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REGULAR ARTICLE

Sharing N resources in the early growth of rapeseed intercropped with faba bean: does N transfer matter?

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Abstract
Background and aims Legume-brassica intercrops may help to reduce N fertilizer input. We tested whether (*i*) intercropping with faba bean can improve N status of rapeseed, and (*ii*) root complementarity and/or N transfer is involved in such performance.
Methods Pre-germinated rapeseed and faba bean were grown either together or in monospecific rhizotrons (2 plants per rhizotron). Root growth was recorded. N rhizodeposition of the crops and N transferred between species were assessed using a ^{15}N stem-labelling method.
Results Intercropped rapeseeds accumulated 20 % higher amounts of N per plant than monocultures. Up to 32 days after sowing, root distribution in the rhizotrons was favourable to physical sharing of the soil N: 64 % of faba bean root length was located in the upper part, as 70 % was in the lower part for rapeseed. At late flowering of the faba bean (52 days after sowing), N rhizodeposition of the two crops were similar and reached 8 to 9 % of the plant N. N transferred from the faba bean to the rapeseed was similar to that transferred from the rapeseed to the faba bean.
Conclusions Niche complementarity benefits more intercropped rapeseed than net N fluxes between species in the early growth.

Keywords Legume · Facilitation · Niche separation · Intercropping · Biological fixation · Root complementarity

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Introduction

The increasing use of chemical fertilisers has affected the nitrogen (N) cycle, resulting in a dramatic elevation of the amount of reactive nitrogen in the biosphere, which has negative consequences on climate, ecosystem resilience, and health (Galloway et al. 2003). Biological N fixation (BNF) by legumes can act as a sustainable source of N and can complement or replace some fertiliser inputs (Garg and Geetanjali 2007). Growing a legume (Fabaceae) with a non-fixing species leads to a more efficient use of light and soil resources and has a positive effect on plant productivity (Hauggaard-Nielsen and Jensen 2005; e.g., Malézieux et al. 2009). Thus, intercropping with legumes has the potential to combine high economic performance and low environmental impact in systems by reducing the amount of fertiliser supplied (Pelzer et al. 2012).

For 10 years, substantial information about the N fluxes and interactions between legume species and either perennial grasses or cereals has been collected (Høgh-Jensen and Schjoerring 2001; Corre-Hellou et al. 2007; Bedoussac and Justes 2010). However, although brassicas require large amounts of N for growth (Malagoli et al. 2005), the practice of brassica-legume intercropping remains poorly documented (Banik et al. 2000; Schröder and Köpke 2012). More research in this area would guide decisions regarding which species and genotypes should be associated to improve intercrop management.

In annual cereal-legume intercrops, the niche separation effect contributes to higher yields than in monocultures of non-fixing species. Furthermore, in mixtures with non-fixing plants, the percentage of the legumes N derived from biological fixation (%Ndfa) is higher than in monocultures (Corre-Hellou et al. 2006). In addition, the level of fixed N_2 was shown to be higher in low-input systems than in high-input systems and led to more stable yields in constraining environments (Jensen 1996; Corre-Hellou et al. 2007).

Similar results have been obtained in perennial grass-legume mixtures (Carlsson and Huss-Danell 2003; Nyfeler et al. 2011). However, in perennial grass communities, it has also been shown that legumes increase the soil-N pool and that grasses can benefit from N provided by the roots of neighbouring legumes (Gylfadóttir et al. 2007; Pirhofer-Walzl et al. 2012). Such N transfer is thought to occur through different pathways (e.g. Fustec et al. 2010). N transfer through nitrate ammonium and/or amino acids exudated by clover roots followed by N uptake by ryegrass was shown to be a major pathway in 2-months-old plants (under hydroponic conditions; Paynel et al. 2008). In older plants, the turnover of N in belowground parts is thought to be the main source of transferable N between plants (Høgh-Jensen and Schjoerring 2001). In annual crops, the N transfer between the roots of legumes and those of non-fixing species remains poorly documented, especially in the case of brassica-legume intercropping. Using a split-root ^{15}N labelling method, Jensen (1996) showed that after 75 days of growth, the amount of N transferred from field pea to barley grown in the same pot may reach 19 % of the total barley plant-N, depending on photosynthesis intensity, root intermingling and nodule activity.

Recent attention has focused on the faba bean (*Vicia faba* L. spp. *minor*) as a key species for enhancing the sustainability of agroecosystems (Jensen et al. 2010; Köpke and Nemecek 2010). In particular, faba bean has the ability to symbiotically fix high amounts of atmospheric nitrogen under a wide range of agronomic conditions (Carranca et al. 1999; Peoples et al. 2009). Furthermore, several studies have provided evidence of high amounts of belowground nitrogen input to the soil via rhizodeposition (e.g., exudates, root cell decay, root turnover) throughout the plant cycle (Rochester et al. 1998; Khan et al. 2002; Mayer et al. 2003). As a consequence the faba bean may be a suitable candidate for intercropping with non-fixing species with high N needs such as rapeseed. In a preliminary study, we have shown a physical complementarity between the roots of rapeseed and those of faba bean at the early stage of growth (Cortes-Mora et al. 2010). N transfer from the legume to the brassica were also detected. However, in this study, the intermingled below ground parts of the two crops were too difficult to sort, so calculations did not include root-N. Furthermore, N transfer from the brassica to the legume have not been assessed. In the present study, we used a ^{15}N stem-labelling method and a protocol design allowing for estimating the total plant-N, the net N fluxes between the both crops and the N rhizodeposition of each species. Our aims were to test whether *i*) intercropping rapeseed with faba bean can improve yield and N status of rapeseed compared with monoculture and *ii*) root complementarity or/and N transfer are involved in N status of rapeseed.

