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## N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds

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**Abstract** – The nitrogen isotope  $^{15}\text{N}$  was used to quantify and determine the direction of nitrogen transfer between white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.). A micro-lysimeter system allowed the determination of the nature of the compounds exuded. During N privation, transfer was one-way from clover to ryegrass and was mainly explained by the large amounts of ammonium exuded by clover. N compound exudation by legume followed by uptake by companion grass is a highly significant pathway for inter-specific N transfer between young plants. Ammonium was the major N compound exuded. Amino acids were released in lower amounts, serine and glycine being the most abundant in leachates. In the micro-lysimeter, the amounts of ammonium, serine and glycine in the exudate were not altered by the presence of bacteria, providing evidence of the plant origin of these compounds.

**mixed culture / exudation / ammonium / amino acid**

**Résumé** – Transfert d'azote du trèfle blanc vers le ray-grass anglais, via l'exsudation de composés azotés. Le  $^{15}\text{N}$  a été utilisé pour quantifier et déterminer le sens du transfert de l'azote entre le trèfle blanc (*Trifolium repens* L.) et le ray-grass anglais (*Lolium perenne* L.). Un système de micro-lysimètre a permis de déterminer la nature des composés exudés. Pendant une privation en azote, le transfert est mono-directionnel, du trèfle vers le ray-grass, et est dû principalement à une exsudation importante d'ammonium par le trèfle. L'exsudation de composés azotés par une légumineuse, puis l'absorption par la graminée compagne constitue une voie importante du transfert interspécifique de l'azote entre jeunes plantes. L'ammonium est le composé majoritairement exsudé. Des acides aminés sont exsudés en quantités moindres, la sérine et la glycine étant les plus abondants dans les exsudats. En micro-lysimètre, les quantités d'ammonium, de sérine et de glycine dans les exsudats ne sont pas modifiées par la présence de bactéries, ce qui met en évidence l'origine végétale de ces composés.

**culture associée / exsudation / ammonium / acide aminé**

### 1. INTRODUCTION

Mixed cultures, and particularly legume-grass associations, are wide-spread in the world, and it is well known that the association of legume with grass entails an increase in grass production, compared with mono-culture of grass [9, 50]. In such grasslands systems, legume is able to fix atmospheric nitrogen, and then to transfer it to grass [1, 16, 28]. Recently, study of N transfer has received increasing interest because of environmental and economic concern and the need to decrease nitrogenous fertiliser [7, 14].

Underground N transfer between plants can occur in two ways. Either nitrogenous compounds are not transferred by

soil, they go from the roots of the “donor” plant to those of the “receiver” plant, via mycorrhizal fungi interconnecting the root systems of both plants (direct transfer), or N compounds are transferred by soil (indirect transfer). The direct way through mycorrhizal hyphae has been demonstrated for C [8], N [3, 13, 40] and P [17]. The indirect interplant transfer involves either death and decomposition of plant debris or exudation of nitrogenous compounds by the “donor” plant. Products released by either way will then be taken up by the neighbouring plant.

Most exudation studies have concerned C release. Sugars are released mainly at the root tips [23], principally as fructose and glucose [20, 24]. Organic acids, mainly citrate, malate and

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oxalate, have also been found in high amounts in root exudates [19, 22, 41]. Studies on N release from roots show that ammonium, amino acids, ureides, peptides and proteins have been identified in exudates of legumes [4, 33], grasses [21] and crucifers [45, 46, 48]. The different carbohydrates and nitrogenous compounds released in the rhizosphere play important trophic roles. They allow mobilisation of ions such as:  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ , Fe, S and Phosphate [18, 19, 21, 43], detoxification of metals: Al, Hg, Cu, Cd and Ni [19, 22, 41, 53], control of the growth of rhizospheric bacteria [44, 46] and nutrition of neighbouring plants. They can also constitute signals between plants and their environment: soil micro-flora [38], surrounding plants [43] and soil micro-fauna [31]. Conversely, environmental factors alter the release of compounds. Temperature [9], light [34], soil structure, soil insects [33], rhizospheric bacteria [32] and mycorrhiza [11] modify the quality and the amounts of exudates.

