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Does labelling frequency affect N rhizodeposition assessment using the cotton-wick method?

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ABSTRACT

The aim of the present study was to test and improve the reliability of the ^{15}N cotton-wick method for measuring soil N derived from plant rhizodeposition, a critical value for assessing belowground nitrogen input in field-grown legumes. The effects of the concentration of the ^{15}N labelling solution and the feeding frequency on assessment of nitrogen rhizodeposition were studied in two greenhouse experiments using the field pea (*Pisum sativum* L.). Neither the method nor the feeding frequency altered plant biomass and N partitioning, and the method appeared well adapted for assessing the belowground contribution of field-grown legumes to the soil N pool. However, nitrogen rhizodeposition assessment was strongly influenced by the feeding frequency and the concentration of labelling solution. At pod-filling and maturity, despite similar root ^{15}N enrichment, the fraction of plants' belowground nitrogen allocated to rhizodeposition in both Frisson pea and the non-nodulating isoline P2 was 20 to more than 50% higher when plants were labelled continuously than when they were labelled using fortnightly pulses. Our results suggest that when ^{15}N root enrichment was high, nitrogen rhizodeposition was overestimated only for plants that were ^{15}N-fed by fortnightly pulses, and not in plants ^{15}N-fed continuously. This phenomenon was especially observed for plants that rely on symbiotic N_2 fixation for N acquisition, and it may be linked to the concentration of the labelling solution. In conclusion, the assessment of nitrogen rhizodeposition was more reliable when plants were labelled continuously with a dilute solution of ^{15}N urea.

1. Introduction

Legume cropping is considered as an alternative to chemical nitrogen fertilisers for two reasons: (1) legumes are able to use atmospheric N_2, and (2) they provide substantial amounts of N to the soil *via* rhizodeposition. However, the amount of nitrogen released into the soil by legume crops remains difficult to assess, even though it is a critical value for: *i)* estimating the N benefit from biological N_2 fixation by a grain legume crop (Peoples et al., 1995; Rochester et al., 1998; Khan et al., 2003; McNeill and Fillery, 2008), *ii)* understanding soil N turnover (Jensen, 1996; Mayer et al., 2003), and *iii)* predicting N economies for the succeeding crops in legume-based cropping systems (Russell and Fillery, 1996b). Belowground N (root N and N rhizodeposition) inputs remain difficult to assess, as roots are difficult to collect from the soil and a significant amount of rhizodeposition is turned over and incorporated into the organic matter pool during root growth (Schmidtke, 2005a; Wichern et al., 2007). Rhizodeposits include a high diversity of compounds released by roots during growth and occur as the result of several mechanisms such as exudation (Paynel et al., 2001), secretion of mucilage, or decomposition of root materials (Lynch and Whipps, 1990; Merbach et al., 1999). Uren (2001) defined rhizodeposition as "the release of all kinds of compounds lost from living plant roots, including ions and volatile compounds".

For approximately ten years, the quantification of the N flux from rhizodeposition into the soil was improved by the use of ^{15}N labelling methods (Jensen, 1996; Khan et al., 2002b; Wichern et al., 2008; Fustec et al., in press). The general assumption has been that the average ^{15}N enrichment of the rhizodeposits matches the average ^{15}N enrichment of the roots, so the proportion of total soil N derived from rhizodeposition (*P*Ndfr) is calculated by dividing the atom $\%^{15}N$ excess in the soil at harvest by the atom $\%^{15}N$ excess of the root grown in the soil. This approach depends on the whole plant being enriched with a relatively uniform level of ^{15}N and implies that the soil of the root area has not been contaminated

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with ^{15}N tracer solution (Khan et al., 2002b). Different methods can be used to provide ^{15}N to the plant. Several ^{15}N shoot-labelling techniques originally developed for particular purposes or specific species' morphologies are suitable for measuring the amount of soil N derived from rhizodeposition in the field (Wichern et al., 2008; Fustec et al., in press). Plants are usually labelled using a concentrated solution of ^{15}N-enriched urea. Split-root labelling techniques have also been used for assessing N rhizodeposition of grain legume species (Schmidtke, 2005b; Mahieu et al., 2007). A substantial number of experiments undertaken with field peas, labelled either by the split-root or cotton-wick method, revealed that the amount of soil N derived from rhizodeposition varied from 7% to 36% of the plant N when this species was harvested at maturity. Such variability may depend on the method used. For a given plant N content, Mahieu et al. (2007) found that the amount of soil N derived from rhizodeposition was about 10% higher in pea plants labelled by the split-root method as compared to the cotton-wick method. The results of both methods suggest that the quantification of N rhizodeposition could be affected by the level of ^{15}N root enrichment. Several other experiments, which were designed to compare or refine different isotopic labelling methods, have shown that ^{15}N root enrichment can be influenced by the technique used (Khan et al., 2002a; Yasmin et al., 2006), the frequency of the ^{15}N supply (Russell and Fillery, 1996a) as well as the concentration of the labelling solution (Khan et al., 2002a). In addition to the labelling technique, numerous other factors appear to be critical for obtaining reliable results regarding nitrogen rhizodeposition of field peas. Differences in the experimental design, growing conditions, or genetic characteristics of the studied cultivars could explain the variability found in the literature (Wichern et al., 2008).

