Kuzyakov Y, Siniakina SV, Stahr K (2001) Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). J Plant Nutr Soil Sci 164:381–387. https://doi.org/10.1002/1522-2624(200108)164:4%3c381::AID-JPLN381%3e3.0.CO;2-5

Douxchamps S, Frossard E, Uehlinger N, Rao I, van der Hoek R, Mena M, Schmidt A, Oberson A (2012) Identifying factors limiting biomass production in a heterogeneous on-farm environment. Journal of Agricultural Sciences 150:675–690

Douxchamps S, Humbert F-L, van der Hoek R, Mena M, Bernasconi SM, Schmidt A, Rao I, Frossard E, Oberson A (2010) Nitrogen balances in farmers fields under alternative uses of a cover crop legume: a case study from Nicaragua. Nutr Cycl Agroecosystems 88:447–462. https://doi.org/10.1007/s10705-010-9368-2

Elser J (2006) Biological stoichiometry: a chemical bridge between ecosystem ecology and evolutionary biology. The American Naturalist 168. Suppl 6:S25-35

Emmert FH (1959) Loss of Phosphorus-32 by Plant Roots after Foliar Application. 12. Plant Physiol 34:449–454

Farrar J, Hawes M, Jones D, Lindow S (2003) How Roots Control the Flux of Carbon to the Rhizosphere. Ecology 84:827–837. https://doi.org/10.1890/0012-9658(2003)084[0827:HRCTFO]2.0.CO;2

Foyjunnessa McNeill, A., Doolette, A., Mason, S., (2018) Dual-labelling (15N and 33P) provides insights into stoichiometry and release of nitrogen and phosphorus from in situ mature lupin and canola below-ground residues. Plant Soil 426:77–93. https://doi.org/10.1007/s11104-018-3621-x

Foyjunnessa McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., (2014) In situ 33P-labelling of canola and lupin to estimate total phosphorus accumulation in the root system. Plant Soil 382:291–299. https://doi.org/10.1007/s11104-014-2163-0

Foyjunnessa McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., (2015) Quantifying total phosphorus accumulation below-ground by canola and lupin plants using 33P-labelling. Plant Soil 401:39–50. https://doi.org/10.1007/s11104-015-2545-y

Foyjunnessa McNeill, A., Doolette, A., Mason, S., McLaughlin, M.J., (2016) Use of 33P to trace in situ the fate of canola below-ground phosphorus, including wheat uptake in two contrasting soils. Crop Pasture Sci 67:726–738. https://doi.org/10.1071/CP15311

Frossard E, Buchmann N, Bünemann EK, Kiba DI, Lompo F, Oberson A, Tamburini F, Traoré OYA (2016) Soil Properties and Not Inputs Control Carbon : Nitrogen : Phosphorus Ratios in Cropped Soils in the Long Term, SOIL 2:83–99. https://doi.org/10.5194/soil-2-83-2016

Fustec J, Lesuffleur F, Mahieu S, Cliquet J-B (2010) Nitrogen rhizodeposition of legumes. A Review Agron Sustain Dev 30:57–66. https://doi.org/10.1051/agro/2009003

Gardner, M., Mark Peoples, Jason Condon, Guangdi Li, Mark Conyers, Brian Dear, 2012. Evaluating the importance of a potential source of error when applying shoot 15N labelling techniques to legumes to quantify the below-ground transfer of nitrogen to other species, in: Proceedings of the 16th Australian Agronomy Conference. Armidale, NSW, Australia.

Gasser M, Hammelehle A, Oberson A, Frossard E, Mayer J (2015) Quantitative evidence of overestimated rhizodeposition using 15N leaf-labelling. Soil Biol Biochem 85:10–20. https://doi.org/10.1016/j.soilbio.2015.02.002

Grimal JY, Frossard E, Morel JL (2001) Maize root mucilage decreases adsorption of phosphate on goethite. Biol Fertil Soils 33:226–230. https://doi.org/10.1007/s003740000312

Hammelehle A, Mayer J, Lüscher A, Oberson A (2024) Nitrogen-15 natural abundance is robust to quantify nitrogen transfer from clover to grass in temporary grassland. Soil Biol Biochem 192:109290. https://doi.org/10.1016/j.soilbio.2023.109290

Hammelehle, A., Oberson, A., Lüscher, A., Mäder, P., Mayer, J., 2018. Above- and belowground nitrogen distribution of a red clover-perennial ryegrass sward along a soil nutrient availability gradient established by organic and conventional cropping systems. Plant Soil 1–19. https://doi.org/10.1007/s11104-018-3559-z

