Review article

Nitrogen rhizodeposition of legumes. A review

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(Accepted 22 January 2009)

Abstract – Because nitrogen is one of the major elements limiting growth of plants in agrosystems, large amounts of N fertilisers have been used in the second half of the twentieth century. Chemical fertilisers have contributed to increasing crop yields and food supply, but they have induced environmental damage such as nitrate pollution and wasting fossil fuel. The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input agrosystems. Here we review agricultural practices, measurement methods and biological pathways involved in N cycling. We show that plant roots interact intimately with soil microflora to convert the most abundant but relatively inert form of N, atmospheric N2, into biological substrates available for growth of other plants, through two consecutive processes; namely, N2 fixation and N rhizodeposition. In intercropping, companion plants benefit from biological fixation by legumes and subsequent transfer of N from legumes to non-legumes. This transfer from legumes to the release of N compounds by legume roots, a process named rhizodeposition, then the uptake by the companion crop. The two main rhizodeposition pathways are (i) decomposition and decay of nodules and root cells, and (ii) exudation of soluble N compounds by plant roots. The contribution of root N and rhizodeposited N to the soil-N pool is difficult to measure, particularly in the field. Firstly, root N is often underestimated because root recovery is problematic. Second, assessment of N rhizodeposition is challenging. Several 15N labelling methods have been performed for different legume species. Rhizodeposition of N, as a percentage of total plant N, varied from 4 to 71%. The high variability of the results illustrates the need for more studies of the environmental and genetic factors influencing the amount of N rhizodeposits released by legumes under field conditions.

N rhizodeposition / legumes / N2 fixation / 15 N / isotopic methods / root exudates / ecological fertilisation

1. INTRODUCTION

Even though N is among the most abundant elements on earth, it is also the major element limiting growth of plants in many agricultural systems because of its unavailability for plants (Hartwig, 1998; Vance, 2001). N fertilisers have been considered for many years as a reasonable insurance against yield loss and have been used extensively (Vance, 2001) but contribute substantially to environmental pollution (Deutsch et al., 2006; Umar and Iqbal, 2007). It is now established that excessive use of these fertilisers affects the balance of the nitrogen cycle in soils, causes eutrophication because of nitrate leaching and has contributed to global warming because of gaseous loss as N2O. The non-stop use of N fertilisers would also accelerate the depletion of stocks of non-renewable energy resources required for fertiliser production. Furthermore, there are vast areas in the developing world where N fertilisers are neither available nor affordable due to weak infrastructure, poor transportation and high cost.

These problems explain why biological alternatives using diazotrophic prokariots have become of increasing interest in agricultural practices in the last few years, particularly for low-input systems. Biological N fixation can act as a sustainable source of N and can complement or replace fertiliser inputs (Garg and Geetanjali, 2007). The two main agricultural practices to benefit from biological N fixation, crop rotation and intercropping legumes (Fabaceae), and non-fixing plants, were practised in ancient times, even if the basis for the benefit derived was not understood (Burris, 1974). Most of the fixed N in legumes is harvested and fed to animals, but evidence from a number of experiments using different methodologies indicates that legumes can deposit significant amounts of N in the soil during growth (Jensen, 1996a, b; McNeill et al., 1998; Khan et al., 2002a; Mahieu et al., 2007; Wichern et al., 2007a, b). Fixed N can also be transferred to intercropped non-legumes in the case of mixed cropping systems, or to following crops in the case of crop rotation.

In addition to the use of legumes in agriculture, other technologies to take advantage of N2-fixing micro-organisms include the utilisation of the symbiosis between the fern Azolla...
2. BIOLOGICAL N₂ FIXATION OF LEGUMES

2.1. Processes

Nitrogen fixation is carried out by a small number of diazotrophic prokaryotic microorganisms, belonging to a wide range of eubacteria and archaea bacteria. Diazotrophs are usually divided into free-living and symbiotic forms, though some cyanobacteria are able to fix N either independently or in symbiotic association. Symbiotic diazotrophs include a number of genera of the Rhizobiaceae, which form the well-documented symbioses with legumes (Gordon et al., 2001; Garg and Geetanjali, 2007), where nitrogen fixation takes place in specialised organs, the nodules. Most of these nodules are formed on legume roots but some rhizobia such as *Azorhizobium caulinodans* are able to form stem nodules. Symbiotic N₂ fixation in legumes is the result of a structurally and physiologically highly organised, host-specific mutualistic interaction between rhizobia and legumes. Biological N fixation is catalysed by an anaerobic enzyme, nitrogenase, which carries a complex metallocluster on its active site. The most abundant nitrogenase contains iron and molybdenum at this position but others contain iron and vanadium, or iron only when abundant nitrogenase contains iron and molybdenum at this position. Because nitrogenase is inhibited upon exposure to oxygen, nitrogen-fixing organisms have certain adaptations. In the case of the legume—Rhizobium symbiosis, the two main adaptations are the formation of the oxygen diffusion barrier into the nodule and the synthesis of the oxygen carrier protein in the symbiosome, leghaemoglobin (Gordon et al., 2001). A number of other non-legume plants, mainly woody species, also produce N₂-fixing nodules, in symbiosis with the actinomycete, *Frankia* (Uselman et al., 1999).

2.2. Benefits and use of legumes in agrosystems

Because of their ability to fix N₂, legumes are considered to be involved in ecological facilitation processes in all ecosystems (Loreau and Hector, 2001; Rochon et al., 2004; Padilla and Pugnaire, 2006). A wide range of legumes are grown around the world, for production of protein-rich seeds or for harvest of the whole shoot. In agrosystems, legumes contribute nitrogen benefits in two main ways:

(i) Legumes are N-rich plants which can be used in crop rotations to increase the soil-N pool (Chalk, 1998). For this purpose, several legume species such as clovers (*Trifolium* sp.), alfalfa and vetches (*Vicia sativa* L. and other *Vicia* genera), fenugreek (*Trigonella foenum-graecum* L.), lupin (*Lupinus angustifolius* L.), velvet bean (*Mucunia pruriens* Bak.), *Crotalaria spectabilis* Roth., or *Sesbania rostrata* Brem. are included in rotations and used as green manure. They contribute to nutrient cycling, soil organic matter conservation, and to the nutrient supply for succeeding crops. However, numerous legumes included in rotations are grain legumes. They are grown worldwide and Crépon (2006) reported production of $241 \times 10^6$ t of dry matter in the 2003/2004 season. Soybean (*Glycine max* L.) is mainly produced in North and South America and in Asia. Pea (*Pisum sativum* L.), fababean (*Vicia faba ssp minor* L.) and dry bean (*Phaseolus vulgaris* L.) are mainly produced for feed in the northern hemisphere, since in the southern hemisphere, the most common grain legumes are mainly grown for food and are dry bean, chickpea (*Cicer arietinum* L.) and cowpea (*Vigna unguiculata* L.). Lentil (*Lens esculenta* L.), pigeon pea (*Cajanus cajan* L.) and peanut (*Arachis hypogea* L.) are also commonly used for human food. Nitrogen harvest indices of grain legumes such as soybean, pea, fababean or lupin are often high; for instance, N accumulated in the seeds may represent more than 85% of plant N for soybean (Toomsan et al., 1995), and more than 75% for pea plants (Mahieu et al., 2007).