Material and methods

Experimental design

Two experiments were undertaken in a greenhouse in Angers, France (47°28′0″ N, 0°47′31″W), in 2 successive years. *Brassica napus* L. cv Licapo and *V. faba* L. spp. *minor* cv. Divine were grown in rectangular rhizotrons (60 × 25 × 5 cm) made of transparent Altuglass® (Fig. 1). The rhizotrons were first filled with sand at the bottom to prevent water retention (5-cm layer) and then with N-poor sandy soil (8 kg dry weight (DW) per rhizotron) that had been carefully mixed, passed through a 2-mm sieve and inoculated

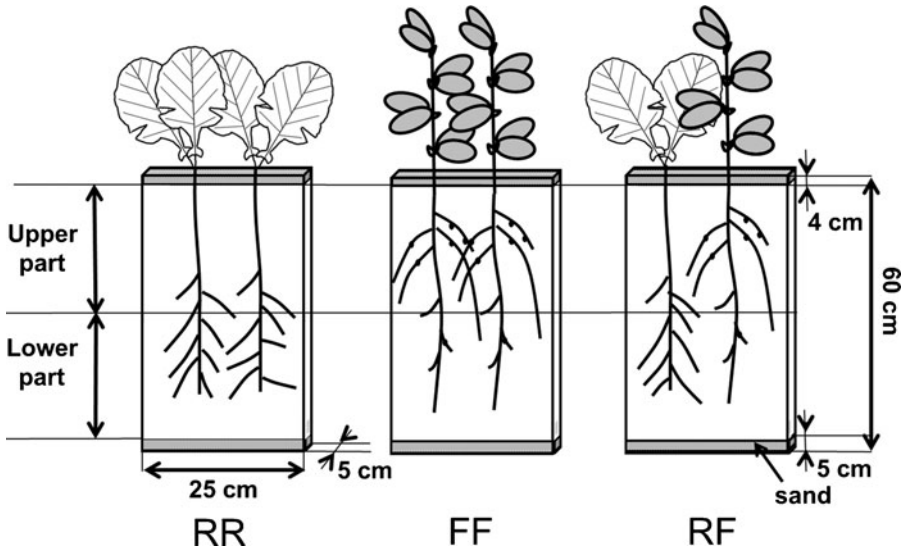

Fig. 1 Schematic representation of rapeseed (R) and faba bean (F) either in monoculture or intercropped in rhizotrons. FF and RR: two plants of faba bean or rapeseed in the same rhizotron; RF: one plant of each species intercropped in the same rhizotron. The rhizotrons were inclined at 45° and covered with black plastic during the experiments

with *Rhizobium leguminosarum* bv. *viciae* to enhance BNF.

In the rhizotrons, a 4-cm thick layer of sand was added to the top of the soil to prevent temporary seedling hypoxia after watering. Two plants were grown in each rhizotron. Seeds were first pre-germinated in moist vermiculite before being transplanted to the rhizotrons 8 cm apart (8 cm from the rhizotron edge). The rhizotrons were inclined at 45° to force the roots to grow towards the underside, and covered with black plastic during the experiments to avoid exposing the roots to light. Three kinds of rhizotrons were installed: *i*) monospecific rapeseed (RR), *ii*) monospecific faba bean (FF), and *iii*) intercropped rapeseed-faba bean (RF ; Fig. 1).

In the first experiment (Exp. 1), the ^{15}N label was carefully mixed into the soil of each rhizotron (isotopic dilution method - ID method) with the aim of assessing N uptake from the soil, BNF and interspecific N net transfer (Høgh-Jensen and Schjoerring 2001; Dahlin and Stenberg 2010). For each kind of rhizotron, the plants were harvested at three stages based on faba bean development (n=5; Fig. 2): 21 DAS (days after sowing, vegetative stage, 443°-days, with a base temperature of 0 °C; McMaster and Wilhelm 1997), 36 DAS (early flowering, 693°-days), and 85 DAS (maturity, 1,890°-days). The soil contained 10.9 % clay, 22.1 % silt, 66.2 % sand, 0.8 % organic matter, 0.47 % total C, 0.04 % total N, 70 mg P kg^{-1}, 150 mg K kg^{-1}, 140 mg Mg kg^{-1}, 8.0 mg NO_3^- kg^{-1}, 0.8 mg NH_4^+ kg^{-1}, and (average pH_{H2O} 7.0).

In the second experiment (Exp. 2), N rhizodeposition, and N transfer between plants were assessed using a ^{15}N stem-labelling method adapted from Russell and Fillery (1996). The plants were harvested at late flowering of the faba bean (52 DAS, 1,087°-days; Fig. 2) because of a sudden oidium attack on the rapeseed. The soil contained 7.9 % clay, 19.5 % silt, 60.2 % sand, 1.5 % organic matter, 0.86 % total C, 0.07 % total N, 75 mg P kg^{-1}, 77 mg K kg^{-1}, 54 mg Mg kg^{-1}, 14.8 mg NO_3^- kg^{-1} and 1.2 mg NH_4^+ kg^{-1} (average pH_{H2O} 7.8).

Labelling methods

Exp. 1 (ID method) One day before the start of the experiment, 500 g of fine sand was enriched with ^{15}N by mixing a 25 ml solution containing 20 mg $^{15}NO_3{}^{15}NH_4$ (99 % atom ^{15}N) and 43 mg ^{15}N-urea (98 % atom^{15}N) into the soil of each rhizotron.

Exp. 2 (stem-labelling method) At 25 DAS (503°-days), the labelled plants were stem-fed with ^{15}N urea using the cotton-wick method (Russell and Fillery 1996). The ^{15}N urea solution was taken up from a 1.5-ml vial by a wick, which was protected by silicon tubes sealed against the stem with putty to prevent desiccation and ensure waterproofness before passing through a hole into the base of the stem. During the experiment, the vial was filled with ^{15}N urea solution (99 % atom ^{15}N, dilution 0.2 %, Mahieu et al. 2009). In half of the monospecific rhizotrons, the two plants were labelled following this method for assessing the N rhizodeposition of both crops (F_LF_L, R_LR_L, n=5).

