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Estimating N transfers between N_2-fixing actinorhizal species and the non-N_2-fixing *Prunus avium* under partially controlled conditions

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Abstract Two methods of N transfer between plants—by litter decomposition and root-to-root exchange—were examined in mixed plantations of N-fixing and non-fixing trees. Nitrogen transfers from decaying litters were measured by placing ^{15}N-labelled litters from four actinorhizal tree species around shoots of containerized *Prunus avium*. Nitrogen transfers by root-to-root exchanges were measured after foliar NO_3-^{15}N fertilization of *Alnus subcordata* and *Elaeagnus angustifolia* growing in containers in association with *P. avium*. During the first 2 years of litter decomposition, from 5–20% of the N, depending on the litter identity, was released and taken up by *P. avium*. N availability in the different litters was strongly correlated with the amount of water-soluble N, which was highest in leaves of *E. angustifolia*. In the association between fixing and non-fixing plants, 7.5% of the *A. subcordata* N and 25% of *E. angustifolia* N was transferred to *P. avium* by root exchange. These results showed that the magnitude of N transfers by root exchange depended on the associated N_2-fixing species. Among the species investigated, *E. angustifolia* displayed the highest capacity for exudating N from roots as well as for releasing N from litters. These qualities make this tree a promising species for enhancing wood yields in mixed stands.

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Introduction

Actinorhizal plants often occur as pioneer vegetation in the early stages of plant succession. Because of their ability to increase the soil N content, these plants are often used to improve impoverished soils (Dawson 1990; Requenal et al. 2001), in both agroforestry systems and sylviculture, especially in mixed stands (Hansen and Dawson 1982; Van Sambeek et al. 1986; Paschke et al. 1989; Vandermeer 1989). The beneficial effect of actinorhizal plants on the growth of their associated trees has been mainly attributed to (1) a transfer of symbiotically fixed N by means of the decomposition of leaves and roots, or (2) a direct excretion of N from root systems. In the first case, considerable amounts of N transferred in soils—ranging from 48–185 kg N ha^{-1} year^{-1} in alder stands (Danière et al. 1986) and up to 236 kg N ha^{-1} year^{-1} in plantations with *Elaeagnus umbellata* (Paschke et al. 1989)—have been reported. For the second transfer modality, N excretions have been estimated to give rise to annual N accretion in soils of around 35–45 kg N ha^{-1} (Berg and Doerksen 1975; Bormann and De Bell 1981).

Explanations for variations in N transfers lie in the litter decomposition process itself. It is known that the release of mineral N from decaying litter results from complex interactions between microbial populations and chemical composition of leaves. Hence, the substrate quality, as defined by its C/N and its lignin and phenolic contents, would be a critical factor determining the rate of litter decay (Swift et al. 1979; Paul and Clarke 1989). Melillo et al. (1982) and Semwal et al. (2003) found that rates of litter decomposition were negatively correlated with the ratio of lignin concentration to initial N concentration in hardwood leaves. The effect of actinorhizal plants on associated non-fixing species was found to depend on the considered species (Dawson

1990). According to Domenach et al. (1994), their leaf contents in water-soluble N, cellulose and lignin may be highly different even when their C/N ratio was similar.

Results of studies on amounts of root-transferred N between fixing and non-fixing plants are markedly different according to authors. Indeed, many field studies on legume–cereal intercrops did not show evidence of N transfer (Ofori et al. 1987; Giller et al. 1991; Lehmann et al. 2000), but other studies found that significant amounts may be transferred (Eaglesham et al. 1981; Ledgard 1991; Snoeck et al. 2000). However, because of the limitations imposed by the available methods, field measurements that clearly demonstrated N transfers from roots of N_2-fixing plants to those of associated plants are scarce and were mainly conducted on herbaceous species (Haystead et al. 1988; Bethlenfalvay et al. 1991; Johansen and Jensen 1996; Paynel et al. 2001). To date, studies on root-to-root transfer of N, with actinorhizal tree species as N_2-fixers, have been carried out on microcosms in the laboratory (Kurdali et al. 1990; Arnebrant et al. 1993), and studies under field conditions are still lacking.

