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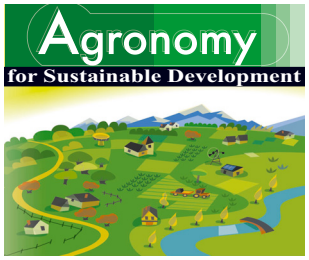

Review article

Nitrogen rhizodeposition of legumes. A review

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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 N_2, into biological substrates available for growth of other plants, through two consecutive processes; namely, N_2 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 ^{15}N 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 / N_2 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 N_2O. 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.

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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 N_2-fixing micro-organisms include the utilisation of the symbiosis between the fern *Azolla*

azollae and the N_2-fixing cyanobacterium *Anabaena azollae* as a green manure in rice wetlands, and the use of free-living N_2-fixing bacteria such as *Azopirillum* inoculated into the rhizosphere of grasses.

2. BIOLOGICAL N_2 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 archaebacteria. 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 symbiosis 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 N_2 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 site but others contain iron and vanadium, or iron only when molybdenum is not available (Curatti et al., 2006). Because of the economic and ecological benefits of N_2 fixation, the genes associated with this process, designated nif, have been extensively studied and are now well characterised (Gordon et al., 2001). 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 N_2-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 N_2, 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.), alfafa 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 241×10^6 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, N_2 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; Høgh-Jensen, 2006; Rasmunssen 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 alfafa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), subterraneum 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):

$$\%\text{NdfR} = [(\text{atom}\%\ ^{15}\text{N excess soil})/(\text{atom}\%\ ^{15}\text{N excess root})] \times 100 \quad (1)$$

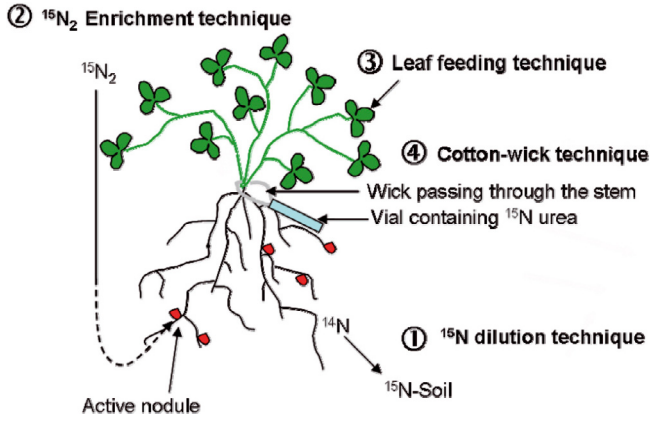

Figure 1. Some techniques used to assess below-ground contribution of legumes to the soil-N pool in the field. (1) – In the ^{15}N dilution technique, ^{15}N is provided directly to the soil, but the differences in ^{15}N natural abundance between air and soil can also be used. (2) – $^{15}N_2$ enrichment technique: nodulated roots are exposed to $^{15}N_2$. (3) and (4) – In the leaf-feeding techniques and in the cotton-wick technique, ^{15}N is provided as urea, nitrate or ammonium contained in a vial., to the above-ground parts. With the cotton-wick technique, ^{15}N-labelling solution is provided to the plant by means of a cotton-wick passing through a hole in the plant stem. (See Fig. 2 for split-root technique).

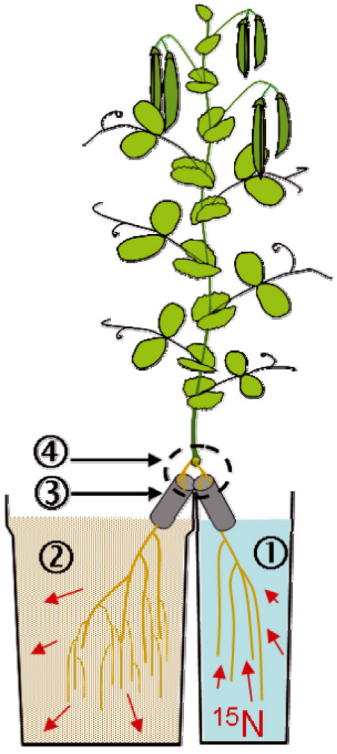

Figure 2. Split-root method applied to a pea plant. Experimental design used by Mahieu et al. (2007). (1) Hydroponic labelling compartment filled with clay marbles and nutrient solution containing $^{15}NO_3$–$^{15}NH_4$. (2) Compartment filled with soil in which net N rhizodeposition was measured. (3) Rubber tubes protecting the upper part of the roots from desiccation. (4) Putty.