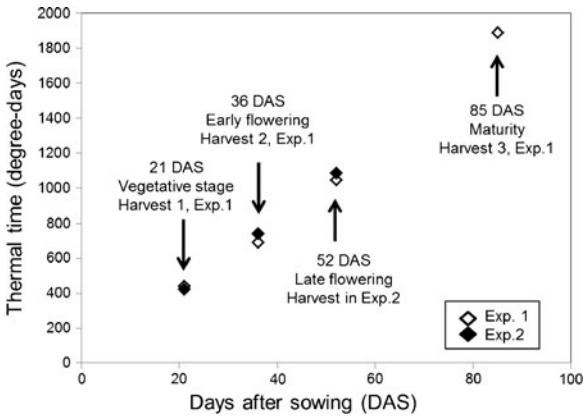

Fig. 2 Relationship between "Days after sowing" and "Thermal Time" for the two experiments. *Arrows* indicate the sampling times for both experiments

Simultaneously, in the faba bean–rapeseed rhizotrons, either the faba bean (RF_L) or the rapeseed (R_LF) was labelled with ^{15}N urea for estimating the bi-directional N transfer between crops (n=5). Unlabelled monospecific and interspecific rhizotrons were used as controls (FF, RR, RF; n=5).

Root growth survey

During Exp. 2, three times per week, the roots were drawn on a transparent acetate sheet fixed with adhesive tape to the lower side of the rhizotron. Different colours were used to distinguish the roots of rapeseed from those of faba bean. At 32 DAS (698°-days), just before flowering, as the roots reached the sand layer at bottom and the rhizotrons (Fig. 1), the last drawings were made.

Sampling and measurements

At harvest, in Exps. 1 and 2, the aboveground parts were separated from the roots by cutting the stem at the base. The rhizotrons were opened by sliding out their removable side, and all visible roots were recovered. In the monocultures, the roots of the two plants were pooled in the same sample. In intercrops, the roots of each plant were sorted into two different samples. A third sample ('root mixture') was made with the roots that could not be easily attributed to rapeseed or faba bean. All the soil was passed through a 2-mm sieve several times to collect the visible roots. The roots were washed with water at 4 °C, and the rinse waters of the different root samples were pooled. Additionally, in Exp. 2, the soil was passed through an automatic sampler to obtain 64 sub-samples. A 1/64 fraction of the rinse water mixture was added to one of the soil sub-samples before drying at 30 °C. Plant samples were dried at 70 °C. Soil and plant samples were ground into a fine powder and prepared for N and ^{15}N:^{14}N analysis (EA3000 EuroVector, mass spectrometer Horizons Nu Instruments).

Calculations and statistics

Experiment 1 (ID method) From the early flowering of the faba bean (36 DAS) to maturity (85 DAS), the percentage of N derived from fixation (%Ndfa) in the faba bean grown either in intercrop (RF) or monocrop (FF) systems was calculated with the ^{15}N-dilution method using monospecific rapeseed (RR) as a reference crop, as in Eq. 1 (Larue and Patterson 1981):

$$\text{Ndfa} = \left[1 - \left(\text{atom\%}^{15}\text{N excess faba bean} / \text{atom\%}^{15}\text{N excess RR}\right)\right] \times 100 \quad (1)$$

where atom%^{15}N excess was atom%^{15}N sample minus atom%^{15}N air N_2 (atom%^{15}N of air N_2 was 0.3663). At sowing, the faba bean seed provides a substantial amount of N for root and cotyledon growth; thus, ^{15}N and N derived from the seed were discounted from the plant ^{15}N content for calculations (López-Bellido et al. 2010). The ID method also allows for the assessment of the net N transfer between the two intercropped species according to Ta and Faris (1987).

Experiment 2 (Stem-labelling method) At harvest (52 DAS; Fig. 2), the BNF was calculated based on the natural abundance of ^{15}N in faba bean grown in unlabelled rhizotrons (Shearer and Kohl 1986; Eq. 2):

$$\%\text{Ndfa} = \left[\left(\delta^{15}\text{N RR} - \delta^{15}\text{N faba bean}\right) / \left(\delta^{15}\text{N RR} - \beta\right)\right] \times 100 \quad (2)$$

where $\delta^{15}N$ RR and $\delta^{15}N$ faba bean are parts per 1000 ^{15}N enrichment of N in unlabelled rapeseed grown in

monospecific rhizotrons and of faba bean in unlabelled rhizotrons, respectively. ß is a measure of isotopic fractionation during biological fixation, which was determined from the $\delta^{15}N$ (‰) of the aboveground parts of faba bean grown in pure sand and watered with an N-free diluted nutrient solution during the experiment (n=10). As in Exp. 1, the ^{15}N derived from the seed was discounted from the plant contents before calculations, and β was readjusted according to López-Bellido et al. (2010) (β readjusted = −0.54 ‰).

The N transfer (%Ndft) from the donor crop to the receiver was calculated, as described by Gylfadóttir et al. (2007), using Eq. 3:

$$\%\mathrm{Ndft} = \left[\left(\mathrm{N_{receiver}} \times \mathrm{atom\%^{15}N\ excess_{receiver}}\right)/\left(\left(\mathrm{N_{receiver}} \times \mathrm{atom\%^{15}N\ excess_{receiver}}\right) + \left(\mathrm{N_{donor}} \times \mathrm{atom\%^{15}N\ excess_{donor}}\right)\right)\right] \times 100 \quad (3)$$

where atom%^{15}N excess corresponds to the isotopic enrichment of either the donor or the receiver (RF_L or R_LF, respectively) grown in the labelled rhizotrons minus the isotopic natural abundance of the donor or receiver grown in unlabelled intercrops (RF). Then the amount of N transferred (Nt) was calculated from the total donor N. The %N in the receiver (%$N_{receiver}$) derived from transfer was calculated using Eq. 4:

$$\%\mathrm{N}_{receiver} = (\mathrm{Nt} \times 100)/\mathrm{N}_{receiver} \quad (4)$$