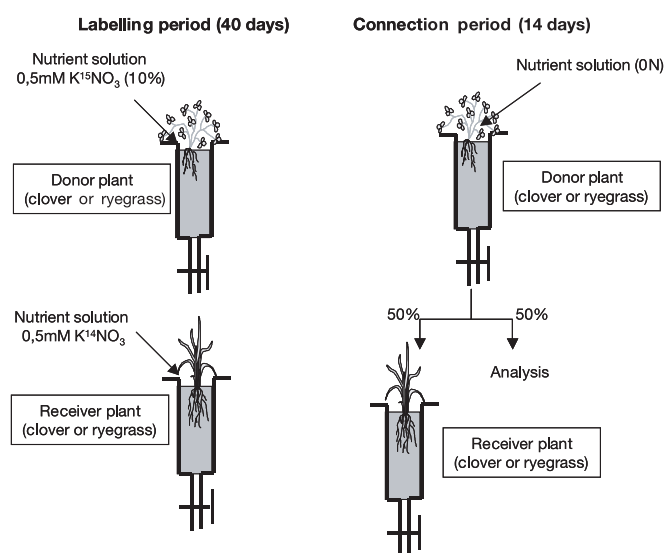
Different methods have been used to quantify transfer between  $\text{N}_2$  fixing legumes and grasses: the  $^{15}\text{N}$  natural abundance method, direct exposure to  $^{15}\text{N}_2$  [12, 51], and the  $^{15}\text{N}$  dilution method [10, 14, 27, 51]. In the  $^{15}\text{N}$  dilution method, roots are fed with  $^{15}\text{N}$ -nitrate or  $^{15}\text{N}$ -ammonium and N derived from the atmosphere is estimated by the difference between the  $^{15}\text{N}$  abundance of grasses grown in association with the legume or alone. Because of its higher sensitivity than the  $^{15}\text{N}$  natural abundance method and its lower cost than exposure to  $^{15}\text{N}_2$ , this last method was used in this study.

The aims of the present work were to determine the direction of nitrogenous transfer between white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.), to quantify it and to determine the nature of the nitrogenous compounds released by both species with micro-lysimeter experiments. The experiments were conducted with white clover and perennial ryegrass, which are major components of grassland in Europe. A complementary experiment with axenically-grown plants was designed to verify the plant origin of exuded compounds.

## 2. MATERIALS AND METHODS

### 2.1. Plant material, germination and experimental conditions

Seeds of perennial ryegrass (*Lolium perenne* L.) cv Bravo and white clover (*Trifolium repens* L.) cv Huia were surface-sterilised with sodium hypochlorite solution, rinsed 3 times and germinated in Petri-dishes containing demineralised water. Ryegrass seeds were germinated 2 weeks later than clover to achieve plants of a comparable size. Clover seedlings were inoculated with *Rhizobium trifolii* when they were transplanted. The plants were kept in a controlled environment room at 20/15 °C day/night with a photoperiod of 16 h and a PAR of  $250 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  and supplied with a nutrient solution [26] with a nitrogenous source that varied with the experiments. Two weeks after germination, one plant was transferred to one micro-lysimeter composed of a 60 mL syringe containing 100 g of sterilised sand as the growth medium. Although a micro-lysimeter system is an artificial environment for growing plants compared with pots, with a



**Figure 1.** Experimental procedure designed to allow quantification of nitrogen transferred from clover to ryegrass and from ryegrass to clover and identification of compounds released. During the labelling period, 10 mL of nutrient solution containing 0.5 mM  $\text{K}^{15}\text{NO}_3$  (10%) was supplied three times a week to donor plants and 10 mL of nutrient solution containing 0.5 mM  $\text{K}^{14}\text{NO}_3$  was supplied three times a week to receiver plants. During the connection period, donor plants were fed with 10 mL of N-free nutrient solution three times a week and receiver plants were fed with one-half of the exudate from donor plants, while the other half was analysed for nitrate and ammonium content.

relatively low volume of sand for root development, the volume was not limiting for the growth of roots and shoots of clover and ryegrass for up to 11 weeks [36]. Two different micro-lysimeter experiments are shown in this paper.