To monitor the transfer of N without directly affecting soil N pools, studied trees can be labelled with ^{15}N. Several methods have been tested, including fixation of $^{15}N_2$ (McNeil et al. 1994), stem injection (Swanston and Myrold 1998), and foliar fertilization with ^{15}N (Gonzalez Prieto et al. 1995). The latter appears to be the most suitable for labelling small tress since, in this case, no special equipment is required and the trees are not damaged.

In this paper our objectives were (1) to highlight the possible relationships between the litter quality of different actinorhizal species, as defined by their chemical composition, and their capacity to supply N assimilable by *Prunus avium*, an associated non-fixing tree, and (2) to evaluate the relative contribution of the two methods of N transfer (N release by litter decays or root-to-root exchange) within each tree association.

Material and methods

N transfer from litter of different actinorhizal species

Experiments were conducted to investigate N transfer from litter of different actinorhizal plants to the non-actinorhizal species *Prunus avium*. In this study, ^{15}N-enriched litters from *Alnus subcordata*, *Alnus incana*, *Elaeagnus angustifolia* and *Hippophae rhamnoides* were used as substrate for trees of *P. avium* growing in individual pots. To obtain labelled litters, 2-year-old actinorhizal trees previously inoculated with compatible active strains of *Frankia* were transplanted in individual 3-l pots containing an artificial substrate of dried clay. Trees were fertilized every 3 days during a period of 25 weeks, with an ^{15}N-enriched KNO_3 solution (5.5 atom $\%^{15}N$, 75 mg N supplied over the entire period). Because individuals of *H. rhamnoides* were the smallest among the actinorhizal species, their N supply was only 40 mg N per plant. Leaves were collected and oven-dried at 60 °C for 48 h. Samples were milled to a fine powder and $\delta^{15}N$, total C and N concentrations were measured using a Finningan MAT delta S mass spectrometer (Finningan, Bremen, Germany) coupled with a CN elemental analyser (SCA CNRS, Vernaison, France) (Pachiaudi et al. 1991). Water-soluble hemicelluloses, cellulose and lignin contents were measured from the four actinorhizal species leaves as described by Bigois et al. (1991) and Domenach et al. (1994) in order to assess the quality of the substrate, which is a critical factor determining the rate of litter decay (Swift et al. 1979; Paul and Clark 1989).

Twenty five individuals of 1-year-old *P. avium* trees were grown under natural climatic conditions in 75-l individual pots containing a 1:3 (v/v) dehydrated clay-silty-loam soil mixture (pH=6.5). Aliquots of labelled leaves (100 mg N-equivalent) from each of the four actinorhizal species (*A. subcordata*, *A. incana*, *E. angustifolia* and *H. rhamnoides*) were placed in the surface of the soil around *P. avium* shoots (five replicates per actinorhizal species, five individuals without litter supply as controls) in April of the first experimental year.

All *P. avium* leaves were collected at the end of the autumn during the first year. Leaves, twigs and roots were harvested at the end of the summer during the second year, and dry mass, N concentration and $\delta^{15}N$ of samples were measured.

Root-to-root transfer

Experiments were conducted on mixed plantations of 2-year-old *P. avium* (non-N_2-fixing tree) and *A. subcordata* or *E. angustifolia* (N_2-fixing trees, five replicates per association) during the period from spring to summer. Paired trees were transplanted in 125-l pots containing a 1:3 (v/v) soil (used in experiment 1) dried clay-perlite mixture. In each set, N_2-fixing trees were labelled according to the method of Gonzalez Prieto et al. (1995) by a gentle spraying up to foliar saturation with a ^{15}N-enriched KNO_3 solution (15 atom $\%$ ^{15}N, 8 ml per tree at 0.1 mg N ml^{-1}). The gradual enrichment in ^{15}N in the plant after foliar spraying has been discussed by Gonzalez Prieto et al. (1995): ^{15}N is incorporated into the different organic fractions and translocated within the plant as the ^{15}N assimilated by roots or fixed by nodules.