Using (3) and (4), N transfer was calculated from the labelled faba bean to rapeseed and from the labelled rapeseed to faba bean, considering the whole plants. For this calculation, the ^{15}N and N of the roots of rapeseed and faba bean grown together were assessed from N and ^{15}N measurements of the three root samples: faba bean, rapeseed and a mixture of undetermined roots collected from the soil (Corre-Hellou and Crozat 2005). The percentages of N in the root mixture derived from rapeseed (%NR_{roots}) and from faba bean (%NF_{roots}) were calculated following Eqs. 5 and 6, respectively:

$$\%\mathrm{NR_{roots}} = \left[\left(\mathrm{atom\%^{15}N\ excess\ root\ mixture} - \mathrm{atom\%^{15}N\ excess\ faba\ bean\ roots}\right)/\left(\mathrm{atom\%^{15}N\ excess\ rapeseed\ roots} - \mathrm{atom\%^{15}N\ excess\ faba\ bean\ roots}\right)\right] \times 100 \quad (5)$$

$$\%\mathrm{NF_{roots}} = (100 - \%\mathrm{NR_{roots}}) \quad (6)$$

Additionnally, nitrogen derived from the rhizodeposition of faba bean (%NdfrF) was calculated from F_LF_L using Janzen and Bruinsma's equation (1989; Eq. 7):

$$\%\mathrm{NdfrF} = \left[\left(\mathrm{atom\ \%15N\ soil}F_LF_L - \mathrm{atom\ \%^{15}N\ background\ A}\right)\right]/\left[\left(\mathrm{atom\%15N\ roots}F_LF_L - \mathrm{atom\%15N\ background\ B}\right)\right] \times 100 \quad (7)$$

where the natural abundance of the soil and the root atom %^{15}N of unlabelled faba bean FF were selected as backgrounds A and B, respectively. Equation 7 was also used for assessing the N rhizodeposition of rapeseed (%NdfrR) from R_LR_L (with the natural abundance of the soil and of the root atom%^{15}N of RR as backgrounds A and B, respectively). The amount of N deposited to the soil by the plant roots was assessed either from the %NdfrF or %NdfrR value and the amount of total soil N. This method assumes that *i*) the ^{15}N enrichment of the roots was homogeneous, ii) the ^{15}N enrichment of the roots was the same during plant growth and *iii*) there was no difference between the ^{15}N enrichment of the rhizodeposits and that of the roots.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 Software© (2010). When possible, an analysis of variance was used for multiple comparisons of means using Newman and Keuls post-hoc tests with a confidence level of 0.05. When assumptions of data normality or equality of variances were not met, comparisons of medians were carried out using non-parametric Kruskal–Wallis or Mann–Whitney tests with a confidence level of 0.05.

Results

Dry weight, N accumulation and biological fixation

From 21 DAS to 36 DAS (Exp. 1), rapeseed and faba bean DWs increased significantly ($p<0.0001$ for rapeseed and $p<0.01$ for faba bean; Fig. 3a and b), and there was no difference between monocrops and intercrops for either of the species ($p>0.05$). From 36 to 85 DAS (Exp. 1), the DWs of rapeseed and faba bean increased markedly ($p<0.0001$). At 85 DAS, the DWs of the aboveground parts of rapeseed were significantly higher in intercrops (RF) than in monocrops (RR) ($p<0.01$ and $p>0.05$ aboveground parts and roots, respectively, Fig. 3a). In Exp. 2, at 52 DAS, intercropping with faba bean significantly increased rapeseed total DW compared with monospecific RR ($p<0.005$, Fig. 4a). At 85 DAS in Exp. 1, the DWs of the aboveground parts and of the roots of faba bean were significantly higher in intercropped faba bean (FR) than in FF ($p<0.005$, Fig. 3b); such difference was not found at 52 DAS in Exp. 2 ($p>0.05$, Fig. 4a).

In Exp. 1, from 21 to 36 DAS, rapeseed N increased markedly in the aboveground parts ($p<0.005$, Fig. 3c), and less significantly in the roots ($p<0.05$). From 36 to 85 DAS, rapeseed continued to accumulate N in the roots ($p<0.001$). Possibly because of a lack of soil mineral N, there was no significant difference between aboveground N at the two harvest stages. However, at 85 DAS, the N content of intercropped rapeseed (RF) was higher than that of RR ($p<0.05$ for the plant N and aboveground parts, Fig. 3c). In Exp. 2, N accumulation in the rapeseed at 52 DAS was also higher in RF than in RR ($p<0.005$; Fig. 4b). In Exp. 1, faba bean N increased markedly from 36 to 85 DAS, especially in the aboveground parts; at 85 DAS, there was no significant difference for N accumulation between plant FF and FR (Fig. 3d).

At 85 DAS (Exp. 1), mean total N accumulated in the rapeseed was lower than 100 mg plant^{-1}, though it was greater than 100 mg plant^{-1} at 52 DAS in Exp. 2 (Figs. 3b and 4b). Conversely, faba bean reached more than 500 mg N per plant at the end of Exp. 1 (85 DAS, Fig. 3d), but accumulated less than 200 mg N per plant in Exp. 2 (52 DAS, Fig. 4b). BNF obtained in the two experiments cannot be compared as they have been assessed with two different methods and soils (ID method in Exp. 1 and Natural abundance in Exp. 2). However, at the end of Exp. 1, the %Ndfa was 9 % greater in RF than in FF ($p<0.0001$, Table 1), and in Exp. 2, the %Ndfa of the faba bean RF was also found to be higher than that of FF (63.9 %±3.3 % and 58.0 %±SE 1.6 % respectively, $p<0.05$).