### Experiment 1

In order to study the direction of N transfer, clover and ryegrass plants were grown in independent micro-lysimeters with 0.5 mM of  $\text{K}^{14}\text{NO}_3$  or  $\text{K}^{15}\text{NO}_3$  10 atom % (Fig. 1). When plants were 40 days old, the sand of the micro-lysimeter was abundantly rinsed with demineralised water. For 2 weeks, one “donor” plant (clover or ryegrass, the plant grown on  $^{15}\text{N}$ ) was connected with one “receiver” plant (the corresponding species, the plant grown on  $^{14}\text{N}$ ). Ten mL of N-free nutrient solution was added to the “donor” plant three times per week. Then, the direction of the N transfer was studied during N privation of the nutrient solution lasting 2 weeks, the only N available for receiver plants being the N compounds exuded by donor plants and N from fixation. Half of the leachate containing nutrient solution and exudates from the “donor” plant went to the “receiver” plant and half was collected for analysis (Fig. 1). In order to take into account the possible contamination by  $^{15}\text{N}$  left in the sand in spite of rinsing, 10 micro-lysimeters of each species were rinsed and the plants removed from the sand. These control micro-lysimeters were connected as described previously. The absence of  $^{15}\text{N}$  in receiver plants showed that rinsing was effective. After

2 weeks of connection, plants were harvested, separated into roots and shoots, freeze-dried, weighed, and total N and  $^{15}\text{N}$  were analysed.

### Experiment 2

To study the effect of micro-organisms on exudation, three levels of sterility were compared. All seeds were surface-sterilised by soaking them for 30 s in 95%  $\text{H}_2\text{SO}_4$ , 5 min in 95% ethanol and 30 min in 10%  $\text{H}_2\text{O}_2$  and then rinsed with sterile demineralised water. After 2 weeks of germination, plants were supplied with a nutrient solution containing 0.5 mM  $\text{KNO}_3$ . Three culture conditions were compared:

- 1- the micro-lysimeters, sand and nutrient solution were sterilised. The root system was maintained under sterile conditions, isolated from the atmosphere with cotton [2] and the nutrient solution was added through a tube across the cotton (noted "sterile" in the results);
- 2- the same as 1, but roots were not protected with cotton (noted "semi-sterile" in the results);
- 3- nothing, except seeds and sand were sterilised (noted "non-sterile" in the results).

An aliquot of the exudates was used to count the bacterial population present in the micro-lysimeters by culturing them in a nutrient agar 1.5% (Sigma) plate. No bacteria were found in the sterile exudate, while the number of Colony Forming Units reached  $10^4$  and  $10^5 \text{ mL}^{-1}$  exudate for the semi-sterile and non-sterile treatments, respectively. When the ryegrass and clover were 39 and 67 days old, respectively, the exudates were collected and analysed.

## 2.2. Analysis

### 2.2.1. Calculation of N transfer

Total N and  $^{15}\text{N}$  were determined by a Carlo Erba N analyser linked to an Isotope Ratio Mass Spectrometer (IRMS, Europa Scientific, Crewe, UK). The  $^{15}\text{N}$  dilution technique allows determination of the percentage of N in the plant parts derived from the atmosphere (% Ndfa) [30]:

$$\%Ndfa = 1 - \frac{\%^{15}\text{N atom excess in clover}}{\%^{15}\text{N atom excess in grass alone}} \times 100 \quad (1)$$

The percentage (% NT) and amounts of N in the grass originating from clover was calculated as described by Ta et al. [51]:

$$\%NT = \frac{\%^{15}\text{N grass in mixture} - \%^{15}\text{N grass alone}}{\%^{15}\text{N clover} - \%^{15}\text{N grass alone}} \times 100 \quad (2)$$

### 2.2.2. Amino acids

The exudates were filtered through a 0.45  $\mu\text{m}$  filter. The amino acids were quantified by HPLC as *o*-phthaldialdehyde derivatives on a C-18 column using the Gold System 8.0

(Beckman Instruments, San Ramon, Ca, USA) as described by Murray et al. [33].

### 2.2.3. Ammonium and nitrate

Ammonium and nitrate of the exudates were measured using a continuous-flow analyser, after filtration through a 0.45  $\mu\text{m}$  filter (Bran + Luebbe, Norderstedt, Germany). The buffered solution containing ammonium was reacted with salicylate 8% (w/v) and dichloro-isocyanurate 0.18% (w/v). Nitrate was measured by reduction to nitrite on a column of cadmium at pH 8. Nitrite reacted with sulfanilamide, N-1-naphthylethylene-diamine dihydrochloride.

## 2.3. Statistical analysis

Levels of significance of differences between different treatments were determined by using the *T*-test of the analysis of variance and standard errors are shown on the graphs. Only significant differences ( $P < 0.05$ ) are discussed.