Hirte J, Leifield J, Abiven S, Oberholzer H-R, Mayer J (2018) Below ground carbon inputs to soil via root biomass and rhizodeposition of field-grown maize and wheat at harvest are independent of net primary productivity. Agric Ecosyst Environ 265:556–566. https://doi.org/10.1016/j.agee.2018.07.010

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792. https://doi.org/10.1038/35081058

Iijima M, Griffiths B, Bengough AG (2000) Sloughing of cap cells and carbon exudation from maize seedling roots in compacted sand. New Phytol 145:477–482

Janzen HH, Bruinsma Y (1989) Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoots to 15N-labelled ammonia. Soil Biol Biochem 21:189–196. https://doi.org/10.1016/0038-0717(89)90094-1

Jensen ES (1996) Rhizodeposition of N by pea and barley and its effect on soil N dynamics. Soil Biol Biochem 28:65–71. https://doi.org/10.1016/0038-0717(95)00116-6

Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480. https://doi.org/10.1111/j.1469-8137.2004.01130.x

Kahle, R., Rao, I., Cadisch, G., 2012. Response of different tropical forage legumes under stress factors of acid soil and drought. Paper presented at the Deutsche Tropentag, 19–21 September 2012, Gottingen, Germany.

Kätterer T, Bolinder MA, Andrén O, Kirchman H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agric Ecosyst Environ 141:184–192

Keel SG, Hirte J, Abiven S, Wust-Galley C, Leifield J (2017) Proper estimate of residue input as condition for understanding drivers of soil carbon dynamics. Glob Change Biol 23:4455–4456. https://doi.org/10.1111/gcb.13822

Kerbiriou PJ, Stomph TJ, van Bueren ETL, Struik PC (2013) Influence of transplant size on the above- and below-ground performance of four contrasting field-grown lettuce cultivars. Front Plant Sci 4:379

Khan WDF, Peoples MB, Herridge DF (2002) Quantifying below-ground nitrogen of legumes. Plant Soil 245:327–334. https://doi.org/10.1023/A:1020407006212

Kong, A.Y.Y., Six, J., 2010. Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems. Soil Sci. Soc. Am. J. 74, 1201. https://doi.org/10.2136/sssaj2009.0346

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1925. https://doi.org/10.1016/S0038-0717(01)00117-1

Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. Review J Plant Nutr Soil Sci 163:421–431. https://doi.org/10.1002/1522-2624(200008)163:4%3c421::AID-JPLN421%3e3.0.CO;2-R

Kuzyakov Y, Schneckenberger K (2004) Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. Arch Agron Soil Sci 50:115–132. https://doi.org/10.1080/03650340310001627658

Ledgard SF, Freney JR, Simpson JR (1985) Assessing nitrogen transfer from legumes to associated grasses - ScienceDirect. Soil Biol Biochem 17:575–577

Lemming, C., Oberson, A., Hund, A., Jensen, L.S., Magid, J., 2016. Opportunity costs for maize associated with localised application of sewage sludge derived fertilisers, as indicated by early root and phosphorus uptake responses. Plant Soil 1–17. https://doi.org/10.1007/s11104-016-2865-6

Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50–6:847–853

Lynch JP (2019) Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. New Phytol 223:548–564. https://doi.org/10.1111/nph.15738

Mahieu S, Fustec J, Faure M-L, Corre-Hellou G, Crozat Y (2007) Comparison of two 15N labelling methods for assessing nitrogen rhizodeposition of pea. Plant Soil 295:193–205. https://doi.org/10.1007/s11104-007-9275-8

Mahieu S, Fustec J, Jensen ES, Crozat Y (2009) Does labelling frequency affect N rhizodeposition assessment using the cotton-wick method? Soil Biol. Biochem 41:2236–2243. https://doi.org/10.1016/j.soilbio.2009.08.008

Maitra P, Al-Rashid J, Barman NC, Khan MMN, Mandal D, Rasul NM, Chowdhury A, El-Sappah AH, Li J (2021) Sand particle size and phosphorus amount affect Rhizophagus irregularis spore production using in vitro propagated spore as a starter inoculum in rhizosphere of maize (Zea mays) plantlets. J Fungi 7:846. https://doi.org/10.3390/jof7100846