The atom% ^{15}N excess values were obtained by correcting the ^{15}N enrichments with background values.

$$\%NdfR = [(atom\%\ ^{15}N soil\ enriched - soil\ background)/ (atom\%\ ^{15}N root\ enriched - root\ background)] \times 100 \quad (2)$$

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 % 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/root 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 Wanek, 2004). After a spray application of ^{15}N-urea, Zebarth 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 trifoliolate 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 subterraneum clover and serradella (*Ornithopus compressus* 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*	Field	Leaf spray	–	–
	Medicago sativa				
Sawatsky and Soper (1991)	*Pisum sativum*	Growth chamber	Split-root $(^{15}NH_4)_2S0_4$ *	–	8–12%
Jensen (1996a, b)	*Pisum sativum*	Growth chamber	Split-root $KN0_3-^{15}N$ **	–	7%
Russel 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*	Field (mesotrons)	Leaf feeding (^{15}N-urea)	85%	10% (fr)
	Ornithopus compressus			76%	20% (fr)
McNeill et al. (1998)	*Trifolium subterraneum*	Greenhouse (pots)	Leaf feeding (^{15}N-urea)	42%	40% (fr)
	Ornithopus compressus			64%	57% (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*	Greenhouse (pots)	Shoot feeding (^{15}N-urea)	90%	23.5%
	Cicer arietinum,			76%	43.9%
	Vigna radiata,			100%	16.5%
	Cajanus cajan			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*	Cover hall (pots)	Cotton-wick (^{15}N-urea)	84.8%	13%
	Pisum sativum			83.2%	12%
	Lupinus albus			84.5%	16%
Schmidtke (2005a, b)	*Pisum sativum*	Greenhouse	Split-root $KN0_3-^{15}N$ ***	–	10.5%
	Lathyrus sativus				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%
	Pisum sativum	Greenhouse (pots)	Split-root $^{15}NO_3-^{15}NH_4$ **	–	14.3–17.3%
		Field		–	27.5%
Gylfadóttir et al. (2007)	Mixture	Field (mesotrons)	Leaf feeding (^{15}N-urea)	–	
	Trifolium repens				47%
	Poa pratensis				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 ^{15}N 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 ^{15}N 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 ^{15}N was 42% in subterranean clover and 64% for serradella. In leaf-feeding methods, ^{15}N enrichment of above-ground parts is often higher than that of below-ground parts (McNeill et al., 1997, 1998; Yasmin et al., 2006). ^{15}N 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 ^{15}N solution can be fed directly to a *leaf petiole*. Rochester et al. (1998) attached vials containing ^{15}N-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 ^{15}N-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% ^{15}N) at each pulse. Leaf and petiole feeding led to higher ^{15}N enrichment of above- than below-ground parts in all tested species except in piegeonpea, 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 ^{15}N, the ^{15}N enrichment of the roots was only between 0.11 and 0.90 atom% ^{15}N 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). ^{15}N-labelling 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 ^{15}N 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 ^{15}N-urea (99 atom% ^{15}N), 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 ^{15}N content of 2.5 atom% ^{15}N excess of the plant N during the growing demand. All experiments undertaken on pea showed that ^{15}N 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 ^{15}N 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 ^{15}N-enriched than roots. Root enrichment ranged between 1.1 and 1.4 atom% ^{15}N excess in Russell and Fillery (1996a), Mayer et al. (2003) and Wichern et al. (2007a) but reached up to 3.6 atom% ^{15}N 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 ^{15}N-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 ^{15}N-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 $(^{15}NH_4)_2SO_4$ containing 66.7% ^{15}N: root ^{15}N enrichment was 9.92 atom% ^{15}N excess. Jensen (1996a), Schmidtke (2005a) and Mahieu et al. (2007) used a 5% or 10% ^{15}N-enriched KNO_3-N, and 10% ^{15}N-enriched NO_3-NH_4, respectively; root enrichments comprised between 0.2 and 3.5% atom% ^{15}N 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 ^{15}N (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

57% (Tab. I). Using leaf feeding with ^{15}N-urea, the ratio of rhizodeposited N: plant N differed markedly among species (from 10% in subterraneum clover to 57% in serradella); values obtained in subterraneum 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 Whipps, 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 and 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 pseudoaccacia* 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

et al., 2005). The reverse is true for other amino acids such as asparagine and glutamine, which are recovered in low proportions in exudates but in high 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 ^{15}N-labelled amino acids has shown that efflux of glycine and serine from roots of legumes is higher than from roots of grasses (Lesuffleur et 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 CO_2 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-wick techniques 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.

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