The foliar fertilization was applied three times a week on four individuals per set (one non-labelled control pot per set), *P. avium* were not labelled and risks of ^{15}N contamination during spraying were prevented by protecting trees with a plastic bag, until leaves of the labelled trees had completely absorbed the solution. Soil ^{15}N contamination in pots was also avoided by placing a permanent plastic film on the soil surface.

Plant shoots, leaves and roots were sampled in September and analyzed for their N concentration and $\delta^{15}N$. Only roots still connected to shoots or well recognizable by their color were kept. Soils from the rhizosphere of each plant and from the pot subsurface were also analyzed.

Calculations

Since labelled plants displayed $\%^{15}N$ values close to natural abundance, results were expressed in $\delta^{15}N$ rather than in $\%^{15}N$ excess (Tables 1 and 5):

$$\delta^{15}N_{sample} = 1000\left(\text{atom}\%^{15}N_{sample} - 0.3663\right)/0.3663 \quad (1)$$

where 0.3663 is the atom $\%^{15}N$ of the atmosphere, which is constant (Mariotti 1983).

Calculations used in assessing N litter transfer

Fertilizer use efficiency (FUE), the percentage of N released by each litter and used by *P. avium*, was calculated in different plant parts (pp: leaves, twigs and roots) as follows:

$$FUE_{pp} = 100\left(\%NdffL_{pp}.N_{pp}/N_{litter}\right) \quad (2)$$

N_{pp} is total N content in the considered plant part and N_{litter} is total N content in litter (100 mg N). $\%NdffL_{pp}$ is the percentage of N derived from litter in the considered plant part:

$$\%NdffL_{pp} = 100\left(atom\%^{15}N_{labelled\,pp} - atom\%^{15}N_{non-labelled\,pp}\right)/\left(atom.\%^{15}N_{labelled\,litter} - atom\%^{15}N_{non-labelled\,pp}\right) \quad (3)$$

where $atom\%^{15}N_{labelled\ pp}$ is the labelled considered plant part and $atom\%^{15}N_{\ non\text{-}labelled\ \ pp}$ is the non-labelled considered plant part.

Using Eq. (1), this percentage can be expressed in $\delta^{15}N$ values instead of $atom\%^{15}N$:

$$\%NdffL_{pp} = 100\left(\delta^{15}N_{labelled\,pp} - \delta^{15}N_{non-labelled\,pp}\right)/\left(\delta^{15}N_{labelled\,litter} - \delta^{15}N_{non-labelled\,pp}\right) \quad (4)$$

FUE in *P. avium* = $\sum FUE_{pp.}$

Calculations used in assessing N root-to-root transfer

^{15}N content in each plant part ($^{15}N_{pp}$) is calculated as follows:

$$^{15}Npp = \%^{15}N_{pp}.p_{pp}.N_{pp} \quad (5)$$

Total plant ^{15}N content ($^{15}N_{Tp}$) is calculated thus:

$$^{15}N_{Tp} = \sum \%^{15}N_{pp}.p_{pp}.N_{pp} \quad (6)$$

where p_{pp} is plant part dry mass and N_{pp} is N concentration in the plant part.

The percentage of N transferred from N_2-fixing trees to *P. avium* (% N_T) was calculated following the Ledgard et al. (1985) equation:

$$\%N_T = 100.{}^{15}N_{excess\,in\,P.avium}/\left({}^{15}N_{excess\,in\,P.avium} + {}^{15}N_{excess\,in\,N2-fixing\,tree}\right) \quad (7)$$

where $^{15}N_{excess}$ is plant ^{15}N excess content:

$$^{15}N_{excess} = \text{total N content}.atom\%^{15}N_{excess}. \quad (8)$$

with $atom\%^{15}N$ excess = $atom\ \%^{15}N_{sample}$ − $atom\%\ ^{15}N_{control}$.