Since roots and rhizodeposits are so rich in N, including a grain legume in rotations may lead to a positive N-preceding effect on the following crop, despite N losses due to harvest. Hence, compared with a cereal grown in the same conditions, greater levels of inorganic N are recorded after harvesting grain legumes, especially in deeper soil layers (Crozat and Fustec, 2006). However, soil inorganic N measurements do not take into account changes in the organic N pool.

(ii) Legumes grown simultaneously and in the same field as non-fixing species (intercropping) lead to a more efficient use of soil resources in time and space (Loreau and Hector, 2001; Hauggaard-Nielsen and Jensen, 2005; Corre-Hellou et al., 2006). The niche separation effect often results in a higher yield in an intercrop than in a sole crop for the non-fixing species. In mixtures with non-fixing plants, N₂ fixation by legumes is higher than in a monoculture regardless of management or location (Carlsson and Huss-Danell, 2003; Corre-Hellou et al., 2006). Experiments undertaken using mixtures of annual crops (for instance, pea-barley intercropping) have shown that this effect is higher in low-input systems than in others, and leads to more stable yields in problematic environments (Jensen, 2006; Corre-Hellou et al., 2007).

Both the niche complementarity effect (Loreau and Hector, 2001) and soil N-pool increase can benefit perennial cover such as legume-based grasslands (Soussana and Machado, 2000; Hoğh-Jensen, 2006; Rasmussen et al., 2007). Forage legumes are widespread and have the potential to give high yields over a range of climatic conditions; the four major forage legumes alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), subterranean clover (*T. subterraneum* L.) and white clover (*T. repens* L.) together cover grassland of hot and dry regions of the earth (Frame et al., 1998). While white clover is the most widespread clover used in agriculture, birdsfoot trefoil (*Lotus corniculatus* L.) is also abundantly sown in temperate and northern areas, as is, to a lesser extent, alsike clover (*T. hybridum* L.).
3. QUANTIFICATION OF N RHIZODEPOSITION

3.1. Estimation of below-ground N

When legumes are used as green manure, biological fixation of N can be transferred to the soil through decomposition of above- and below-ground legume residues after harvest (Fujita et al., 1992). This is the reason why legumes are used in organic agriculture and are undersown with cereals for subsequent incorporation into the soil as green manure. Additionally, in intercropping systems, legume roots also release a significant proportion of N into the rhizosphere (rhizodeposited N). However, studies dealing with N balance in rotational farming systems including legume crops have long omitted to consider the below-ground contribution of legumes to the soil-N pool (Unkovich and Pate, 2000). The below-ground N pool can be defined as the sum of visible fibrous macro-root N and the part of soil N derived from rhizodeposition (Høgh-Jensen and Schjoerring, 2001). Estimation of soil N derived from rhizodeposition is greatly influenced by the method of measurement. Sampling of the roots and soil has major consequences on the results. Two kinds of methods are available for measuring below-ground N, with or without the use of a $^{15}$N isotope:

(i) The most simple and commonly used approach for assessing below-ground N involves physical removal of the roots from the soil. Using this method, values of below-ground N as a percentage of total plant N are often very low compared with those obtained in the greenhouse. This is probably because sampling the entire root biomass is challenging, as many roots are fine and fragile and difficult to recover by wet sieving (Bergersen et al., 1989; Chapman and Myers, 1987; Toomsan et al., 1995; Russell and Fillery, 1996b; Dalal et al., 1997; Rochester et al., 1998; Unkovich and Pate, 2000). Greenhouse experiments undertaken in pots with limited volume allow a higher recovery of the root compartment (Mahieu et al., 2007). In addition, physical recovery of roots does not take rhizodeposited N into account, though this is also a necessary value for assessing below-ground N (Khan et al., 2002a, b).

Crawford et al. (1997) used a sequential coring and summation technique proposed by Hansson and Steen (1984) designed to assess total root production from repeated and simultaneous measurements of living roots, dead roots and old organic material. This method seems more accurate than assessments based solely on physical recovery of intact roots, but total root biomass remains underestimated.

(ii) Direct labelling of legumes with a tracer such as $^{15}$N provides a means to assess the two components of below-ground N and particularly rhizodeposited N in the soil. $^{15}$N is applied to a part of the plant and transferred to all organs by the sap stream, so rhizodeposits are $^{15}$N-enriched (Figs. 1 and 2). The percentage of NdfR (N derived from rhizodeposition) is usually calculated using equation (1), proposed by Janzen and Bruinsma (1989):

$$\%\text{NdfR} = \left( \frac{\text{atom}\% \ ^{15}\text{N}_{\text{excess soil}}}{\text{atom}\% \ ^{15}\text{N}_{\text{excess root}}} \right) \times 100$$  \hspace{1cm} (1)
The atom% $^{15}$N excess values were obtained by correcting the $^{15}$N enrichments with background values.

$$\%\text{NdfR} = \frac{\text{[atom%}^{15}\text{Nsoil enriched - soil background]} \times 100}{\text{[atom%}^{15}\text{Nroot enriched - root background]}}$$

The $^{15}$N abundance of plants grown in unlabelled soil, or of unlabelled legume or non-legume control plants, has often been used as background (Jensen, 1996a; Russel and Fillery, 1996a, b; Khan et al., 2002a, b; Mayer et al., 2003; Mahieu et al., 2007; Gylfadóttir et al., 2007). Schmidtke (2005a) has demonstrated that the lower the $^{15}$N abundance of the roots, the more important the choice of adequate soil and root background values (Eq. (2)). The best estimation of N derived from rhizodeposition is obtained when the $^{15}$N abundance of soil unlabelled N is used for soil background and the $^{15}$N abundance of unlabelled roots for root background. Non-fixing plants can also be used for soil and root background values. As N re-absorptions are not taken into account, equations (1, 2) correspond to the assessment of net N rhizodeposition. The amount of total N (mg) derived from rhizodeposition is calculated by multiplying the N amount in this pool with the $\%\text{NdfR}$ value.