The cotton-wick method adapted for peas has provided consistent results when plants are grown either in the greenhouse or in the field (Jensen, 1996; Mayer et al., 2003; Mahieu et al., 2007). In this method, ^{15}N is fed directly into the secondary xylem by means of a wick that passes through the stem before being transported to the leaves, which are the major sink for N. The ^{15}N recovered in the roots and allocated to rhizodeposition results from translocation of N assimilated in the leaf to the roots *via* the phloem. Our aim was to optimise this method for the quantification of N rhizodeposition in pea plants with regard to: (1) the frequency of ^{15}N labelling and (2) the concentration of the labelling solution. We also tested whether plant partitioning and nodule production could influence the results.

2. Materials and methods

2.1. Labelling method

Pea plants were stem-fed with ^{15}N urea using the cotton-wick method (Russell and Fillery, 1996a; Mayer et al., 2003; Mahieu et al., 2007). The ^{15}N urea (99 atom% ^{15}N) solution was taken up from a reservoir by a wick, which was passed through a hole into the stem. The hole was made in the middle of the internode located just below the first true leaf. The wick was passed through the hole. It was protected from dessication by two silicon tubes sealed against the stem with Terostat® putty in order to prevent the loss of solution. The two protected extremities were passed through two holes into the top of the reservoir, and the labelling solution was supplied through a third hole with the aid of a syringe.

Labelled urea was supplied either by pulses or continuously. In the pulse technique, the reservoir was supplied with 0.5 ml of 0.6% ^{15}N urea solution once every two weeks. After absorption, the reservoir was washed with 1 ml of deionised water, which was absorbed by the plant. Plants labelled continuously received 3 ml of 0.2% ^{15}N urea solution supplied parsimoniously every 3–4 days during the first four weeks and 1 ml of 0.3% ^{15}N urea during the last two weeks of the ^{15}N feeding period.

2.2. Experimental design

Successive greenhouse experiments were undertaken in the spring of 2006 and 2007 in Angers (France, 47° 37′ N, 0° 39′ W). We used two pea genotypes, Frisson and its non-nodulating isoline called P2, and grew them with various levels of N fertilisation to enhance differences in plant partitioning and to modulate biological N fixation. Peas were grown in 2-l plastic pots (one plant per pot) that were filled with 2 kg of a mixture of ½ sand and ½ clayey sand. The same clayey sand (passed through a 2-mm sieve) was used for both experiments. It contained 12.2% clay, 17.5% silt, 69.4% sand, 0.85% organic matter, 0.63% total C, 3.5 mg inorganic N kg^{-1}, 46.7 mg P kg^{-1}, 215.7 mg K kg^{-1}, and 102.5 mg Mg kg^{-1}, and its pH_{H2O} was 6.2–6.6. The inner walls of the pots were lined with a plastic bag to avoid N loss *via* percolation. Lateral ramifications from the first and second nodes were cut when they reached 1 or 2 cm to avoid heterogeneity in plant structures. Each pot was inoculated with a solution of *Rhizobium leguminosarum* bv. *viciae* before the 4-leaf stage, and plants were labelled from the 6- or 7-leaf stage onward. A net of tulle was fixed around each pot to prevent the mixing of organs shed from differently treated plants. Shed organs were collected daily.

The 2006 greenhouse experiment was carried out from February to May on Frisson peas and P2, a non-nodulating isoline of Frisson. The mean temperature was 18.6 °C, and the relative humidity was around 50%. The experiment was designed in six blocks. Three levels of N fertilisation were applied (low, medium, and high); Frisson and P2 were watered daily with either an N-free, a 5 mM N, or a 15 mM N nutrient solution. All solutions contained: 0.75 mM KH_2PO_4, 0.4 mM $MgSO_4$ $7H_2O$, and 0.35 mM $CaCl_2$ $2H_2O$ with microelements (5% diluted Pokon®), and the pH was adjusted to 5.7. The N-free solution was completed with 0.3 mM K_2SO_4 and 0.2 mM KCl; the 5 mM N solution with 2.5 mM KNO_3, 1.25 mM NH_4–NO_3, 0.2 mM K_2SO_4, and 0.1 mM KCl; and the 15 mM N solution with 7.5 mM KNO_3, 3.75 mM NH_4–NO_3, and 0.15 mM K_2SO_4. The N-free treatment was integrated into the experimental design to enhance nodule activity in Frisson peas since the high level of N (15 mM) should limit nodule production and biological N fixation.

Frisson and P2 pea plants grown with the three mineral N levels were treated using the cotton-wick technique from the 7-leaf stage onward, and the labelled urea solution was applied either continuously or in fortnightly pulses. Regardless of the labelling frequency, an equal amount of ^{15}N urea was supplied to the plants during the 6-week labelling period (9 mg $plant^{-1}$). For each level of N fertilisation, control Frisson pea and P2 plants were grown without cotton-wick labelling. Six plants from each treatment and their controls were collected at maturity, when all aboveground parts had turned yellow. Six plants in the N-free treatment and their controls were also harvested at pod-filling, which was determined as the time at which one seed reached 6 mm in diameter (Ney and Turc, 1993).

In 2007, the experiment was carried out from April to July using unfertilised Frisson peas (one plant per pot, mean temperature 21.5 °C, relative humidity around 57.8%). Plants were labelled by supplying fortnightly pulses of 0.5 ml of a 0.2%, 0.4%, 0.6%, or 0.8% labelling solution (five replicates) over six weeks, and harvested at maturity.

2.3. Sampling and measurements

Plants were sorted into samples hereafter called: *i)* 'seeds', *ii)* 'pods, leaves, and stems', *iii)* 'roots', and *(iv)* 'soil'. Aboveground