Or this can be expressed in $\delta^{15}N$:

$$\%N_T = 100\left(\delta^{15}N_{P.avium\,labelled} - \delta^{15}N_{P.avium\,control}\right)/\left(\left(\delta^{15}N_{P.avium\,labelled} - \delta^{15}N_{P.avium\,control}\right) + \left(\delta^{15}N_{N2-fixing\,tree\,labelled} - \delta^{15}N_{N2-fixing\,tree\,control}\right)\right) \quad (9)$$

Results and discussion

N transfer from litter

The ^{15}N values in actinorhizal tree leaves used as litter were 0.75±0.03 atom $\%^{15}N$ in *A. subcordata*, 0.67±0.02 in *A. incana*, 0.47±0.01 in *H. rhamnoides* and 0.74±0.02 in *E. angustifolia*. These values are low if we consider (1) the relatively high ^{15}N isotopic abundance of the $K^{15}NO_3$ solution (5.5 atom $\%^{15}N$) and (2) the high amounts (75 mg) of NO_3-^{15}N supplied to each plant. It is likely that N requirements of actinorhizal trees were almost entirely satisfied by N_2 fixation, which results in a strong ^{15}N dilution in plants. High N_2-fixation rates in actinorhizal trees species growing in soils exhibiting high N-availability conditions have already been reported (Beaupied et al. 1990; Mead and Preaston 1992). Using the values presented in Table 1 and the method of calculation given above, the FUE of *P. avium* was calculated for different

Table 1 Dry mass (in g), N concentration (in %) and $\delta^{15}N$ (in ‰) in *Prunus avium* trees grown during 2 years with ^{15}N-enriched litters of *A. incana*, *A. subcordata*, *E. angustifolia* and *H. rhamnoides*. *Numbers in parentheses* Standard deviation with *n*=5

	First year	Second year		
	Leaves	Leaves	Twigs	Roots
P. avium without litter				
Dry mass	7.3 (1.3)	6.0 (1.4)	14.9 (1.4)	24.0 (8.5)
N concentration	2.1 (0.1)	1.3 (0.2)	0.4 (0.1)	0.5 (0.1)
$\delta^{15}N$	0.8 (0.3)	–0.7 (1.1)	0.9 (1)	1.6 (0.6)
P. avium with *A. incana* litter				
Dry mass	6.4 (2)	3.1 (1)	8.7 (2.1)	11. (3.2)
N concentration	1.85 (0.2)	1.25 (0.1)	0.45 (0.1)	0.65 (0.1)
$\delta^{15}N$	9.6 (5.6)	9.3 (1.1)	11.8 (1.4)	14.2 (4.8)
P. avium with *A. subcordata* litter				
Dry mass	6.1 (2.8)	3.3 (1.8)	8.9 (6.6)	12.25 (10.8)
N concentration	1.9 (0.2)	1.35 (0.1)	0.5 (0.2)	0.6 (0.1)
$\delta^{15}N$	6.4 (3)	6.5 (1.6)	7.7 (0.7)	9.2 (1.8)
P. avium with *E. angustifolia* litter				
Dry mass	8.3 (2.3)	7.2 (1.9)	19.5 (3.8)	26.7 (5.3)
N concentration	1.8 (0.2)	1.3 (0.2)	0.4 (0.1)	0.6 (0.04)
$\delta^{15}N$	32.3 (6)	25.5 (6.8)	25.7 (7.6)	30.2 (2.7)
P. avium with *H. rhamnoides* litter				
Dry mass	7.7 (2.0)	5.0 (2.6)	13.1 (5.3)	18.7 (9.6)
N concentration	1.7 (0.2)	1.3 (0.2)	0.4 (0.1)	0.65 (0.1)
$\delta^{15}N$	7.1 (2.0)	6.8 (2.6)	9.3 (1.8)	7.9 (1.6)