## 3. RESULTS

In order to determine the direction of N transfer during N privation, clover and ryegrass plants were grown in micro-lysimeters for 40 days with 0.5 mM  $\text{K}^{15}\text{NO}_3$  10 atom % in the nutrient solution ("donor" plants) or with 0.5 mM  $\text{KNO}_3$  at the natural abundance ("receiver" plants). "Receiver" plants were subsequently grown and fed with exudates from "donor" plants for 2 weeks.

Before the N privation, the N contents (% of the dry matter) (Tab. I) were 3.9 and 3.6% for "donor" clover and ryegrass, respectively. These contents decreased strongly for the grass during the first and the second week, and slightly for the legume. Expressed as absolute amounts (Tab. I), the legume was much richer than the grass before N privation ( $J_0$ ) (858  $\mu\text{g}$  of N per plant for clover against 254  $\mu\text{g}$  for ryegrass). After 7 and 14 days of N privation of the nutrient solution, the amount of N in the "donor" ryegrass was not modified, while that of clover increased strongly (from 860  $\mu\text{g}$  to 2.95 mg of N per plant after 14 days of N privation). The  $^{15}\text{N}$  abundance of clover N was already lower than 10% ( $^{15}\text{N}$  abundance of N of the nutrient solution) before the experiment and decreased throughout the privation (Tab. I). That of "donor" ryegrass was stable during the course of the experiment (close to 7.5%). Before the N privation ( $J_0$ ), the percentage of clover N coming from the atmosphere was 55.5% (Tab. II). This percentage increased throughout the privation and reached more than 90% after 2 weeks, while the amount of N coming from the atmosphere increased by more than five times.

The results show that transfer, in the case of N privation of the nutrient solution, was one-directional; from clover to ryegrass (Fig. 2). No nitrogen originating from ryegrass was detected in clover, while  $2.5 \pm 0.7 \mu\text{g}$  N were transferred daily from clover to ryegrass. The amounts were well distributed in the whole plant: 1.3  $\mu\text{g}$  in roots and 1.2  $\mu\text{g}$  in shoots. They represented 4.0% and 6.6% of the total N of the roots and shoots of ryegrass, respectively.

**Table I.** Nitrogen content (expressed as percent of dry matter) and nitrogen amounts (expressed as mg per plant) of “donor” clover and ryegrass and  $^{15}\text{N}$  abundance of “donor” clover and ryegrass nitrogen (experiment 1), just before the N privation of the nutrient solution ( $J_0$ ), 7 days ( $J_7$ ) and 14 days after the N privation ( $J_{14}$ ). ( $\pm$  ES,  $n = 5$ ).

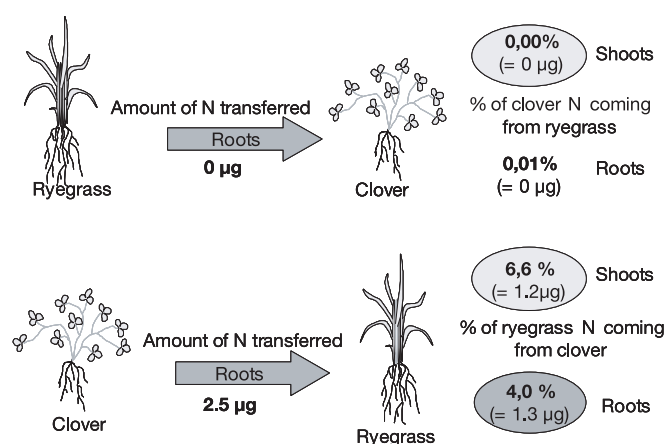
	“donor” clover		“donor” ryegrass	
	N content (% of DM) N amounts (mg·plant <sup>-1</sup> )	$^{15}\text{N}$ abundance of N	N content (% of DM) N amounts (mg·plant <sup>-1</sup> )	$^{15}\text{N}$ abundance of N
$J_0$	3.85% $\pm$ 0.27 (0.858 mg $\pm$ 0.082)	3.60% $\pm$ 0.35	2.08% $\pm$ 0.18 (0.254 mg $\pm$ 0.024)	7.35% $\pm$ 0.15
$J_7$	3.67% $\pm$ 0.20 (1.24 mg $\pm$ 0.18)	1.71% $\pm$ 0.19	1.14% $\pm$ 0.04 (0.228 mg $\pm$ 0.016)	7.82% $\pm$ 0.07
$J_{14}$	3.40% $\pm$ 0.08 (2.95 mg $\pm$ 0.41)	0.85% $\pm$ 0.04	0.87% $\pm$ 0.04 (0.230 mg $\pm$ 0.010)	7.33% $\pm$ 0.06

