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Nitrogen transfer from a legume tree to the associated grass estimated by the isotopic signature of tree root exudates: A comparison of the ^{15}N leaf feeding and natural ^{15}N abundance methods

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Abstract

Nitrogen (N) transfer from legume trees to associated crops is a key factor for the N economy of low-input agroforestry systems. In this work, we presented a new approach to estimate N transfer based on the ^{15}N content of root exudates and N released by root turnover of the donor plant (*Gliricidia sepium*) and the temporal change of the ^{15}N content of the receiver plant (*Dichanthium aristatum*). The study was carried out in greenhouse using two isotopic methods: ^{15}N leaf feeding (LF) and the natural ^{15}N abundance (NA). Measurements of exudate ^{15}N were made at several dates before and after tree pruning. A time-dependent box model was devised to quantify N transfer in time and to make comparisons between the isotopic methods. In NA, although tree roots and exudates presented a similar ^{15}N signature before tree pruning, exudates were strongly depleted in ^{15}N after pruning. In LF, exudates were always depleted in ^{15}N in relation to tree roots. Hence, the current assumption used in N transfer studies concerning the equal $^{15}N/^{14}N$ distribution in tissues of the donor plant and in its excreted N was not confirmed in our study. Before pruning, N transfer functioned as a two-N-source system (soil N and exudates N) and both isotopic methods provided similar estimates: 11–12% for LF and 10–15% for NA. Calculations performed with the model indicated that N transfer occurred with small or nil fractionation of ^{15}N in exudates. After pruning, there was a third N source associated with N released from tree root turnover. During this period, the isotopic signature of the receiver plant showed a transient state due to the progressive decrease of ^{15}N content of that N source. The amount of N derived from the tree represented 65% of the total N content of the grass at the end of the experiments.

Keywords: Agroforestry; Box model; *Gliricidia sepium*; ^{15}N fractionation; Root turnover; Tree pruning

1. Introduction

Below-ground nitrogen (N) transfer from legume trees to the associated crop can be an important though poorly quantified N source in agroforestry systems. Because of the increasing environmental and economic pressures, N transfer from legumes has received particular attention in the last two decades, mainly in crop systems such as legume-based pastures (Høgh-Jensen and Schjoerring, 2000) and annual crop associations (Chu et al., 2004). Transfer of the fixed N by the legume is a complex process involving several mechanisms: root-to-root contact, mycorrrhizal fungi, release of N in exudates and turnover in soil, root and nodule turnover, among others (Høgh-Jensen, 2006). Nevertheless, little information is available on the effect of legume management in pastures and agroforestry (e.g. cut or pruning) on the mechanisms involved in N transfer.

First isotopic techniques developed to quantify the rate of N transfer were based on dilution of soil ^{15}N (Vallis et al., 1967), but these methods may present constraints affecting the accuracy of the estimate (Høgh-Jensen and Schjoerring, 2000). Other isotopic methods were developed to overcome such constraints, and currently the leaf ^{15}N feeding proposed initially by Ledgard et al. (1985) is the most common technique used in this kind of study. Substantial information has been obtained using this method under field (Høgh-Jensen and Schjoerring, 2000) and greenhouse (Roggy et al., 2004) conditions. Compared with

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^{15}N labelling methods, only a few studies of N transfer have been carried out using natural ^{15}N abundance (Høgh-Jensen and Schjoerring, 1994; Snoeck et al., 2000; Moyer-Henry et al., 2006; Sierra and Nygren, 2006). The greatest advantage of this technique is that no ^{15}N needs to be added to the soil or the plant, and so N transfer may be investigated in long-term experiments without disturbing the soil–plant system. The natural isotopic signature of plants reflects the ^{15}N abundance in N sources and the isotope fractionation in the soil–plant system (Högberg, 1997). Thus, the accuracy of the estimates based on this method depends greatly upon a careful assessment of the rates of ^{15}N fractionation. As several pathways may be involved in N transfer, interpretation of natural ^{15}N abundance data requires the development of time-dependant models including ^{15}N fractionation throughout the soil–plant system (Högberg, 1997).

Independently of the method used to quantify N transfer, the estimates are currently based on the assumption that the isotopic signature of the transferred N is equal to that of the tissues of the donor plant (He et al., 2003). Ta et al. (1986) observed that recently fixed N was the main source of N excreted by legumes. This suggests that the isotopic signature of the transferred N may be very different from that measured in organs of leaf-labelled plants.