Table 2 Fertilizer use efficiency (FUE, in %) in *Prunus avium* grown during 2 years in soils enriched with litters from different actinorhizal species (100 mg N litter equivalent) *Numbers in parentheses* Standard deviation with *n*=5

Litters	First year Leaves	Second year				First and second years Total
		Leaves	Twigs	Roots	Total	
E. angustifolia	4.3 (0.9)	4.1 (0.4)	4.2 (0.4)	4.3 (0.4)	12.6 (1.2)	16.9 (2.1)
A. incana	0.91 (0.5)	0.84 (0.3)	0.86 (0.3)	0.88 (0.3)	2.58 (0.9)	3.5 (1.4)
A. subcordata	0.58 (0.41)	0.45 (0.28)	0.45 (0.28)	0.44 (0.27)	1.34 (0.8)	1.92 (1.2)
H. rhamnoides	2.84 (0.86)	0.66 (0.24)	0.64 (0.23)	0.63 (0.23)	1.93 (0.70)	4.8 (1.56)

Table 3 Leaf carbon and N constituents in actinorhizal tree leaves. *Numbers in parentheses* Standard deviation with *n*=2

	Hippophae rhamnoides	*Elaeagnus angustifolia*	*Alnus subcordata*	*Alnus incana*
N total (mg g^{-1})	30.5	22.5	24.7	26.5
C/N	16	20	20.2	19.3
Water-soluble N (mg g^{-1} leaf)	10.0 (0.8)	15.5 (0.7)	6.3 (0.0)	5.4 (0.6)
Percentage cellulose	13.4 (0.4)	22.4 (0.6)	13.4 (1.1)	21.9 (1.7)
Percentage hemicellulose	31.1 (1)	7.9 (0.9)	29.6 (3)	30.2 (0.7)
Percentage lignin+tannin	13 (1.5)	25.6 (0.7)	14.9 (1.8)	8.3 (0.8)

litter species over the years of experiment (Table 2). After 1 and 2 years, the values of FUE in *P. avium* were dependent on the litter species. The FUE values found in leaves of *P. avium* at year 1 were similar to those found at year 2 for each species litter, except for *H. rhamnoides* litter. Indeed, in the case of *H. rhamnoides* litter, the FUE value in leaves of *P. avium* in year 1 was found to be four times higher than the FUE value of year 2. These results suggest that for *A. subcordata*, *A. incana* and *E. angustifolia* litters, the dynamics of litter decay during the first year was not significantly different from that occurring during the second year. Contrastingly, it is likely that the litter decay of *H. rhamnoides* was much more rapid during the first than during the second year. Moreover, the FUE values observed at the end of the experiment (second year) were similar in all the *P. avium* organs. This suggest that the fertilizer was used with a homogeneous efficiency by the different plant organs, an interpretation that could not be proposed from the results of Table 1 (the δ^{15}N values of the different organs being far from similar).

The total FUE in *P. avium* ranged from 2–17 mg N per 100 mg N added over the entire period (Table 2). The highest values were obtained with the litter of *E. angustifolia* and the lowest with those of *A. incana* and *A. subcordata*. It is well known that N mineralization is usually facilitated by high N contents and/or low C/N ratios (Edmonds 1980). However, the variability of these parameters in leaves (N content and C/N) among the four actinorhizal species (Table 3) was not sufficient to explain the observed differences in N transfer. Furthermore, the leaf concentrations in cellulose, hemicellulose and lignin were different between species (Table 3), but were not correlated with the amounts of transferred N. The following trends may nevertheless be highlighted: litters with high lignin concentrations (for instance, *E. angustifolia*) exhibited high ability for releasing N assimilable by plants, while litters with low lignin concentrations (for instance, *A. incana*) did not. These results are not in agreement with what has been usually found, i.e. negative correlations between rates of litter breakdown and lignin concentration in leaves (Palm and Sanchez 1991; Oglesby and Fownes 1992; Tian et al. 1992) or lignin-to-N ratio in leaves (Semwal et al. 2003).