Mayer J, Buegger F, Jensen ES, Schloter M, Heß J (2003a) Estimating N rhizodeposition of grain legumes using a 15N

in situ stem labelling method. Soil Biol Biochem 35:21–28. https://doi.org/10.1016/S0038-0717(02)00212-2

Mayer J, Buegger F, Jensen ES, Schloter M, Heß J (2003b) Residual nitrogen contribution from grain legumes to succeeding wheat and rape and related microbial process. Plant Soil 255:541–554. https://doi.org/10.1023/A:1026081015076

McLaughlin MJ, Alston AM, Martin JK (1987) Transformations and movement of P in the rhizosphere. Plant Soil 97:391–399. https://doi.org/10.1007/BF02383229

McNeill AM, Zhu C, Fillery IRP (1997) Use of in situ 15N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil–plant systems. Aust J Agric Res 48:295–304

Meharg AA (1994) A critical review of labelling techniques used to quantify rhizosphere carbon-flow. Plant Soil 166:55–62. https://doi.org/10.1007/BF02185481

Meharg AA, Killham K (1988) A comparison of carbon flow from pre-labelled and pulse-labelled plants. Plant Soil 112:225–231. https://doi.org/10.1007/BF02139999

Misra RK, Alston AM, Dexter AR (1988) Role of root hairs in phosphorus depletion from a macrostructured soil. Plant Soil 107:11–18. https://doi.org/10.1007/BF02371538

Moore RF (1949) Downward translocation of phosphorus in separated maize roots. Am J Bot 36:166–169. https://doi.org/10.2307/2437784

Nanzer S, Oberson A, Berger L, Berset E, Hermann L, Frossard E (2014) The plant availability of phosphorus from thermo-chemically treated sewage sludge ashes as studied by 33P labeling techniques. Plant Soil 377:439–456. https://doi.org/10.1007/s11104-013-1968-6

Neumann G (2007) Root Exudates and Nutrient Cycling. In: Marschner DP, Rengel PDZ (eds) Nutrient Cycling in Terrestrial Ecosystems, Soil Biology. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 123–157

Neumann, G., Römheld, V., 2007. The Release of Root Exudates as Affected by the Plant Physiological Status, in: The Rhizosphere, Books in Soils, Plants, and the Environment. CRC Press, pp. 23–72. https://doi.org/10.1201/9781420005585.ch2

Nguyen, C., 2009. Rhizodeposition of Organic C by Plant: Mechanisms and Controls, in: Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C. (Eds.), Sustainable Agriculture. Springer Netherlands, pp. 97–123. https://doi.org/10.1007/978-90-481-2666-8_9

Oburger E, Jones DL (2018) Sampling root exudates—Mission impossible? Rhizosphere 6:116–133

Palta J, Fillery I, Mathews E, Turner N (1991) Leaf Feeding of [15N]Urea for Labelling Wheat With Nitrogen. Funct Plant Biol 18:627–636

Pausch J, Kuzyakov Y (2011) Photoassimilate allocation and dynamics of hotspots in roots visualized by 14C phosphor imaging. J Plant Nutr Soil Sci 174:12–19. https://doi.org/10.1002/jpln.200900271

Racz GJ, Rennie DA, Hutcheon WL (1964) The P32 Injection Method for Studying the Root System of Wheat. Can J Soil Sci 44:100–108. https://doi.org/10.4141/cjss64-013

Rao IM, Borrero V, Ricaurte J, Garcia R, Ayarza MA (1996) Adaptive attributes of tropical forage species to acid soils II. Differences in shoot and root growth responses to varying phosphorus supply and soil type. J Plant Nutr 19(2):323–352. https://doi.org/10.1080/01904169609365125

Rasmussen J, Kusliene G, Jacobsen OS, Kuzyakov Y, Eriksen J (2013) Bicarbonate as tracer for assimilated C and homogeneity of 14C and 15N distribution in plants by alternative labeling approaches. Plant Soil 371:191–198. https://doi.org/10.1007/s11104-013-1660-x

Rasse DP, Rumpel C, Dignac M-F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil. 2005;269:341–56.

Russell C, Fillery I (1996) In situ 15N labelling of lupin below-ground biomass. Aust J Agric Res 47:1035–1046>

Sawatsky N, Soper RJ (1991) A quantitative measurement of the nitrogen loss from the root system of field peas (Pisum avense L.) grown in the soil. Soil Biol Biochem 23:255–259. https://doi.org/10.1016/0038-0717(91)90061-N

Sierra J, Daudin D, Domenach A-M, Nygren P, Desfontaines L (2007) Nitrogen transfer from a legume tree to the associated grass estimated by the isotopic signature of tree root exudates: A comparison of the 15N leaf feeding and natural 15N abundance methods. Eur J Agron 27:178–186. https://doi.org/10.1016/j.eja.2007.03.003

Somasegaran, P., Hoben, H.J., 2012. Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer Science & Business Media.