Root/soil sampling methods may also influence the results, since they have direct consequences on $^{15}$N enrichment values of roots and soil. In some studies, roots are separated from the soil by dry gentle sieving (2 mm) before being carefully brushed to give a clean root fraction (McNeill et al., 1997, 1998; McNeill and Fillery, 2008). After root/soil sieving, Yasmin et al. (2006) separated the roots from the remaining adhering soil (called 'rhizosphere soil') by $-40$ °C freeze-drying for 2 d. In other studies (Sawatsky and Soper, 1991; Mayer et al., 2003; Schmidtke, 2005a, b; Mahieu et al., 2007; Wichern et al., 2008), after root collection by gentle dry sieving, visible micro-roots were hand-collected with tweezers. Then all roots were shaken in a closed dish with deionised water, and the rinse solution was pooled with the soil sample. Most experiments are undertaken in a sandy substrate to make soil/roots sorting easier. In the field or under rain shelters, plants are often planted in columns pushed down into the soil (or mesotrons; Russell and Fillery, 1996b; McNeill et al., 1997; Gylfadóttir et al., 2007; Tab. I), or in microplots delimited with plastic or iron sheets (Rochester et al., 1998; Mahieu et al., 2007).

The use of equation (1) assumes a uniform distribution of $^{15}$N label throughout the root system and similar enrichments of both N deposited and of roots, but differences in $^{15}$N enrichment between fine roots, coarse roots and nodules are often observed (Khan et al., 2002a, b; Russell and Fillery, 1996a; McNeill and Fillery, 2008).

3.2. $^{15}$N labelling methods

Isotopic methods should ideally allow a uniform labelling of the whole plant. The $^{15}$N label used to assess below-ground N can be provided to the legume in different ways.

(i) In the $^{15}$N dilution technique, the label is provided directly to the soil and N fixation is estimated by the input of $^{14}$N from the atmosphere (Fig. 1). This method is reliable for measurement of N$_2$ fixation by legumes and transfer to companion plants (Giller et al., 1991; Moyer-Henry et al., 2006; Paynel et al., 2008) but is strongly influenced by small differences in the spatial and temporal distribution of soil $^{15}$N when used for measurement of N rhizodeposition (Hétier et al., 1986; Khan et al., 2007). Poth et al. (1986) used a soil with very low nitrogen content and labelled this soil with $^{15}$NH$_4$ for six years to increase the accuracy of the measurement of rhizodeposition by pigeonpea plants in a greenhouse study.

(ii) The $^{15}$N$_2$ enrichment technique, by which nodulated roots are exposed to $^{15}$N$_2$, is the more direct way to measure the input of fixed N$_2$ into the rhizosphere (Fig. 1; Warembourg et al., 1982; McNeill et al., 1994; Russelle et al., 1994). However, this technique requires specific equipment and cannot be applied easily in the field. Furthermore, free-living N$_2$-fixing bacteria can use $^{15}$N and complicate interpretation of results.

(iii) The leaf-feeding technique involves feeding $^{15}$N as a gas (NH$_3$), or as a solution (urea, ammonium or nitrate; Fig. 1). Janzen and Bruinsma (1989) exposed shoots of wheat plants (Triticum aestivum L.) to $^{15}$NH$_3$ for a relatively short duration (6 h) periodically during the growing season. For this purpose, plants were temporarily placed in a sealed plexiglass enclosure, and the medium was sealed from the atmosphere. This method resulted in a uniform labelling of the above- and below-ground parts (though $^{15}$N enrichment in the roots was lower than in the shoots), but has not been used with legumes. Only limited quantities of $^{15}$N can be absorbed by the plant material because of short exposure time. Longer periods of exposure would require sophisticated and expensive equipment unsuitable for field measurements (Bazot et al., 2008).

Urea is a good $^{15}$N carrier because it is non-polar, highly mobile and has a higher N content than nitrate and ammonium. Leaf-feeding (or leaf-flap) methods involve immersing a part of the foliage in a $^{15}$N-labelled solution contained in a sealed vial for several hours. These have been found to be more accurate than spray applications of $^{15}$N-labelled urea, because of the loss of $^{15}$NH$_3$ occurring after $^{15}$N-urea hydrolysis and runoff from the labelled leaves to the soil in the case of spray applications (Russell and Fillery, 1996a; Hertenberger and Waneck, 2004). After a spray application of $^{15}$N-urea, Zebart et al. (1991) recovered less than 70% of the $^{15}$N applied in the case of alfalfa and 30% in the case of red clover. Using the leaf-feeding technique, Ledgard et al. (1985) labelled pasture legumes by immersing a trifoliate leaf into a glass vial sealed in a plastic bag and filled with 15 mL of a 10% $^{15}$N KNO$_3$ solution (300 mM) for 72 h and measuring N transfers between neighbouring pasture plants. McNeill et al. (1997, 1998) adapted the leaf-feeding technique (Oghoghorie and Pate, 1972; Pate, 1973), to assess below-ground N of subterranean clover and serradella (Onichthopsis compressa L.). They conducted similar experiments in the field and in the greenhouse (McNeill et al., 1997, 1998). After cutting (1997) or not (1998) the 1–2 mm tip, a young expanded leaf was inserted into a 2-mL non-porous vial filled with 1 mL of a 0.25–0.4% (w/v) solution of $^{15}$N-labelled urea (99.6 atom% $^{15}$N).
Table I. N rhizodeposited by various legume species as a percentage of the plant N. Values obtained using different labelling methods. (* injected into labelling compartment soil at the beginning of the experiment, ** continuous labelling in hydroponic compartment, *** injected every two days in vermiculite of labelling compartment, (fr) including fine roots).