Extractable organic substances are considered as the labile pool of tree litters, because they are quickly decomposed by soil microorganisms (Taylor et al. 1989). In our study, the water-soluble N in litters ranged from 5–15‰ (Table 3) depending on the actinorhizal species, *E. angustifolia* exhibiting the highest value. These values were positively correlated with the amounts of N assimilated by *P. avium* during both years of experiment (Fig. 1), suggesting that the amounts of water-soluble N in leaves constitute a strong determinant of the capacity of litters to release N. This may explain why, despite a high concentration in lignin (which is, as stated above, usually known to limit biodegradation), leaves of *E. angustifolia* showed high abilities to release N. Cromack et al. (1976) evaluated that around 4 years is needed to reach total decomposition of red alder leaves. In our case, it seems that only water-soluble N was available during the two experiment years and that the other litter N fractions were

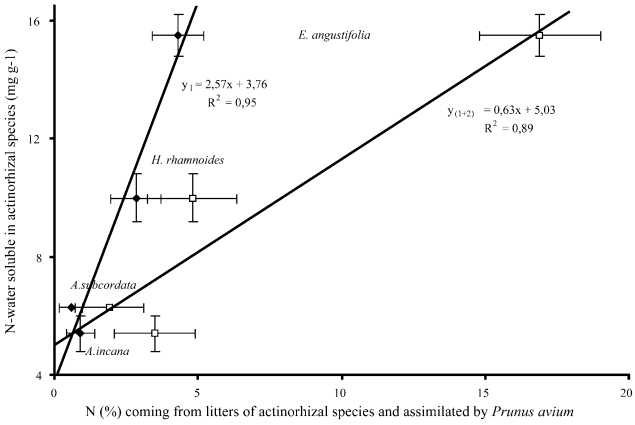

Fig. 1 Relationship between percentage of water-soluble N in litters of *Alnus subcordata*, *Alnus incana*, *Elaeagnus angustifolia* and *Hippophae rhamnoides* and fertilizer use efficiency (FUE) coming from these litters evaluated from leaves of *Prunus avium* in the first year and from the total plant in the second year

only poorly utilized by *P. avium*. It can be hypothesized that these fractions were (1) not actively mineralized or (2) mineralized but quickly immobilized by the microorganisms.

Root-to-root transfer

As similar ^{15}N abundance was measured in soils and rhizospheric soils that support growth of ^{15}N-labelled plants and in control soils (i.e., soil supporting the growth of non-labelled plants) (Table 4), it may be assumed that no contamination occurred during spraying.

From the values of ^{15}N content shown in Table 5, it may be deduced that the ^{15}N incorporated into both actinorhizal species after spraying was translocated within their different organs and also transferred to their associated tree (which exhibited ^{15}N enrichment without ^{15}N supply). However, the ^{15}N isotopic abundances observed in *E. angustifolia* and *A. subcordata* were much lower than the ^{15}N isotopic abundance of the applied fertilizer (15 atom $\%^{15}N$) (Table 5). Gonzalez Prieto et al. (1995) found similar results on *Alnus glutinosa* (^{15}N abundance in leaves of 0.4551 atom $\%^{15}N$ after foliar spraying with a 10-atom $\%^{15}N$ fertilizer). These authors suggested that ^{15}N dilution in plants resulted from high rates of N_2 fixation. Moreover, the duration of leaf exposition to $^{15}NO_{-3}$ was too short to allow the incorporation of a high quantity of fertilizer.