^{15}N enrichment and the distribution of the ^{15}N label

In Exp. 1, in all rhizotrons, plant ^{15}N enrichments increased from 21 to 36 DAS before dropping until 85 DAS (Table 1). Enrichment of the roots was higher ($p<0.05$) or similar to that of the shoots. There was no difference between ^{15}N enrichment of rapeseed grown in RR and RF except at 36 DAS. The change in the amount of ^{15}N in the rapeseed parts as a function of time was comparable to that of the amount of N (Fig. 3c and e); as the soil was poor in organic matter, the difference between 36 and 85 DAS may be primarily linked to the decrease of labelled N availability in the soil. ^{15}N enrichment of the faba bean was lower than that of rapeseed, and there was no difference between FF and RF ($p>0.05$). At 21 DAS, the ^{15}N enrichment of the faba bean was low compared to that of rapeseed but at this early stage, but the large amount of N carried over the seed of faba bean may explain the discrepancy between the species. The amount of ^{15}N in the faba bean increased markedly from 21 to 36 DAS and more slowly from 36 DAS to faba bean maturity (85 DAS, Fig. 3f), possibly due to BNF as suggested by the simultaneous accumulation of N in the plant (Fig. 3d).

In Exp. 2, ^{15}N recovery in the labelled rhizotrons was 92.4±SE 0.6 %. In the faba bean as well as in rapeseed, the natural abundance of ^{15}N in the aboveground parts and roots did not differ between monocrops and intercrops (except in rapeseed RF, Table 2). However, atom %^{15}N excess in all the labelled plants was significantly higher in the aboveground parts than in the roots ($p<0.05$, Table 2).

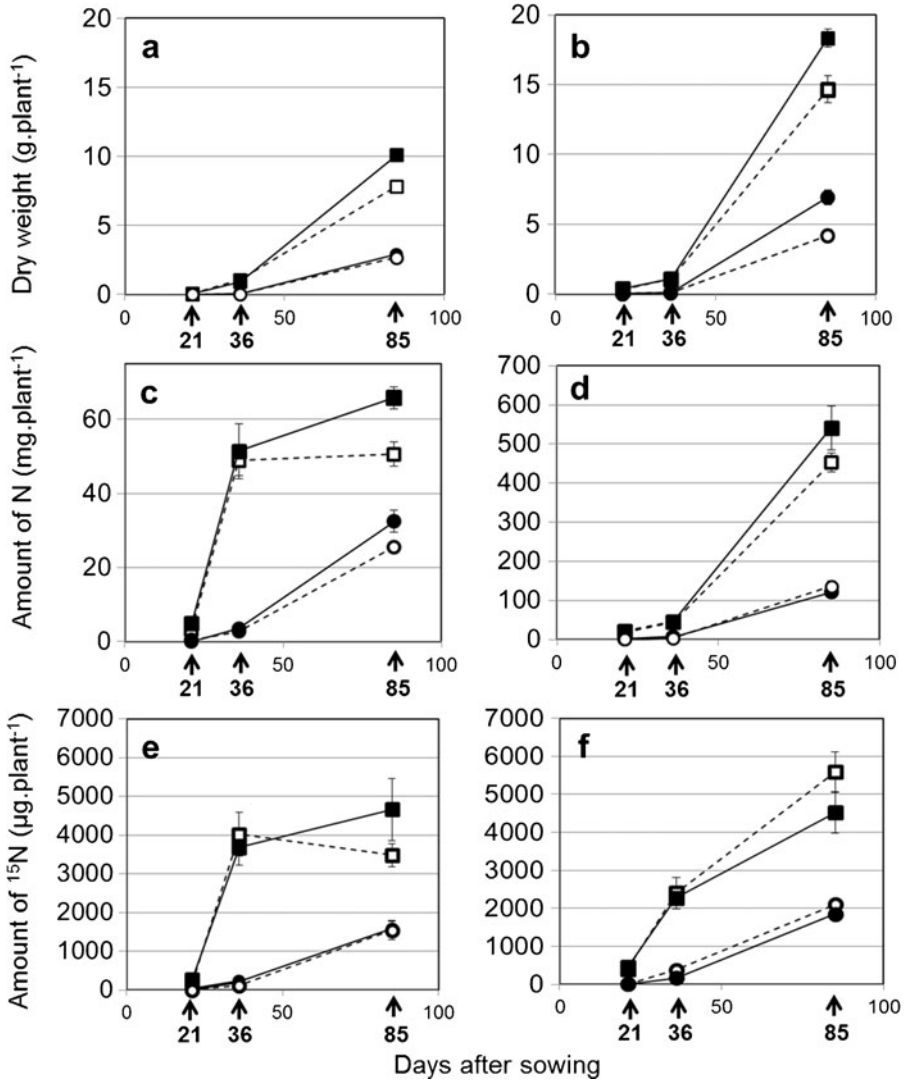

Fig. 3 Partitioning of dry weight (g plant^{-1}), nitrogen (mg plant^{-1}) and ^{15}N in the roots and aboveground parts of rapeseed and faba beans harvested at 21, 36 and 85 days after sowing, in Exp. 1. *White square* monocrops, aboveground parts; *black square* intercrops, aboveground parts; *white circle* monocrops, roots; *black circle* intercrops, roots; **a** and **b** – Dry weight in rapeseed and faba bean parts, respectively; **c** and **d** – Nitrogen partitioning in rapeseed and faba bean, respectively; **e** and **f** – ^{15}N partitioning in rapeseed and faba bean, respectively. Error bars represent SE

Direct N transfer between species

In Exp. 2, when intercropped with a labelled companion species, the atom%^{15}N excess calculated from unlabelled intercropped faba bean or rapeseed indicated that N transfer did occur between the two species. When the faba bean was labelled, the atom%^{15}N of the companion rapeseed increased 0.056 % in the roots and 0.001 % the aboveground parts (Table 2). When rapeseed was labelled, the atom%^{15}N of the associated faba bean increased 0.018 % in the roots and 0.002 % in the aboveground parts. The amounts of N transferred from one species were negligible, failing to reach 1 mg plant^{-1}, and species exchanged similar amounts of N ($p>0.05$, Table 3). Furthermore, there was no difference between the amount of N rhizodeposited by rapeseed (14.3 mg plant^{-1}) and faba bean (11.8 mg plant^{-1}, $p>0.05$, Table 3). At maturity of faba bean, no N transfer was detected with the dilution method used in Exp. 1.