**Table II.** Nitrogen fixation by the “donor” clover grown in micro-simulator (experiment 1), just before N privation of the nutrient solution ( $J_0$ ), 7 days ( $J_7$ ) and 14 days after the N privation ( $J_{14}$ ). Results are expressed as percentage of the clover nitrogen which comes from atmosphere and in amounts of clover nitrogen coming from atmosphere (in mg per plant). ( $\pm$  ES,  $n = 5$ ).

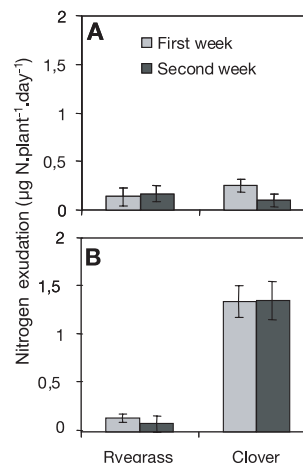
Nitrogen fixation by the “donor” clover	
$J_0$	55.5% $\pm$ 4.8 (0.488 mg $\pm$ 0.077)
$J_7$	82.2% $\pm$ 2.5 (1.03 mg $\pm$ 0.17)
$J_{14}$	93.2% $\pm$ 0.6 (2.75 mg $\pm$ 0.38)

When the exudates were analysed for nitrate and ammonium content (Fig. 3), in the case of N privation, nitrate was scarcely exuded by both species: around 163 ng nitrate  $\text{N}\cdot\text{plant}^{-1}\cdot\text{day}^{-1}$ . In contrast, large amounts of ammonium N were exuded from the legume (1.36  $\mu\text{g}\cdot\text{plant}^{-1}\cdot\text{day}^{-1}$ ). The mean of  $\text{NH}_4^+$  exudation by ryegrass was much lower (Fig. 3).

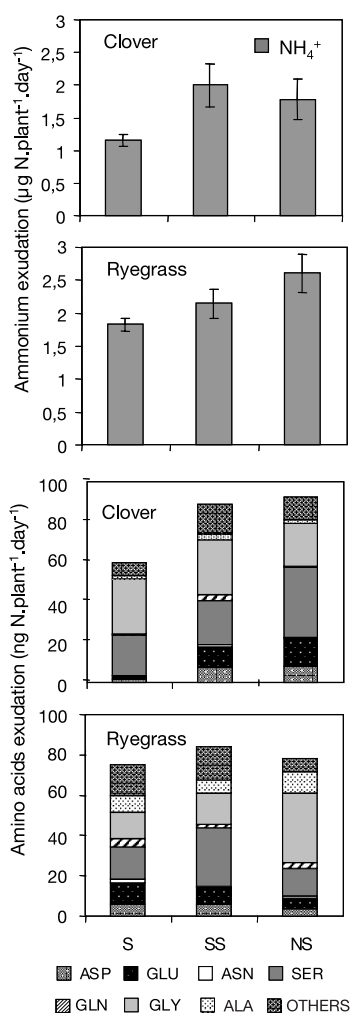
Figure 4 shows the comparison of ammonium and amino acid composition of the exudates of both species grown in 3 culture conditions: sterile, semi-sterile and non-sterile (cf. Sect. 2), with 0.5 mM nitrate in the nutrient solution. The amount of ammonium recovered in clover exudates and ryegrass exudates was found to be independent of the sterilisation procedure. Compared with experiment 1 this amount was of the same order of magnitude (1.65  $\mu\text{g}$  ammonium  $\text{N}\cdot\text{plant}^{-1}\cdot\text{day}^{-1}$ ) for clover while the amount of ammonium exuded from ryegrass was much higher in experiment 2 than in experiment 1 (2.19  $\mu\text{g}$  ammonium  $\text{N}\cdot\text{plant}^{-1}\cdot\text{day}^{-1}$ ). This can be explained by the presence of 0.5 mM nitrate in the nutrient solution in the second experiment, while no N was added to the nutrient solution when the exudates were harvested in the first experiment. No significant differences were observed between the 3 treatments concerning the amounts and profiles of amino acids exuded from ryegrass, but a significant difference was found for clover. More amino acids were recovered in exudates obtained from clover grown in semi- and non-sterile conditions, compared with clover grown in sterile conditions (around 91 ng amino  $\text{N}\cdot\text{plant}^{-1}\cdot\text{day}^{-1}$  against 60). This difference was due to higher amounts of



**Figure 2.** Quantification of N transfer from ryegrass to clover (top of figure) and from clover to ryegrass (bottom of figure), expressed as amounts of N transferred daily per plant and as the N proportion of the “receiver” plant (roots and shoots) coming from the “donor” plant, during 2 weeks of connection while no N was added to the nutrient solution ( $n = 5$ ).