^{15}N contents in excess were higher in *A. subcordata* than in *E. angustifolia*, suggesting a higher retention of the labelled N by the former. This interpretation is strengthened by the fact that the values of $\delta^{15}N$ observed

Table 4 $\delta^{15}N$ and N concentration of soil and rhizospheric soil in mixed plantations of *Prunus avium* with *A. subcordata* or *E. angustifolia*. *Numbers in parentheses* Standard deviation with n=4

Mixed plantation	Without ^{15}N foliar application				With ^{15}N foliar application			
	Soil		Rhizosphere		Soil		Rhizosphere	
	$\delta^{15}N$	%N	$\delta^{15}N$	%N	$\delta^{15}N$	%N	$\delta^{15}N$	%N
A. subcordata with *P. avium*	1.9	0.08	2.4	0.09	2.0 (1.1)	0.06 (0.00)	2.3 (0.7)	0.08 (0.01)
E. angustifolia with *P. avium*	2.3	0.09	2.9	0.12	2.4 (0.6)	0.07 (0.02)	3.7 (2.4)	0.09 (0.02)

Table 5 Dry mass, N concentration, $\delta^{15}N$, N content and $^{1\ 5}N$ content in excess in leaves, stems and roots of trees in two mixed plantings (*P. avium*/*A. subcordata* and *P. avium*/*E. angustifolia*) after $K^{15}NO_3$ foliar application on fixing trees. *Numbers in parentheses* Standard deviation with n=4. *K** is a constant value = $0.3663/10^2 x 10^3$

Associated trees		1 Dry mass (g)	2 N concentration	3 $\delta^{15}N$ (%)	4 $\delta^{15}N$ control without $^{15}NO_3$	1×2 N content (g)	1×2(3–4)xK* ^{15}Ncontent in excess (g K)
A. subcordata/*P. avium*							
A. subcordata	Leaves	62 (14)	2.2 (0.4)	23.4 (3.3)	−1.6	136	3,410
	Stems	85 (14)	0.86 (0.01)	23.9 (1.2)	−1.8	73	1,878.7
	Roots	78 (18)	1.2 (0.2)	13.9 (3.5)	−1.1	94	1,404
P. avium	Leaves	10 (2)	1.6 (0.3)	7.03 (0.5)	0.2	16	109.3
	Stems	34 (17)	0.5 (0.1)	7.5 (0.1)	–0.7	17	139.4
	Roots	30 (0.5)	0.9 (0.1)	7.7 (1)	0.75	35	244
E. angustifolia/*P. avium*							
E. angustifolia	Leaves	24 (3)	3.3 (0.2)	14.1 (3.1)	–1.6	79	1,116.7
	Stems	43 (8)	2.0 (0.06)	8.12 (1.5)	–2	86	870.3
	Roots	37 (4)	2.9 (0.1)	3.9 (0.7)	–1.2	107	547.2
P. avium	Leaves	13 (4)	1.6 (0.1)	12.9 (4.2)	1.65	21	234
	Stems	40 (8)	0.5 (0.03)	12.2 (2)	0.6	20	232
	Roots	50 (3)	0.8 (0.1)	11.9 (3.8)	1.3	40	424

in *P. avium* were higher with the *E. angustifolia* pairing than with *A. subcordata* pairing (Table 5). It is noticeable that *P. avium* exhibited higher $\delta^{15}N$ values than the associated *E. angustifolia*, which directly received the labelled fertilizer. This result confirms that the ability of *E. angustifolia* to retain N is low, resulting in a high capacity of this plant to transfer N to trees growing in association.