| Reference               | Species                                      | Culture conditions | Method                           | $^{15}$N recovery | Rhizodeposited N / plant-N |
|-------------------------|----------------------------------------------|--------------------|----------------------------------|-------------------|-----------------------------|
| Zebarth et al. (1991)   | *Trifolium pratensis* Medicago sativa        | Field              | Leaf spray                       | –                 | –                           |
| Sawatsky and Soper (1991) | *Pisum sativum*                                 | Growth chamber     | Split-root $^{15}$NH$_4$SO$_4$ * | –                 | 8–12%                       |
| Jeensen (1996a, b)      | *Pisum sativum*                                 | Growth chamber     | Split-root $\text{KNO}_3$–$^{15}$N * | –                 | 7%                          |
| Rosel and Fillery (1996b) | *Lupinus angustifolius*                        | Field (mesotrons)  under rain shelter | Cotton-wick $(^{15}$N-urea) | 81–102% | 18.5%                       |
| McNeill et al. (1997)   | *Trifolium subterraneum* Ornithopus compressus | Field (mesotrons)  | Leaf feeding $(^{15}$N-urea) | 85%               | 10% (fr)                    |
| McNeill et al. (1998)   | *Trifolium subterraneum* Ornithopus compressus | Greenhouse (pots)  | Leaf feeding $(^{15}$N-urea) | 42%               | 40% (fr)                    |
| Rochester et al. (1998) | *Vicia faba* ssp minor, Glycine max, Lens culinaris, *Lupinus angustifolius, Vigna radiata, V. angularis, V. unguiculata, Cajanus cajan, Arachis hypogaea, Lablab purpureus, Pisum sativum* | Field              | Petiole feeding $(^{15}$N-urea) | –                 | –                           |
| Khan et al. (2002a, b)  | *Vicia faba* Cicer arietinum, Vigna radiata, Cajanus cajan | Greenhouse (pots)  | Shoot feeding $(^{15}$N-urea) | 90%               | 23.5%                       |
|                         |                                              |                    |                                  | 76%               | 43.9%                       |
|                         |                                              |                    |                                  | 100%              | 16.5%                       |
|                         |                                              |                    |                                  | 102%              | 35.5%                       |
| Chalk et al. (2002)     | *Sesbania rostrata*                           | Greenhouse (pots)  | Leaf feeding $(^{15}$N-urea) | 35%               | –                           |
|                         |                                              |                    | Stem injection $(^{15}$N-urea)   | 45%               | –                           |
|                         |                                              |                    | Adventitious root feeding $(^{15}$N-urea) | 101% | –                           |
| Mayer et al. (2003)     | *Vicia faba* Pisum sativum Lupinus albus      | Cover hall (pots)  | Cotton-wick $(^{15}$N-urea)     | 84.8%             | 13%                         |
|                         |                                              |                    |                                  | 83.2%             | 12%                         |
|                         |                                              |                    |                                  | 84.5%             | 16%                         |
| Schmidtke (2005a, b)    | *Pisum sativum* Lathyrus sativus              | Greenhouse (pots)  | Split-root $\text{KNO}_3$–$^{15}$N *** | –                 | 10.5%                       |
|                         |                                              |                    |                                  |                   | 9.2%                        |
| Yasmin et al. (2006)    | *Cicer arietinum*                              | Greenhouse (pots)  | Leaf feeding                     | –                 | –                           |
|                         |                                              |                    | Petiole feeding Cotton-wick      | –                 | –                           |
| Mahieu et al. (2007)    | *Pisum sativum*                                | Greenhouse (pots)  | Cotton-wick $(^{15}$N-urea)     | 65–85%            | 9.7–11.7%                   |
|                         |                                              | Field              |                                  | 70%               | 34.2%                       |
|                         |                                              | Greenhouse (pots)  | Split-root $^{15}$NO$_3$–$^{15}$NH$_4$ ** | –                 | 14.3–17.3%                  |
|                         |                                              | Field              |                                  | –                 | 27.5%                       |
| Gylfadóttir et al. (2007) | *Mixture* Trifolium repens Poa pratensis     | Field (mesotrons)  | Leaf feeding $(^{15}$N-urea)     | –                 | 47% (of total N for both species) |
|                         |                                              |                    |                                  |                   | 10% (of total N for both species) |
| Wichern et al. (2007a)  | *Pisum sativum*                                | Field (mesotrons)  | Cotton-wick $(^{15}$N-urea)     | 59–77%            | 32–36%                      |
| McNeill and Fillery (2008) | *Lupinus angustifolius*                      | Field (mesotrons)  | Cotton-wick $(^{15}$N-urea)     | 69–76%            | 35–65% (fr)                 |
The system was sealed with inert plastic putty to avoid 15N loss. To avoid leaf damage, the concentration of the urea solution must not be too high. In the field, mean total recovery of the fed 15N in the entire plant-soil system at the late vegetative stage was 85% for subterranean clover and 76% for serradella, but was more than 92% in both species after feeding at maturity (Tab. I). In the greenhouse, mean recovery of the fed 15N was 42% in subterranean clover and 64% for serradella. In leaf-feeding methods, 15N enrichment of above-ground parts is often higher than that of below-ground parts (McNeill et al., 1997, 1998; Yasmin et al., 2006). 15N leaf-feeding techniques used both by Ledgard et al. (1985) and by McNeill et al. (1998) were also used to measure N compounds deposited in the soil by mixtures of common grassland species in the field and N transfer from legumes to the neighbouring non-fixing plant (Bardgett et al., 1999; Høgh-Jensen and Schjoerring, 2001; Ayres et al., 2007; Rasmussen et al., 2007).

The 15N solution can be fed directly to a leaf petiole. Rochester et al. (1998) attached vials containing 15N-urea to petioles of eleven different species of grain legume. Khan et al. (2002b) compared the use of leaf-feeding and petiole-feeding methods in the field with four different species. They concluded that 15N leaf-flap feeding was best for fababean, mungbean and pigeonpea, but petiole feeding was best for chickpea. The best compromise to enable comparison of results between species was to apply short pulses of labelled urea to the lower third or fourth stem-node using 0.2 mL of 0.5% urea (98 atom% 15N) at each pulse. Leaf and petiole feeding led to higher 15N enrichment of above- than below-ground parts in all tested species except in pigeonpea, where shoot enrichment was about 30% lower than root enrichment (Ledgard et al., 1985; Russell and Fillery, 1996a; b; McNeill et al., 1997, 1998; Khan et al., 2002a; Chalk et al., 2002). In leaf and petiole feeding, although the urea was highly enriched in 15N, the 15N enrichment of the roots was only between 0.11 and 0.90 atom% 15N excess (McNeill et al., 1997; Høgh-Jensen and Schjoerring, 2001; Khan et al., 2002a, b).

(v) The cotton-wick technique was proposed by Russell and Fillery (1996a). 15N-labelled solution is provided to the plant by means of a cotton-wick passing through a hole in the plant stem (Fig. 1). These authors have shown that the transfer of solutions into young lupin plants is more effective using the cotton-wick method than the leaf-feeding method. N uptake by the cotton-wick technique is mainly driven by the transpiration stream, avoiding active mechanisms occurring with root or leaf immersion. Results reported by Russell and Fillery (1996b) and McNeill and Fillery (2008) confirm that this method seems accurate for assessing below-ground N of field-grown lupin and provides a more homogeneous 15N distribution in the plants compared with leaf-feeding techniques (Mayer et al., 2003). It has also been confirmed for fababean, chickpea, mungpea (Vigna radiata (L.) R. Wilcz.), pigeonpea, pea and white lupin (Russell and Fillery, 1996b; Mayer et al., 2003; Mahieu et al., 2007). Fortnightly pulses of high 15N-urea (99 atom% 15N), were found to be more efficient than a weekly application (Russell and Fillery, 1996a) and provide similar results to pulses applied at given growing stages (6-leaf stage, flowering and pod-filling; Mahieu et al., 2007). In Mayer et al. (2003) the amount of urea applied to pea plants at each pulse was calculated from dilution curves, to keep an average 15N content of 2.5 atom% 15N excess of the plant N during the growing demand. All experiments undertaken on pea showed that 15N recovery was around 90% (84–94%) in the greenhouse and 50–76% in the field (Tab. I; Mayer et al., 2003; Mahieu et al., 2007; Wichern et al., 2007a). Furthermore, the longer the experiment, the lower 15N recovery in the plant-soil system (Russell and Fillery, 1996a; Mayer et al., 2003; Mahieu et al., 2007). In cotton-wick, as in leaf-flap and petiole feeding, above-ground parts are markedly more 15N-enriched than roots. Root enrichment ranged between 1.1 and 1.4 atom% 15N excess in Russell and Fillery (1996a), Mayer et al. (2003) and Wichern et al. (2007a) but reached up to 3.6 atom% 15N excess in Mahieu et al. (2007). However, cotton-wick cannot be used with thin-stemmed species such as chickpea (Yasmin et al., 2006). Few attempts have been made to inject 15N-urea directly into the stem with a syringe. Chalk et al. (2002) did not obtain reliable results with S. rostrata, probably because of its hollow stem.