Distribution of roots in the rhizotrons

At 32 DAS, for each species, the root length per plant, the number of secondary roots, and their distribution in the lower and upper parts of the rhizotrons were not significantly different in monocrops compared to RF ($p<0.05$, Table 4). Root drawings showed that the number of secondary roots and the total root length per plant did not differ between RR and RF rapeseed or between

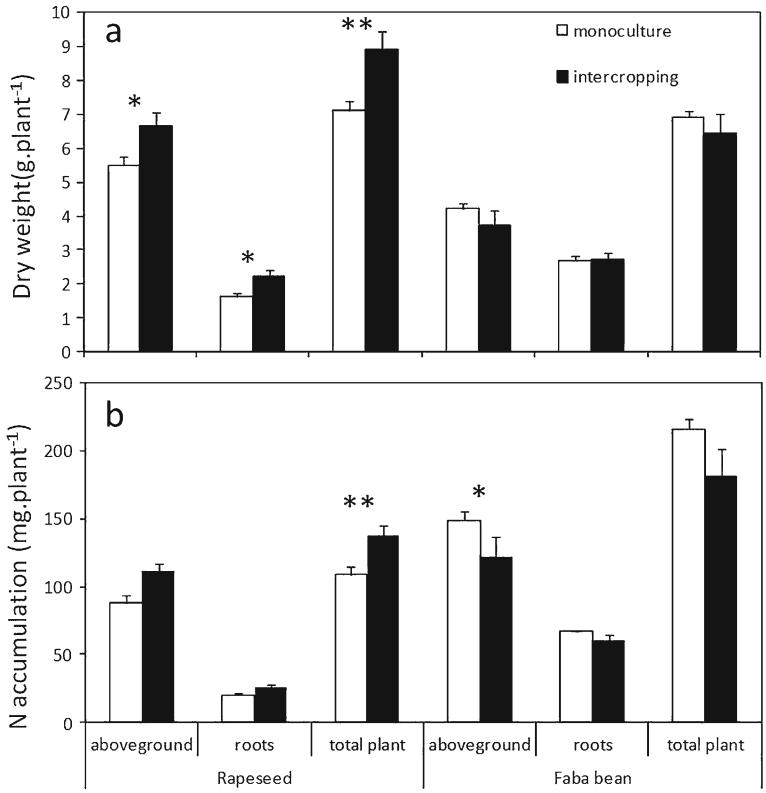

Fig. 4 Dry weight (**a**) and N accumulation (**b**) in the aboveground parts and roots of rapeseed and faba bean grown either in monoculture or intercropped in Exp. 2 (harvested at late flowering of the faba bean, 52 DAS). Values are means ± SE (*bars*). Rapeseed monoculture (n=11), faba bean monoculture (n=11), intercrops (n=13). * and ** indicate significant differences between monoculture and intercropping, with * $p<0.05$ and ** $p<0.005$

Table 1 Plant atom ^{15}N % enrichments of rapeseed (R) and faba bean (F) grown either together or in monoculture in labelled soil, harvested at 21, 36 and 85 days after sowing (DAS), and part of the plant N from BNF (%), in Exp. 1

	Rapeseed			Faba bean		
	R-R	R-F	*P*	F-F	R-F	*P*
Shoot enrichment (%)						
21 DAS	5.383±0.049 c	5.474±0.212 b	n.s.	2.095±0.206 b	2.085±0.183 b	n.s.
36 DAS	8.114±0.332 a	7.100±0.185 a	*	5.542±0.335 a	4.790±0.547 a	n.s.
85 DAS	6.835±0.296 b	7.077±0.355 a	n.s.	1.279±0.210 b	0.850±0.082 c	*
$P_{Harvest}$	****	**		***	****	
Root enrichment (%)						
21 DAS	6.215±0.037 b ••	6.377±0.400 b	n.s.	2.817±0.255 b	2.958±0.177 b ••	n.s
36 DAS	10.006±0.337 a ••	9.534±0.273 a ••	n.s.	5.869±0.350 a	4.630±0.402 a	n.s
85 DAS	6.208±0.289 b	6.853±0.493 b	n.s.	1.833±0.223 b	1.134±0.134 c	*
$P_{Harvest}$	**	**		***	****	
N derived from fixation (%)						
21 DAS	–	–	–	–	–	
36 DAS	–	–	–	33.2±2.8 b	27.5±7.5 b	n.s.
85 DAS	–	–	–	83.9±0.7 a	91.5±0.5 a	****
$P_{columns}$				*	*	

P indicates statistical significance between monocrops and intercrops or across treatments ; P_{column} and a, b and c indicates statistical significance across harvest stages; *P<0.05, **P<0.005 ***P<0.0005, ****P<0.0001, *n.s.* not significant ; • indicates significant difference between enrichment of the shoots and that of roots. Mean ± SE

Table 2 Natural abundance, atom %^{15}N excess in the shoots, roots and soil, and assessment of the contribution of BNF (%Ndfa), N from the companion crop (%Nfcc), N from the seed (%Nfseed) and N from the soil (%Nfsoil) to the plant N, in Exp. 2 (harvest at 52 DAS)