**Figure 3.** Exudation of nitrate (A) and ammonium (B) by clover and ryegrass, expressed as  $\mu\text{g}$  per plant per day, during the first and the second week of N privation. Exudates were collected three times a week, and each point is the average of 5 independent plants.



**Figure 4.** Comparison of ammonium and amino acid composition of exudate of white clover and ryegrass in 3 conditions of culture (S: Sterile; SS: Semi-sterile; NS: Non-sterile). Exudates were analysed from 67-day-old clover and 39-day-old ryegrass. ( $\pm$  se,  $n = 5$ ).

GLU and ASP in non-axenic conditions (semi-sterile and non-sterile). In addition, GLY and SER were the most abundant amino acids in root exudates of both species. They represented around 50% of ryegrass exudates and 65% of clover exudates.

#### 4. DISCUSSION

In the first micro-lysimeter experiment, designed to determine if nitrogenous transfer between white clover and perennial ryegrass was one-way or in both directions, the “donor” plants were fed with  $^{15}\text{N}$  and the “receiver” plants were fed with  $^{14}\text{N}$  for 40 days of pre-treatment. Our results show that transfer occurred only from clover to ryegrass during the course of the experiment, when no N was added to the nutrient solution. This is in contrast with previous data concerning short-term and long-term bi-directional transfer

between legumes and grasses [52]. This difference could be due to the species studied (alfalfa and bromegrass) or the nature of connection (a pot where the roots of the plants are allowed to co-exist and, in this study, a micro-lysimeter which removes direct contact between root systems). Moreover, clover contained more N than ryegrass in our experiment, and Bethlenfalway et al. [3] have shown, in a soybean-maize mixture, that nitrogenous transfer from soybean to maize is governed by a source-sink relationship. This could explain the net transfer from clover to ryegrass obtained in a mixture [7, 28]. The direction of the N transfer could be due to this greater difference in N content between both species during N privation. In the present experiment, N privation increased the difference in N content (% of N content and amounts of N). While the amount of N per plant of “donor” ryegrass did not vary during N privation, that of clover increased, because this legume could use atmospheric nitrogen. The proportion of N originating from fixation increased in clover from 55% before N privation to 93% at the end of the two weeks. This confirms previous data obtained with low N concentration in soil [14, 54]. As recently-fixed nitrogen may be the nitrogen preferentially transferred from a legume to the neighbouring plant [50], N privation, increasing fixation, may increase nitrogenous transfer from clover to ryegrass.

The nitrogenous transfer in this micro-lysimeter system occurred only in the indirect way: release of nitrogenous compounds by clover followed by uptake by ryegrass. The very low nitrate exudation of clover and ryegrass roots confirms previous results concerning alfalfa [49]. However, Macduff and Jackson [29] showed that nitrate exudation was proportional to the difference in nitrate concentration between sap and the soil solution. In the present study, the  $\text{NO}_3^-$  concentration of the soil solution being near zero should facilitate  $\text{NO}_3^-$  exudation. This suggests that nitrate content was low in the roots and was reduced to form organic nitrogenous compounds.

Ammonium exudation by grass during N privation was very low, while that of legume was high (nearly 100 times more). This was probably due to the absence of N in the nutrient solution. When ryegrass was fed with nitrate in the second experiment, ammonium was found in the exudate; this suggests that the exudation of this ion came from nitrate reduction in the grass. Clover is able to reduce  $\text{N}_2$  to ammonium that may be released. As exudation of  $\text{NH}_4^+$  is a passive efflux [35], ammonium release followed a concentration gradient between the cytoplasmic pool and the soil solution [4, 21]. This may explain the differential ammonium exudation between ryegrass and clover in this experiment. Consequently, during nitrogen privation, the one-way N transfer from clover to ryegrass was partially explained by a high ammonium exudation by clover. However, the mono-directional transfer observed here could also be due to the absence of N in the nutrient solution increasing the N difference between the legume and grass. Transfer of nitrogenous compounds may occur in both directions [51], the way being favoured by the relation of the N source-sink, varying according to the environment, development stage and growth of both species. During 2 weeks of N privation,  $7.35 \mu\text{g}$  of N per week were transferred to the ryegrass grown in a micro-lysimeter. In a similar micro-lysimeter experiment with a nutrient