(vi) The split-root technique was proposed by Sawatsky and Soper (1991) to quantify the amount of N lost from the root system of pea plants. Before the beginning of the experiments, seedlings of pea were raised in moist sand or vermiculite, and the radicle was cut 1 cm below the seed after seedling emergence to enhance the development of adventitious roots. Then the root system was split between two different soil compartments. One of them, filled with soil (Sawatsky and Soper, 1991), with clay marbles (Jensen, 1996a, b; Mahieu et al., 2007) or vermiculite (Schmidtke, 2005a, b) was labelled with a mineral 15N-enriched source, and the other part of the root system growing in the unlabelled soil compartment could be monitored (Fig. 2). Sawatsky and Soper (1991) used a solution of (15NH4)2SO4 containing 66.7% 15N: root 15N enrichment was 9.92 atom% 15N excess. Jensen (1996a), Schmidtke (2005a) and Mahieu et al. (2007) used a 5% or 10% 15N-enriched KNO3-N, and 10% 15N-enriched NO3-NH4 respectively; root enrichments comprised between 0.2 and 3.5% atom% 15N excess.

This technique can also be used to assess N transfer between a legume and a non-fixing species (Jensen, 1996b). It allows continuous labelling during plant growth and N uptake follows a natural pathway. A disadvantage of the split-root method is that it substantially disturbs the root system and plant development, particularly for species with a taproot (McNeill et al., 1997; Khan et al., 2002a). In addition, roots of the labelling compartment tend to keep more than 50% of the applied 15N (Schmidtke, 2005b; Mahieu et al., 2007), leading to lower enrichment in N of the other plant parts. Furthermore, estimation of N derived from rhizodeposition accounts for only a part of the root system. This technique is difficult to adapt to field conditions (Mahieu et al., 2007).

### 3.3. Amounts of N rhizodeposited by legumes

Among all reviewed studies, N derived from rhizodeposition as a percentage of the mature plant N varied from 7% to
Using leaf feeding with $^{15}$N-urea, the ratio of rhizodeposited N: plant N differed markedly among species (from 10% in subterranean clover to 57% in serradella); values obtained in subterranean clover and serradella were markedly higher in the field than in the greenhouse (McNeill et al., 1997, 1998).

Several studies have investigated N rhizodeposition of mature pea crops using split-root or cotton-wick methods (Sawatsky and Soper, 1991; Jensen, 1996a, b; Mayer et al., 2003; Schmidtke, 2005a, b; Mahieu et al., 2007; Wichern et al., 2007a). Harvesting at different stages indicates that N rhizodeposition increases as plants mature, probably because of the increase in senescing roots and nodules (see Wichern et al., 2008). However, Wichern et al. (2007b) measured high levels of rhizodeposition at early vegetative stages of growth (71% of the plant N at the 3–6 leaf stage). For a pea plant, the ratio of rhizodeposited N: plant N was 4 to 71% and the ratio of the below-ground N: plant N varied from 14 to 74%. At maturity, in greenhouse conditions, rhizodeposited N and below-ground N often represented around 15% and 25% of plant N, respectively (Mahieu et al., 2007; see Wichern, 2008). In the field, below-ground N represented around 30% of plant N and rhizodeposited N often accounted for 88–97% of below-ground N. Mahieu et al. (2007) showed that the ratio of rhizodeposited N: plant N obtained with split-root was 10% higher than that obtained with cotton-wick. Furthermore, the values were higher in the field than in the greenhouse experiments, though the root-to-shoot ratios were markedly lower in the field than in the greenhouse. Consistently with other studies, roots represented less than 5% of the total plant weight in the field (Voisin et al., 2002), since they represented at least 10–20% of the plant weight in the greenhouse pots. In their greenhouse study, Mahieu et al. (2007) found a significant relationship between the amount of N rhizodeposited by a pea plant and the plant-N content that could contribute to explain this difference, since plant-N contents of field peas were higher than those of greenhouse plants.

4. N RHIZODEPOSITION PATHWAYS

4.1. General considerations

The term rhizodeposition was first used to describe carbon loss from roots (Lynch and Whipp, 1990) but also includes N loss, as most organic compounds lost by roots also contain N (but see Wichern et al., 2008). Less N than C is rhizodeposited, but deposition of both elements cannot be distinguished (Bais et al., 2006) as in both cases, the potential pathways for rhizodeposition are (1) senescence, death and decay of roots and nodules; (2) exudation of soluble compounds; (3) sloughing-off of root border cells, and (4) secretion of mucilage. Quantitative data providing reliable estimation of these pathways are sparse but a recent review concerning carbon rhizodeposition showed that sloughing-off of border cells and secretion of mucilage represent a very small proportion of carbon rhizodeposition (N’guyen, 2003). This proportion must be even smaller for N rhizodeposition, as little N is present in mucilages.

4.2. Senescence of roots and nodules

Several studies have demonstrated that death of nodules and roots is a major source of biological fixation of N for the soil (Dubach and Russelle, 1994; Russelle et al., 1994). Its importance is undisputed but reliable quantitative data are sparse, as no methodology is available to clearly distinguish rhizodeposition due to death and decay of below-ground tissues from rhizodeposition due to exudation of soluble compounds. By comparing the accumulation of biologically fixed $^{15}$N$_2$ in fine roots and nodules of alfalfa and birdsfoot trefoil with soil surrounding the roots, Dubach and Russelle (1994) have estimated that decomposition of these tissues is the main pathway for N rhizodeposition. Though little quantitative data are available concerning fixed nitrogen in legume rhizospheres, quantification of underground N transfer from legumes to intercropped grasses is now well documented. Because transfer increases with plant age, it is often proposed that N release from senescence of below-ground residues of legumes coupled with grass uptake is the dominant factor in N exchange (Høgh-Jensen and Schjoerring, 1997; Moyer-Henry et al., 2006). Release of N through degradation of above-ground tissues is highly dependent on numerous factors such as mycorrhizal fungi, bacteria, root herbivory or defoliation (Ta and Faris, 1988; Johansen and Jensen, 1996; Ayres et al., 2007). Numerous studies have established that N transfer between plants can also occur between young plants, through mycorrhizal networks interconnecting plants or through exudation of N compounds by legumes coupled with uptake by grasses (Paynel et al., 2001; Moyer-Henry et al., 2006).