Soong JL, Reuss D, Pinney C, Boyack T, Haddix ML, Stewart CE, Cotrufo MF (2014) Design and operation of a continuous 13C and 15N labeling chamber for uniform or differential, metabolic and structural, plant isotope labeling. J vis Exp 16:1–8

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press. http://www.jstor.org/stable/j.ctt1jktrp3.

Stevenel P, Frossard E, Abiven S, Rao IM, Tamburini F, Oberson A (2019) Using a Tri-Isotope (13C, 15N, 33P) Labelling Method to Quantify Rhizodeposition. In: Reinhardt D, Sharma AK (eds) Methods in Rhizosphere Biology Research, Rhizosphere Biology. Springer Singapore, Singapore, pp 169–195. https://doi.org/10.1007/978-981-13-5767-1_10

Studer MS, Siegwolf RTW, Abiven S (2014) Carbon transfer, partitioning and residence time in the plant-soil system: a comparison of two 13CO2 labelling techniques. Biogeosciences 11:1637–1648. https://doi.org/10.5194/bg-11-1637-2014

Swinnen J (1994) Rhizodeposition and turnover of root-derived organic material in barley and wheat under conventional and integrated management. Agric. Ecosyst. Environ. Soil Ecology of Conventional and Integrated Arable Farming Systems 51:115–128. https://doi.org/10.1016/0167-8809(94)90038-8

Swinnen J, Van Veen JA, Merckx R (1994) 14C pulse-labelling of field-grown spring wheat: An evaluation of its use in rhizosphere carbon budget estimations. Soil Biol Biochem 26:161–170. https://doi.org/10.1016/0038-0717(94)90159-7

Thonar C, Schnepf A, Frossard E, Roose T, Jansa J (2011) Traits related to differences in function among three arbuscular mycorrhizal fungi. Plant Soil 339:231–245. https://doi.org/10.1007/s11104-010-0571-3

Tiessen H, Moir J (1993) Characterisation of available P by sequential extraction. In: Carter MR (ed) Soil Sampling and Methods of Analysis. CRC Press Inc, Boca Raton Florida USA, pp 75–86

Unkovich M, Pate J, Sanford P, Armstrong E (1994) Potential precision of the δ15N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. Aust J Agric Res 45:119–132

Uren N (2007) Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants, in: the Rhizosphere—biogeochemistry and organic substances at the soil–plant interface. Marcel Dekker, New York, pp 19–40. https://doi.org/10.1201/9781420005585.ch1

Van Veldhoven PP, Mannaerts GP (1987) Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem 161:45–48. https://doi.org/10.1016/0003-2697(87)90649-X

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:423–447. https://doi.org/10.1046/j.1469-8137.2003.00695.x

Weigl J (1968) Austausch-Mechanismus des Ionentransports in Pflanzen am Beispiel des Phosphat- und Chloridtransports bei Maiswurzeln. Planta 79:197–207. https://doi.org/10.1007/BF00396027

Wichern F, Eberhardt E, Mayer J, Joergensen RG, Müller T (2008) Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. Soil Biol Biochem 40:30–48. https://doi.org/10.1016/j.soilbio.2007.08.010

Zang, H.D., Yang, X.C., Feng, X.M., Qian, X., Hu, Y.G., Ren, C.Z., Zeng, Z.H., 2015. Rhizodeposition of nitrogen and carbon by mungbean (*Vigna radiata* L.) and its contribution to intercropped oats (*Avena nuda* L.). PLoS ONE, 10, e0121132.

Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, Peñuelas J, Richter A, Sardans J, Wanek W (2015) The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecol Monogr 85:133–155

Zhang, W., Liu, K., Wang, J., Shao, X., Xu, M., Li, J., Wang, X., Murphy, D.V., 2015. Relative contribution of maize and external manure amendment to soil carbon sequestration in a long-term intensive maize cropping system. Sci. Rep. 5, 10791; https://doi.org/10.1038/srep10791

Zhou J, Zang H, Loeppmann S, Gube M, Kuzyakov Y, Pausch P (2020) Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. Soil Biol Biochem 140:107641

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