N transferred was evaluated from values obtained from Table 5 by applying Eq. (9):

- From *A. subcordata* to *P. avium* = 7.5% (2%) of the total N of *A. subcordata*
- From *E. angustifolia* to *P. avium* = 25% (5%) of the total N of *E. angustifolia*

(in parenthesis, the standard errors of the means calculated on four replicates)

Our results were close to those of Arnebrant et al. (1993) who calculated that around 20% of the N in pines was derived from atmospheric N_2 fixed by *A. glutinosa*. As experimental conditions were similar for both associations, the quantity of transferred N appears to depend on the species of the associated plant. This observation is in agreement with several studies on mixed and intercropping systems with legumes. Indeed, (1) Ikram et al. (1994) found that no significant amount of N was transferred between *Pueraria* and *Hevea*, (2) Burity et al. (1989) estimated N transfers from alfalfa of up to 38% of the total annual brome grass N yield, and (3) below-ground transfers of fixed N by white clovers to grasses were estimated to be around 60 kg N ha^{-1} $year^{-1}$ by Ledgard (1991), and from 29–70 kg N ha^{-1} $year^{-1}$ (depending on the year) by Elgersma et al. (2000). According to Hamel et al. (1991), the extent of the root contact between N_2-fixing legumes and maize was the main factor affecting N transfers. Moreover, these authors indicated that N transfers increased with time, particularly between weeks 12 and 15. In our study, after the 4-month experiment, roots of fixing trees were closely intertwined with those of *P. avium*, suggesting that conditions for optimal N transfers had occurred.

Transferred N can come from (1) root dead cells, (2) exudates assimilated after immobilization-mineralization and/or (3) direct transfers from associated N_2-fixing plants. However, only the two latter hypotheses may explain the fact that rhizospheric soils supporting labelled as well as unlabelled plants exhibited similar $\delta^{15}N$ values (Table 4): N was directly transferred, or the receiver plant is able to efficiently assimilate the N released by the donor plant, after a quick mineralization which allows minimization of losses of ^{15}N into the soil.

Mycorrhizal interconnections between trees are well known for enhancing N transfers (Van Kessel et al. 1985; Arnebrant et al. 1993), allowing a direct N transfer through roots. Leyval and Berthelin (1993) and Grayston et al. (1996) showed that N exudation was stimulated by the presence of mycorrhizae. Additionally, it was demonstrated that the direction and the extent of nutrient movement through the mycelium were influenced by a source/sink relationship (Bethlenfalvay et al. 1991) or by the mycorrhizal fungus species (Ek Blad and Huss-Danell 1995). Mycorrhizal interconnections between trees were also observed in our experiment, and it is likely that the quantity of N transferred between plants was enhanced by the presence of the fungus. N movements between plants were also probably enhanced by the high source/sink relationship imposed in the experiment, or, in other words, by the limited soil-N availability (Table 4). Thus, N movements from actinorrhizal trees with high rates of N_2 fixation (i.e. *E. angustifolia*) (see Rodriguez-Barrueco and Moiroud 1989 for review) to non-N_2-fixing plants were expected to be greatly enhanced in poor N soils.

Conclusion

Nitrogen contents as well as biomass production of non-N_2-fixing trees are often higher in interplanted or mixed stands with N_2-fixing species than in pure stands. Actinorhizal species are well suited to interplantings due to their high N contents in leaves and their high production of litter. These are considered as the main factors for enhancing N nutrition in associated trees (Dawson 1990). Large differences between amounts of N transferred from the actinorhizal plant to the companion trees were observed. These are due to the fact that the actinorhizal species considerably differ in their ability to improve the growth of the associated non-fixing plants. In turn, such ability depends on the litter composition and, especially, on the content of water-soluble N (at least during the first stages of litter decomposition).

Additionally, below-ground translocations of N seem to be efficient in interplantings, as it was observed with *A. subcordata* and even more clearly with *E. angustifolia* pairings. This may be due to mycorrhizal connections between N_2-fixing and non-N_2-fixing trees, which enhance the amount of N transferred.

Elaeagnus angustifolia, which presents all the characteristics for supplying large amounts of N to other associated plants, may be considered as a promising species for enhancing wood yields in mixed stands.

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