4.3. Exudation of soluble compounds

The N released from roots and nodules as low-molecular-weight substances, such as soluble root exudates, amino acids, hormones and enzymes, is also poorly quantified in soil conditions. Most of the experiments on N exudation have been carried out in the laboratory, in hydroponically grown plants or in sand cultures. Between 3% (Ta et al., 1986) and 4.5% of the fixed N is released by alfalfa to the solution as soluble compounds, while between 10 (Brophy and Heichel, 1989) and 30% (Ofosu-Budu et al., 1990) of fixed N is released to the nutrient solution. Robinia pseudoacacia L., which is an N$_2$-fixing tree, has also been observed to release a significant, but minor, proportion of fixed N to the solution, as dissolved organic nitrogen (Uselman et al., 1999).

The main N compound released is generally ammonium, which is the main product of the nitrogenase enzyme, but significant proportions of ureides and amino acids are also recovered in root exudates of alfalfa, soybean and clover (Ta et al., 1986; Brophy and Heichel, 1989; Paynel and Cliquet, 2003). Among amino acids found in root exudates of various species including white clover and alfalfa, glycine and serine have often been recovered in high proportions (Svenningsson et al., 1990; Paynel et al., 2001; Hertenberger and Wanek, 2004; Lesuffleur et al., 2007) despite also constituting a major amino acid in rhizospheric soils (Kielland, 1995; Jones...
The reverse is true for other amino acids such as asparagine and glutamine, which are recovered in low proportions in root extracts, showing that amino acid root exudation is a selective process. Ammonium and amino acids are also recovered in root exudates of non-fixing plants (Paynel and Cliquet, 2003), but use of 15N-labelled amino acids has shown that efflux of glycine and serine from roots of legumes is higher than from roots of grasses (Lesufleuret al., 2007). Like the other components of rhizodeposition, root exudation is altered by numerous biotic factors, such as mycorrhizal fungi and root herbivores (Murray et al., 1996; Bais et al., 2006) and abiotic factors, such as defoliation and CO2 enrichment (Ayres et al., 2007; Bazot et al., 2008).

5. CONCLUSION

In conclusion, biological fixation of N can act as a sustainable source of N and can complement or replace fertiliser inputs. This review highlights that numerous agricultural practices have been developed all around the world to take advantage of the biological reduction of atmospheric N to ammonia realised by some prokariots. N fixation is performed by these prokariots alone or in symbiosis with plants. Legumes form a symbiosis with Rhizobium but release a substantial part of the biologically fixed N into the rhizosphere. As a consequence, biological N fixation can act as a sustainable source of N and contribute to decreasing fertiliser inputs. However, the part of this N available for non-fixing crops remains difficult to assess. N rhizodeposition is mainly due to senescence and decay of roots and nodules, and exudation of N compounds by living roots. The main N compounds released by legume roots are ammonium, amino acids and ureides, but a wide range of organic compounds released by plant roots remain to be determined. A significant effort has been made in the last decade to develop tracer methods suitable for quantifying N rhizodeposition in realistic conditions. Long-term studies using the split-root and the cotton-technique have shown that N rhizodeposition increases with plant age and plant N content, but more information is lacking concerning the effects of plant-N partitioning and of root characteristics. Ecological functions of these rhizodeposits are still unknown, but they may constitute a rapidly incorporating source of C and N for soil microorganisms and neighbouring plants. Further investigations combining assessments of C and N rhizodeposition are needed to obtain a better understanding of these fluxes in the rhizosphere of legumes.

REFERENCES

Ayres E., Dromph K.M., Cook R., Ostle N., Bardgett R.D. (2007) The influence of below-ground herbivory and defoliation of a legume on nitrogen transfer to neighbouring plants, Funct. Ecol. 21, 256–263.

Bais H.P., Weir T.L., Perry L.G., Gilroy S., Vivanco J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms, Ann. Rev. Plant Biol. 57, 233–266.

Bazot S., Blum H., Robin C. (2008) Nitrogen rhizodeposition assessed by a 15NH3 shoot pulse-labelling of Lolium perenne L. grown on soil exposed to 9 years of CO2 enrichment, Environ. Exp. Bot. 63, 410–415.

Bergersen F.J., Brockwell J., Gault R.R., Morthorpe L., Peoples M.B., Turner G.L. (1989) Effects of available soil nitrogen and rates of inoculation on nitrogen fixation by irrigated soybeans and evaluation of δ15N methods for measurements, Aust. J. Agr. Resour. Ec. 40, 763–780.

Brophy L.S., Heichel G.H. (1989) Nitrogen release from roots of alfalfa and soybean grown in sand culture, Plant Soil 116, 77–84.

Burris R.H. (1974) Biological nitrogen fixation, Plant Physiol. 54, 443–449.

Carlsson G., Huss-Danell K. (2003) Nitrogen fixation in perennial forage legumes in the field, Plant Soil 253, 353–372.

Chapman A.L., Myers R.J.K. (1987) Nitrogen contribution by grain legumes to rice grown in rotation on the Cununurra soils the Ord irrigation area, Western Australia, Aust. J. Agr. Resour. Ec. 48, 1139–1150.

Chalk P.M. (1998) Dynamics of biologically fixed N in legume-cereal rotations; a review, Aust. J. Agr. Resour. Ec. 49, 303–316.

Chalk P.P., Ladha J.K., Padre A. (2002) Efficacy of three 15N labelling techniques for estimating below ground N in Sesbania rostrata, Biol. Fert. Soils 35, 387–398.

Corre-Hellou G., Fustec J., Crozat Y. (2006) Interspecific competition for soil N and its interaction with N2 fixation, leaf expansion and crop growth in pea-barley intercrops, Plant Soil 282, 195–208.

Corre-Hellou G., Brisson N., Launay M., Fustec J., Crozat Y. (2007) Effect of root depth penetration on soil N sharing and dry matter in pea-barley intercrops given different soil N supplies, Field Crop. Res. 103, 76–85.

Crawford M.C., Grace P.R., Bellotti W.D., Oades J.M. (1997) Root production of a barrel medic (Medicago truncatula) pasture, a barley grass (Hordeum leporium) pasture, and a faba bean (Vicia faba) crop in Southern Australia, Aust. J. Agr. Resour. Ec. 48, 1139–1150.

Crépon K. (2006) Protein supply in Europe and the challenge to increase grain legumes production: a contribution to sustainable agriculture, in: Grain legumes and the environment: how to assess benefits and impacts. Proceedings of the AEP workshop, (Ed.) AEP, 18–19 November 2004, Zürich, Switzerland, pp. 13–16.

Crozat Y., Fustec J. (2006) Assessing the role of grain legumes in crop rotation: some agronomic concepts that can help!, in: Grain legumes and the environment: how to assess benefits and impacts. Proceedings of the AEP workshop, (Ed.) AEP, 18–19 November 2004, Zürich, Switzerland, pp. 55–60.

Curatti L., Ludden P.W., Rubio L.M. (2006) NifB-dependent in vitro synthesis of the iron-molybdenum cofactor of nitrogenase, PNAS 103, 5297–5301

Dalal R.C., Strong W.M., Doughton J.A., Weston E.J., McNamara G.T., Cooper J.R. (1997) Sustaining productivity of a vertisol at warra, Queensland, with fertilizer, no-tillage or legumes. 4. Nitrogen fixation, water use and yield of chickpea, Aust. J. Exp. Agr. 37, 667–676.