	Rapeseed				Faba bean			
Natural abundance (unlabelled rhizotrons)								
	R - R	R - F		*p*	F - F	R - F		*P*
Shoots	0.368±0.000	0.369±0.001		n.s.	0.367±0.000	0.367±0.000		n.s.
Roots	0.368±0.000	0.368±0.000		n.s.	0.367±0.000	0.367±0.000		n.s.
Psr	n.s.	*			n.s	n.s.		
Soil	0.370±0.000	0.370±0.000			0.370±0.000	0.370±0.000		
Atom %15N excess (labelled rhizotrons)								
	R_L - R_L	R_L - F	R - F_L	*p*	F_L - F_L	R - F_L	R_L - F	*P*
Shoots	2.506±0.267 a	2.345±0.498 a	0.001±0.000 b	***	1.444±0.219 b	2.377±0.382 a	0.002±0.001 c	**
Roots	1.563±0.091 a	1.592±0.315 a	0.056±0.024 b	***	0.588±0.027 a	0.825±0.166 a	0.018±0.004 b	**
Psr	*	n.s.	*		*	*	*	
Soil	0.0064±0.002	0.003±0.001	0.002±0.001		0.003±0.001	0.002±0.001	0.001±0.000	
Contribution of the different N sources to the plant N (%)								
	R - R	R - F			F - F	R - F		
%Ndfa (1)	0	–			58.0	63.9		
%Nfcc (2)	0	0.9			0	0.3		
%Nfseed	–	–			8.7	12.2		
%Nfsoil	100.0	99.1			33.3	23.6		

P and letters (a, b, c) indicate statistical significance across treatments within a row; *Psr* corresponds to statistical difference between shoots and roots within the plant; *P<0.05, **P<0.01, ***P<0.001; n.s: not significant. n=4–5. The atom %^{15}N excess in the different compartments of the labelled rhizotrons (mean ± SE) was calculated using unlabelled controls as background. (1) was calculated from Eq. 2; (2) was calculated from Eqs. 3 and 4

FF and RF faba bean (Table 4). In RR and RF rapeseed, the branches of the taproot were significantly more numerous (p<0.01), and the root length was higher (p<0.001) in the lower part than in the upper part of the rhizotron (Fig. 1; Table 4). Thus, 70 % of the total root length of rapeseed measured from the drawings was located in the lower part of the rhizotron. Conversely, 64 % of the total root length of the faba bean was located in the upper part of the rhizotron.

Discussion

The effect of intercropping on yield and N acquisition

In annual crops, the benefit of intercropping a cereal with a legume to increase its N acquisition in low-input systems has often been demonstrated during the past 10 years (e.g., Malézieux et al. 2009; Hauggaard-Nielsen et al. 2009). However, studies focusing specifically on brassica-legume

Table 3 The amounts of N deposited in the soil (N rhizodeposition) and taken up by the companion plant in intercropped at harvest, in Exp. 2 (52 DAS)

	Amount of N (mg plant^{-1})			Part of the plant N (%)		
	Rapeseed	Faba bean	*p*	Part of the rapeseed N (%)	Part of the faba bean N (%)	*p*
N deposited by the crop (1)	14.2±1.2	11.8±1.9	n.s.	9.1±1.0	8.3±1.3	n.s.
N taken up by the companion crop (2)	0.7±0.1	0.9±0.1	n.s.	0.5±0.1	0.6±0.1	n.s.

P indicates statistical significance between species, *n.s*: not significant; mean ± SE; n=5. (1) was calculated from monocrops and (2) from intercrops

Table 4 The distribution of rapeseed and faba bean roots in the lower and upper parts of the rhizotrons at 32 DAS (Exp. 2): root length (cm.plant^{-1}) and the number of secondary roots developed from the taproot per plant

	Rapeseed		Faba bean		p_{row}
	R - R	R - F	R - F	F - F	
Total root length (cm/plant)					
Upper part	88.2±4.2 b	85.4±4.4 b	356.3±28.2 a	348.8±13.9 a	****
Lower part	208.6±15.9	211.8±17.8	206.8±22.5	177.7±18.3	n.s.
p	***	***	**	****	
Number of secondary roots					
Upper part	24.7±1.7 c	29.6±0.7 bc	37.5±3.8 ab	46.6±2.4 a	****
Lower part	32.6±2.0 ab	39.5±1.7 a	28.2±3.9 bc	22.5±4.1 c	**
p	**	***	n.s.	***	

P_{row} and letters (a, b, c) indicate statistical significance across treatments within a row. *P* indicates significant differences between the upper and lower parts of the rhizotrons; *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$; *n.s* not significant. $n=8$ (intercrops) or $n=20$ (monocrops)

intercropping are scarce and have generally failed to demonstrate any benefit of intercropping for brassica yield or land equivalent ratio (LER) compared with brassica monoculture grown under the same conditions (Waterer et al. 1994; Banik et al. 2000). Conversely, there have been reports of pea-brassica intercropping leading to N overyielding depending on N fertilisation and weed management (Andersen et al. 2007; Szumigalski and van Acker 2006). Our experiments were conducted in two different N-poor soils to enhance the interspecific interactions involved in sharing N resources. Such conditions were favourable for BNF by the faba bean (López-Bellido et al. 2010), which is a key point in the dynamics of interactions between plant species. In our study, at the end of both assays, the DW per plant of intercropped rapeseed was approximately 20 % higher than that of monocrops. N accumulation in rapeseed was also improved by intercropping, and the benefit was approximately 30 % greater than that of the monocropped rapeseed in Exp.1 at maturity. This positive effect of intercropping on N acquisition in rapeseed only became effective after the flowering stage of faba bean. The higher percentage of organic matter in the soil of Exp. 2 compared to that of Exp. 1 may contribute to explain the differences in rapeseed and faba bean N accumulation between the two assays. Thus, in Exp. 1, the low mineral availability combined with the low percentage of organic matter may have limited N accumulation in the aboveground parts of rapeseed (Gombert et al. 2010), especially in RR, and forced the faba bean to rely on BNF in RF. Our results suggest that rapeseed was affected by the lack of N early after the beginning of the assay (36 DAS). In Exp. 2, rapeseed may have benefit from mineralisation of the organic matter, explaining the higher N accumulation in the aboveground parts until 52 DAS than in the first assay. In Exps. 1 and 2, the forms of N in the soil were quiet different (mineral N, added urea, organic N), and the two species may have used differently these resources according to their physiology and soil dynamic, with consequences on soil N uptake and accumulation in the crop parts, and on BNF.