In this study, we developed a new approach to quantify N transfer based on the isotopic signature of exudates of the donor plant. The aims of the study were: (i) to compare the performance of two different isotopic methods (^{15}N leaf feeding and natural ^{15}N abundance) for estimating N transfer from the legume tree *Gliricidia sepium* (Jacq.) Kunth ex Walp. to the associated grass *Dichanthium aristatum* (Poir.) C.E. Hubb. in a greenhouse experiment and (ii) to assess the effect of tree pruning on N transfer and on the isotopic signature of the grass. For this, we made direct measurements of tree root exudates before and after pruning. Also, we devised a box model of ^{15}N flux in order to carry out quantitative comparisons between the two isotopic data sets.

2. Materials and methods

2.1. Design of the greenhouse experiments

The soil used in this study was a Vertisol with 80% clay. The soil was taken from a *G. sepium–D. aristatum* agroforestry plot established in 1989 in the Godet Experimental Station of the Institut National de la Recherche Agronomique in Guadeloupe (French Antilles). This plot was used in earlier work on symbiotic N_2 fixation by the tree and the transfer of the fixed N to the grass (Nygren et al., 2000; Dulormne et al., 2003; Sierra and Nygren, 2006). Some characteristics of the 0–0.2 m layer of the soil were: organic carbon 33.1 g kg^{-1}; organic N 3.1 g kg^{-1}; pH 7.7 (1:1, soil:water); cation exchange capacity 50.8 cmol$_c$ kg^{-1}. Soil was taken by removing the 0–0.2 m layer from a quadrat of 10 m^2 at 2 m from the nearest tree row. The soil was manually ground to reduce soil aggregates to less than 1 cm. Grass and tree roots were removed carefully, and the soil was put into 34 15-l pots in a greenhouse. The δ^{15}N of the sampled soil was 7.16 ± 0.15, and soil mineral N was 17 mg N kg^{-1}. Phosphorus and K fertilizers equivalent to 100 kg P ha^{-1} and 100 kg K ha^{-1} were applied to each pot and mixed with the first 10 cm of the potting soil.

A cutting of *G. sepium* was planted in each of twenty-eight pots. Six pots were kept without *G. sepium* for measuring the natural ^{15}N abundance in grass without contact with tree roots or litter. These were used as the reference grass for calculations. Twenty pots containing a tree were kept without grass and used for measuring root exudates: 12 pots for the ^{15}N leaf feeding experiment (LF) and 8 pots for the natural ^{15}N abundance experiment (NA). Grass was planted in the remaining eight pots containing a tree for measuring N transfer: four pots for the LF experiment and four pots for the NA experiment. Grass was planted fourteen weeks after *G. sepium* planting. All sampling times will be referred to the time of grass planting. At this time, swards of *D. aristatum* were gently transplanted from the field into the eight pots containing a *G. sepium*, and the six pots designed for the reference grass experiment. Five 15-cm-high grasses were planted in each pot.

Swards of *D. aristatum* used in the reference grass experiment were taken from the agroforestry plot described above at 2 m from the nearest tree row. Initial δ^{15}N value of the aboveground grass biomass was 1.62 ± 0.42 ($n = 5$). Swards of the grass to associate with *G. sepium* in the LF and the NA experiments were taken from a plot kept as pure *D. aristatum* grassland for the last 20 years, and located 150 m from the agroforestry plot. The initial δ^{15}N value of the aboveground biomass was 7.19 ± 0.24 ($n = 5$). The isotopic analyses of the sampled grass were performed separately on the shoots and a part of the root system of each plant in order to assess the ^{15}N fractionation within the grass. The aim of using grass swards of different plots was to maximize the differences between the initial value of grass δ^{15}N and the putative δ^{15}N values during each experiment.

G. sepium for the LF experiment were leaf-labelled with ^{15}N at week 12 after grass planting: 4 trees for the tree–grass association experiment and 12 trees for the measurements of root exudates. Trees were pruned by removing all the leaves and by cutting the branches to one third of their initial length at week 24 in the LF experiment and at week 25 in the NA experiment. Grass shoots were harvested by cutting at 1 cm height. In the LF and the NA experiments, grass was harvested every 4–8 weeks from week 12 to week 70 (12 sampling dates for LF and 11 for NA). For the reference grass experiment, grass was harvested every 8–12 weeks from week 17 to week 83 (7 sampling dates). This experiment was longer in order to make a number of measurements large enough to ensure a reliable estimate of the reference δ^{15}N value of the grass. A part of grass root system was sampled in the last grass sampling of each treatment. The δ^{15}N value of soil mineral N was also measured at this time using the procedure described below for the solution of root exudates. Soil moisture in the pots was maintained between 0.3 and 0.4 kg kg^{-1} (60–80% of water holding capacity; Sierra and Nygren, 2006). Daily mean temperature in the greenhouse varied from 25 to 29 °C during the experiment.