Deutsch B., Kahle P., Voss M. (2006) Assessing the source of nitrate pollution in water using stable N and O isotopes, Agron. Sustain. Dev. 26, 263–267.

Dubach M., Russelle M.P. (1994) Forage legume roots and nodules and their role in nitrogen transfer, Agron. J. 86, 259–266.
Frame J., Charlton J.F.L., Laidlaw A.S. (1998) Temperate forage legumes, CAB International, Wallingford, UK, 327 p.

Fujita K., Ofosu-Budu K.G., Ogata S. (1992) Biological nitrogen fixation in mixed legume-cereal cropping systems, Plant Soil 141, 155–175.

Garg N., Geetanjali (2007) Symbiotic nitrogen fixation in legume nodules: process and signaling. A review, Agron. Sustain. Dev. 27, 59–68.

Giller K.E., Ormesher J., Awah F.M. (1991) Nitrogen transfer from legumes to grasses in mixed crops, Plant Soil 140, 281–288.

Gilles K.E., Ormesher J., Awah F.M. (1991) Nitrogen transfer from legumes to grasses in mixed crops, Plant Soil 140, 281–288.

Gyldadóttir T., Helgadóttir A., Høgh-Jensen H. (2007) Consequences of above-ground rhizodeposition of nitrogen by perennial legumes, Plant Soil 297, 93–104.

Hansson A.C., Steen E. (1984) Methods of calculating root production and nitrogen uptake in an annual crop, Swedish J. Agr. Res. 14, 191–200.

Hartwig U.A. (1998) The regulation of symbiotic N2 fixation: a conceptual model of N feedback from the novel ecosystem to the gene expression level, Perspect. Plant Ecol. Evol. Syst. 1(1), 92–120.

Hauggaard-Nielsen H., Jensen E.S. (2005) Facilitative root interactions in intercrops, Plant Soil 274, 237–250.

Herteningen G., Wanek W. (2004) Evaluation of methods to measure differential 15N labeling of soil and root-N pools for studies of root exudation, RCM 18, 2415–2425.

Hétier J.M., Andreux F., Schouller E., Marol C. (1986) Organic matter inputs after growth of carbon 14-N-labeled maize, Soil Sci. Soc. Am. J. 50, 76–80.

Hög-Hansen H. (2006) The nitrogen transfer between plants: An important but difficult flux to quantify, Plant Soil 282, 1–5.

Hög-Hansen H., Schjoerring J.K. (1997) Interaction between white clover and ryegrass under contrasting nitrogen availability: N2 fixation, N fertilizer recovery, N transfer and water-use efficiency, Plant Soil 197, 187–199.

Hög-Hansen H., Schjoerring J.K. (2001) Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys, Soil. Biol. Biochem. 33, 439–448.

Janzen H.H., Bruinsma Y. (1989) Methodology for the quantification of root and rhizosphere N dynamics, Soil. Biol. Biochem. 21, 189–196.

Jensen E.S. (1996a) Rhizodeposition of N by pea and barley and its effect on soil N dynamics, Soil. Biol. Biochem. 28, 65–71.

Jensen E.S. (1996b) Barley uptake of N deposited in the rhizosphere of associated field pea, Soil. Biol. Biochem. 28, 159–168.

Jensen E.S. (2006) Grain legume functions in crop rotations, in: Grain legumes and the environment: how to assess benefits and impacts. Proceedings of the AEP workshop, (Ed.) AEP, 18–19 November 2004, Zürich, Switzerland, pp. 49–54.

Johansen A., Jensen E.S. (1996) Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus, Soil. Biol. Biochem. 28, 73–81.

Jones D.L., Healey J.R., Willett V.B., Farrar J.F., Hodge A. (2005) Dissolved organic nitrogen uptake by plants, an important N uptake pathway? Soil Biol Biochem 37, 413–423.

Khan D.F., Peoples M.B., Chalk P.M., Herridge D.F. (2002a) Quantifying below-ground nitrogen of legumes. 1. A comparison of 15N and non-isotopic methods, Plant Soil 239, 277–289.

Khan D.F., Peoples M.B., Herridge D.F. (2002b) Quantifying below-ground nitrogen of legumes. 1. Optimising procedures for 15N shoot-labelling, Plant Soil 245, 327–334.

Khan D.F., Herridge D.F., Peoples M.B., Shah S.H., Khan T., Madani M.S., Ibrar M. (2007) Use of isotopic and non-isotopic techniques to quantify below-ground nitrogen in fababean and chickpea, Soil Environ. 26, 42–47.

Kielland K. (1995) Landscape patterns of free amino acids in Arctic tundra soils, Biogeochemistry 31, 85–98.

Ledgard S.F., Freney J.R., Simpson J.R. (1985) Assessing nitrogen transfer from legumes to associated grasses, Soil. Biol. Biochem. 17, 575–577.

Lesuffleur F., Paynel F., Bataille M.P., Le Deunff E., Cliquet J.B. (2007) Root amino acid exudation: Measurement of high efflux rates of glycine and serine from six different plant species, Plant Soil 294, 235–246.

Loreau M., Hector A. (2001) Partitioning selection and complementarity in biodiversity experiments, Nature 412, 72–76.

Lynch J.M., Wipps T.M. (1990) Substrate flow in the rhizosphere, Plant Soil 129, 1–10.

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.

Mayer J., Buegger F., Jensen E.S., Schloter M., Hefti G. (2003) Residual nitrogen contribution from grain legumes to succeeding wheat and rape and related microbial process, Soil. Biol. Biochem. 35, 21–28.

McNeill A.M., Fillery I.R.P. (2008) Field measurement of lupin below-ground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate, Plant Soil 302, 297–316.

McNeill A.M., Hood R.C., Wood M. (1994) Direct measurement of nitrogen fixation by Trifolium repens and Alnus glutinosa using 15N2, J. Exp. Bot. 45, 749–755.

McNeill A.M., Zhu C., Fillery I.R.P. (1997) Use of in situ 15N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems, Aust. J. Agr. Resour. Ec. 48, 295–304.

McNeill A.M., Zhu C., Fillery I.R.P. (1998) A new approach to quantifying the N benefit from pasture legumes to succeeding wheat, Aust. J Agr. Resour. Ec. 49, 427–436.

Moyer-Henry K.A., Burton J.W., Israel D.W., Rufty T.W. (2006) Nitrogen Transfer Between Plants: A 15N Natural Abundance Study with Crop and Weed Species, Plant Soil 282, 7–20.

Murray P.J., Hatch D.J., Cliquet J.B. (1996) Impact of insect herbivory on the growth, nitrogen and carbon contents of white clover (Trifolium repens L.), Can. J. Bot. 74, 1591–1595.