solution containing 0.5 mM of KNO<sub>3</sub>, 12.5 µg of N per plant per week were transferred from clover to ryegrass [37]. These results are in agreement with an increase in N transfer with the N concentration of the nutrient solution, which was observed by Paynel et al. [37] and by Høgh-Jensen and Schjoerring [15]. This stimulation of N transfer by N fertilisation could be due to an increase in N exudation by clover and/or a stimulation of uptake by ryegrass. Further studies on the effect of the N concentration of the nutrient solution in a micro-lysimeter system are needed to test these hypotheses.

In a pot, roots are allowed to have direct contact, while in a micro-lysimeter system, direct contact between the root systems of both species is not possible. Release of nitrogen by a legume implies the death and decay of nodules and roots [25] or exudation of N compounds from the legume's roots [49]. The micro-lysimeter system was designed to measure these nitrogenous compounds. Death of legume organs is unlikely to be significant in the present study concerning young plants. Moreover, an experiment including the filtration or not of exudates gave similar results (data not shown).

The higher ammonium exudation by both clover and ryegrass, compared with amino acids, confirms previous results with soybean [34] and alfalfa [4]. These results were low compared with measurements from hydroponically-grown alfalfa [49], soybean [34], forage rape [45] and maize [21], but at the same level as those of plants grown in sand: soybean and alfalfa [4]. The difference observed between plants grown either in hydroponic conditions, or in a matrix (sand, for example), could be due to differences in the experimental procedures.

Exudation of N compounds is altered by the chemical [46, 47], physical and biotic environment [24, 32]. Rhizosphere micro-flora can modify exudation. Indeed, these organisms can constitute a sink for N compounds coming from root exudation and increase root release [32]. In this context, a complementary experiment in a micro-lysimeter was designed, to study the plant or bacterial origin of N compounds found in the exudates. Three conditions of growth were compared (cf. Sect. 2). The amounts of ammonium found in the bathing solution did not vary significantly with the presence of bacteria. This shows that the NH<sub>4</sub><sup>+</sup> found in the exudates really originated from the plants. Nevertheless, the same results obtained in the 3 treatments do not show conclusively that bacteria are not involved. This could be explained by a balance between ammonium release by plants and consumption of soil ammonium by bacteria, leading to the same ammonium concentration in the soil. Comparison of total amino acids found in the exudates of ryegrass showed no difference between treatments, while less amino acids were found in the sterile exudates of clover compared with both of the non-axenic treatments. This difference was explained by a significant presence of ASP and GLU only in semi- and non-sterile clover exudates. These higher amounts in the presence of bacteria suggest subsequent microbial transformations of exudates in both non-axenic treatments [32]. Moreover, the comparison of the rate of amino acid release by maize (Rr) and the consumption rate by rhizosphere micro-organisms (Rc) shows the highest Rc/Rr for ASN and ARG. This suggests a degree of selectivity for N-rich amino acids by micro-organisms [45, 46].

SER and GLY were the major amino acids found in the exudates. They constituted more than 50% of the total amino acids released. SER and GLY were also shown to be the major amino acids released by sterile forage rape [46] and sterile alfalfa [39], while steppes grasses released mainly ARG, SER and CYS [24]. The difference between the profiles of the amino acids of roots (ASN and GLN are the major amino acids of clover and ryegrass, respectively) and the exudates supports the observation by Ta et al. [49] and Ofusu-Budu et al. [33] on selective exudation by root systems.

The different results obtained in this study show that short-term inter-specific N transfer (with young plants) can occur between young plants through the soil solution and implies exudation of N compounds by the donor plant (legume) and uptake by the receiver plant (grass). While the mechanisms of N uptake have been studied [5, 35], the mechanisms of N exudation have been little documented. Moreover, most of the exuded compounds are recaptured by the plant [6, 21]. In order to separate exudation (efflux) and uptake (influx), more research is needed to study net exudation.

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