In this study, we showed rapeseed and faba bean are complementary in their use of N resources. First, interspecific competition for soil N enhances niche separation between the two crops, forcing faba bean to rely on atmospheric N. The lower the availability of the mineral soil N, the higher the BNF is (Salon et al. 2009), even if BNF of faba bean is less affected by nitrate than other legume species. Consistent with other studies dealing with either legume-cereal intercrops (Corre-Hellou and Crozat 2005) or clover-grass mixtures (Nyfeler et al. 2011), BNF was higher in intercrops than in monocrops. This enhancement of BNF may be due to the decrease in the concentration of mineral nitrogen in the vicinity of the roots of the faba bean because of rapeseed N soil uptake (Corre-Hellou et al. 2006), and BNF appears to increase with

root intermingling (Xiao et al. 2004). As a consequence, intercropping with faba bean leads to a soil N sparing effect. In the very early growth stages, interspecific competition may also be attenuated because faba bean seed contains a substantial amount of N (López-Bellido et al. 2010).

In our study, root development appeared to be unaffected by the neighbour. However, the early growth stages of both species exhibited a strong taproot and secondary roots developed in the opposite parts of the rhizotrons: in rapeseed, 70 % of the secondary roots grew first in the lower part of the rhizotron, whereas approximately 64 % of the roots of the faba bean were located in the upper parts. As a consequence, the roots of rapeseed and faba bean harbour complementary architecture, leading to physical niche complementarity. Such soil sharing occurred in the very early growth stages and remained effective until the BNF became active in later growth stages. Consequently, the root traits of rapeseed and faba bean are well suited for shared resource utilisation and thus contribute markedly to an improved N acquisition of both species when intercropped in comparison with their growth as monocrops.

N transfer

In Exp. 2, the cotton-wick method was used for the first time for assessing N transfer between plants and revealed the net N transfer from faba bean to rapeseed and vice versa. This method allowed for a good control and recovery of the supplied ^{15}N, and had no consequence on root architecture compared to the split root method (Mahieu et al. 2007). Furthermore, the labelling revealed significant differences between the enrichments of the labelled and unlabelled associated plants, making it possible to estimate the root-N and DW of each crop in intermingled root systems, by using isotopic methods. Consequently, our calculations were performed with the entire plants and not just the shoots as is generally the case in other studies. However, such calculations include approximations, as the assumption of label homogeneity in the plant roots is not ensured (Wichern et al. 2011). Similarly to other shoot labelling and to the split-root method as well, cotton-wick leads to higher enrichments of the shoots compared to roots. This uneven distribution of the label was not obtained with the isotopic dilution method and may have consequences on the results. Furthermore, in Exp. 2, as labelling did not start before 25 DAS, N transfer may have been underestimated.

Comparing our Exp. 2 with that of Jensen (1996), who used the split-root technique to measure N transfer in pea-barley intercropping, isotopic enrichment of the roots and aboveground parts of the faba bean as donor and rapeseed as receiver were similar to that of the pea as donor at the flowering of the legume crop. At this stage, considering the amount of N accumulated in the donor legume in each study, similar percentages of the pea-N (0.43 % to 0.89 % during flowering in Jensen 1996) or faba bean-N (0.58 % at late flowering in our study) were transferred to the companion plant. However, the pea-N at maturity was 30 % higher than that of faba bean at late flowering; thus, the amount of N transferred from pea to barley was higher than that observed in our Exp. 2. In Jensen (1996), the percentage of pea-N transferred to barley increased with time, reaching 1.16 % at the maturity of the pea. With the mean faba bean-N of 730 mg measured at maturity in our first experiment, applying a value of %N transfer from faba bean to rapeseed ranging from 0.58 % to 1.16 % would lead to 4.2 to 8.5 mg N plant^{-1} transferred; this result would be consistent with Jensen (1996) and Xiao et al. (2004). The part of rapeseed-N received from faba bean would then be between 3.8 and 8.9 %, which is much lower than the 19 % reported for barley by Jensen (1996) or 15 % reported for wheat associated with faba bean by Xiao et al. (2004).

Using the cotton-wick method, we detected N transfer from rapeseed to faba bean, which was not reported in other studies (Jensen 1996; Xiao et al. 2004). Up to late flowering, the associated plants exchanged similar amounts of N. Such result is in agreement with the fact that the amounts of N rhizodeposed by the rapeseed and the faba bean were not different. Nevertheless, rhizodeposition calculated with Janzen and Bruisma's equation must be considered with caution because of the assumptions supporting calculations (Wichern et al. 2008; Rasmussen 2011). In our study, the amount of N transferred from faba bean to rapeseed at late flowering (0.6 mg plant^{-1}) does not explain why N accumulation in rapeseed was approximately 20 mg higher in intercrops than in monocrops (Exp. 2). In addition, a net

N flux of less than 10 mg from faba bean-N would not be sufficient to explain the similar difference observed at maturity in Exp. 1. Furthermore, in our study, the dilution method did not confirm the existence of such positive and substantial N flux at maturity of the faba bean..

Under low-N conditions, intercropping faba bean with rapeseed has a positive effect on DW and N accumulation in rapeseed. This result, which was observed from the late flowering stage of faba bean, mainly relies on the complementarity between the species for sharing N resources, as N transfer was negligible at this stage. In addition to niche separation due to BNF, rapeseed and faba bean harbour complementary root architecture that reduces plant competition for the soil-N at the beginning of their development. Deep rhizotrons may have been more favourable than pots to show this effect. Before late flowering, N transfer did not contribute to the gain of N measured in intercropped rapeseed compared with monocrops: first, the amounts transferred were negligible, and second, similar amounts of N were exchanged in both directions. Our study did not assess N transfer between plants from late flowering of faba bean to maturity but pointed to the importance of BNF for increasing net N flux from faba bean to rapeseed from flowering to maturity.

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