N’guyen C. (2003) Rhizodeposition of organic C by plant: mechanisms and controls, Agronomie 23, 375–396.

Ofosu-Budu K.G., Fujita K., Ogata S. (1990) Excretion of ureide and other nitrogenous compounds by the root system of soybean at different growth stages, Plant Soil 128, 135–142.

Oghoghorie C.G.O., Pate J.S. (1972) Exploration of the nitrogen transport system in a nodulated legume using 15N, Planta 104, 35–49.

Padilla F.M., Pugnaire F.I. (2006) The role of nurse plants in restoration of degraded environments, Front. Ecol. Environ. 4, 196–202.

Pate J.S. (1973) Uptake, assimilation and transport of nitrogen compounds by plants, Soil Biol. Biochem. 5, 109–119.

Paynel F., Cliquet J.B. (2003) 15N transfer from white clover to perennial ryegrass, via exudation of nitrogenous compounds, Agronomie 23, 503–510.
Paynel F., Murray P., Cliquet J.B. (2001) Root exudates: a pathway for short-term N transfer from clover and ryegrass, Plant Soil 229, 235–243.

Paynel F., Lesuffleur F., Bigot J., Diquelou S., Cliquet J.B. (2008) A study of 15N transfer between legumes and grasses, Agron. Sustain. Dev. 28, 281–290.

Poth M., La Favre J.S., Focht D.D. (1986) Quantification by direct 15N dilution of fixed N2 incorporation into soil by Cajanus cajan (pigeon pea), Soil Biol. Biochem. 18, 125–127.

Rasmussen J., Eriksen J., Jensen E.S., Esbensen K.H., Høgh-Jensen H. (2007) In situ carbon and nitrogen dynamics in ryegrass clover mixtures: transfers, deposition and leaching, Soil Biol. Biochem. 39, 804–815.

Rochester L.J., Peoples M.B., Constable G.A., Gault R.R. (1998) Fababean and other legumes add nitrogen to irrigated cotton cropping systems, Aust. J. Exp. Agr. 38, 253–260.

Rochon J.J., Doyle C.J., Greef J.M., Hopkins A., Motte G., Sitzia M., Scholefield D., Smith C.J. (2004) Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects, Grass Forage Sci. 59, 197–214.

Russell C.A., Fillery I.R.P. (1996a) In situ 15N labelling of lupin below ground biomass, Aust. J. Agr. Resour. Ec. 47, 1035–1046.

Russell C.A., Fillery I.R.P. (1996b) Estimates of lupin below ground biomass nitrogen, dry matter, and nitrogen turnover to wheat, Aust. J. Agr. Resour. Ec. 47, 1047–1059.

Russelle M.P., Allan D.L., Gourley C.J.P. (1994) Direct assessment of symbiotically fixed nitrogen in the rhizosphere of alfalfa, Plant Soil 159, 233–243.

Sawatsky N., Soper R.J. (1991) A quantitative measurement of the nitrogen loss from the root system of field peas (Pisum avenese L.) grown in the soil, Soil. Biol. Biochem. 23, 255–259.

Schmidtko K. (2005a) A model to predict the accuracy of measurements of legume N rhizodeposition using a split-root technique, Soil Biol. Biochem. 37, 829–836.

Schmidtko K. (2005b) How to calculate nitrogen rhizodeposition: a case study in estimating N rhizodeposition in the pea (Pisum sativum L.) and grasspea (Lathyrus sativus L.) using a continuous 15N labelling split-root technique, Soil Biol. Biochem. 37, 1893–1897.

Soussana J.F., Machado O. (2000) Modelling the temperate grasses and legumes in cut mixtures, in: Grassland Ecophysiology and Grazing Ecology, Lemaire et al. (Eds.), CAB International London, UK, pp. 169–190.

Svenningsson H., Sundin P., Liljenberg C. (1990) Lipids, carbohydrates and amino acids exuded from the axenic roots of rape seedlings exposed to water-deficit stress, Plant Cell Environ. 13, 155–162.

Ta T.C., Faris M.A. (1988) Effects of environmental conditions on the fixation and transfer of nitrogen from alfalfa to associated timothy, Plant Soil 107, 25–30.

Ta T.C., Macdonald F.D.H., Faris M.A. (1986) Excretion of nitrogen assimilated from N2 fixed by nodulated roots of alfalfa (Medicago sativa), Can. J. Bot. 64, 2063–2067.

Toomsan B., McDonagh J.F., Limjumrutana V.J.H.A., Giller K.E. (1995) Nitrogen fixation by groundnut and soyabean and residual nitrogen benefits to rice in farmers’ fields in Northeast Thailand, Plant Soil 175, 45–56.

Umar A.S., Iqbal M. (2007) Nitrate accumulation in plants, factors affecting the process, and human health implications. A review, Agron. Sustain. Dev. 27, 45–57.

Unkovich M.J., Pate J.S. (2000) An appraisal of recent field measurements of symbiotic N2 fixation by annual legumes, Field Crop. Res. 65, 211–228.

Uselman S.M., Qualls R.G., Thomas R.B. (1999) A test of a potential short cut in the nitrogen cycle: The role of exudation of symbiotically fixed nitrogen from the roots of a N-fixing tree and the effects of increased atmospheric CO2 and temperature, Plant Soil 210, 21–32.

Vance C.P. (2001) Symbiotic nitrogen fixation and phosphorus acquisition: plant nutrition in a world of declining renewable resources, Plant Physiol. 127, 390–397.

Voisin A.S., Salon C., Munier-Jolain N.G., Ney B. (2002) Effect of mineral nitrogen nutrition and biomass partitioning between the shoot and roots of pea (Pisum sativum L.), Plant Soil 242, 251–262.

Wichern F., Mayer J., Joergensen R.G., Müller T. (2007a) Rhizodeposition of C and N in peas and oats after 13C,15N double labelling under field conditions, Soil Biol. Biochem. 30, 2527–2537.

Wichern F., Mayer J., Joergensen R.G., Müller T. (2007b) Release of C and N from roots of peas and oats and their availability to soil microorganisms, Soil Biol. Biochem. 39, 2829–2839.

Wichern F., Eberhardt E., Mayer J., Joergensen R.G., Müller T. (2008) Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects, Soil Biol. Biochem. 40, 30–48.

Warembourg F.R., Montagne D., Bardin R. (1982) The simultaneous use of 14CO2 and 15N2 labelling techniques to study the carbon and nitrogen economy of legumes grown under natural conditions, Physiol. Plant. 56, 46–55.

Yasmin K., Cadish G., Bags E.M. (2006) Comparing 15N-labelling techniques for enriching above- and below ground components of the plant-soil system, Soil. Biol. Biochem. 38, 397–400.

Zebarth B.J., Alder V., Sheard R.W. (1991) In situ labeling of legume residues with a foliar application of a [15N-enriched urea solution, Commun. Soil Sci. Plant Anal. 22, 437–447.