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Nitrogen transfer from *Lupinus albus* L., *Trifolium incarnatum* L. and *Vicia sativa* L. contribute differently to rapeseed (*Brassica napus* L.) nitrogen nutrition

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Abstract

Nitrogen (N) transfer is well documented in legume-cereal intercropping but this is less often reported for legume-*Brassica* intercrops even though *Brassica* crops require higher levels of N fertilizers. The present study was carried out to quantify N transfer from legumes (*Lupinus albus* L., *Trifolium incarnatum* L. or *Vicia sativa* L.) to rapeseed (*Brassica napus* L.) using the split-root ^{15}N-labelling method. After three months we observed that legumes did not alter the growth of rapeseed. Vetch showed the lowest growth and demonstrated low ^{15}N shoot to root translocation and no significant N transfer to rapeseed. In contrast, significant ^{15}N enrichment was found in lupine and clover and ^{15}N was transferred to the associated rapeseed plants (around 6 and 4 mg N plant^{-1}, respectively), which contributed 2 to 3% of the rapeseed total N. Additionally, the data revealed that N_2 fixation dominated the N nutrition in lupine despite the high N level provided in the donor compartment,

suggesting a greater niche segregation between companion plants. Based on the results of this study we suggest that intercropping can be a relevant contributor to rapeseed N nutrition. Among the three legumes tested, clover and lupine seemed to be the best intercropping candidates.

Keyword: Plant biology

1. Introduction

To reduce the use of nitrogen (N) fertilizers and their adverse economic and environmental impacts, N_2-fixing legumes grown in rotations or under intercropping are considered an alternative and sustainable way to introduce N into agroecosystems (see review of Fustec et al., 2010). Indeed, legumes contribute to the enrichment of soil N *via* biological N_2-fixation and N rhizodeposition and facilitate N acquisition in companion plants through the transfer of N (Ledgard and Steele, 1992; Høgh-Jensen and Schjoerring, 2000). Indeed, some authors have shown that in grass-legume mixtures, legumes increase the soil-N pool and that the grasses can benefit from the N provided by legumes (Gylfadóttir et al., 2007; Pirhofer-Walzl et al., 2012). Nitrogen can be transferred within plant mixtures through different pathways (Fustec et al., 2010). Except for *Brassicaceae* and *Plantaginaceae* which are never mycorrhized, N transfer can occur *via* mycorrhizal fungi interconnecting the root systems of both species and indirectly through N rhizodeposition and root litter decomposition into the soil followed by uptake by the grass (Johansen and Jensen, 1996; Høgh-Jensen and Schjoerring, 2001). N rhizodeposition mainly occurs through mineralization of legume litter, or through N root exudation (Ledgard and Steele, 1992; Paynel et al., 2001). Whichever process, the proportion of N transferred from legume to non-legume plants is different between species because they have different N_2-fixation abilities (Ta and Faris, 1987).

In ryegrass-clover mixtures it has been shown that 10% of the N fixed by clover is transferred to the grass and accounts for up 50% of the N in ryegrass (Høgh-Jensen and Schjoerring, 2000; Rasmussen et al., 2007). The N transfer from white clover to perennial ryegrass has been assessed at between 11 and 113 kg N ha^{-1} year^{-1} with a mean of 70 kg N ha^{-1} year^{-1} (Ledgard and Steele, 1992; Elgersma et al., 2000). N transfer through exudation of ammonium and amino acids by clover roots followed by N uptake by ryegrass was shown to be a major pathway in young plants (two-months-old) (Paynel et al., 2001; Paynel and Cliquet, 2003). 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). Giller et al. (1991) estimated in a greenhouse experiment that up to 15% of the N in N_2-fixing beans could be transferred to intercropped maize.

The transfer of N between the legumes and non-fixing plants is usually quantified by using ^{15}N-enriched methodologies, since any tracer incorporated into the legume and detected in the non-legume receiver plant shows evidence of transfer. Donor legume plants can be labelled by different labelling methods such as [15]N$_2$-labelling (Ta et al., 1989), foliar labelling (Ledgard et al., 1985; Giller et al., 1991), labelling roots induced on the legume stem (Hamel and Smith, 1991), transplanting labelled plants into soil with receiver plants (Tomm et al., 1994), the cotton-wick method in which ^{15}N is provided through the stem (Jamont et al., 2013) or split-root labelling of the legume donor (Jensen, 1996; Van Kessel et al., 1985; Purnamawati and Schmidtke, 2003). For example, using a split-root ^{15}N labelling method Jensen (1996) estimated that almost 19% of the N produced by field peas was transferred to barley grown in mixture. Martensson et al. (1998) also estimated that transfer from pea or red clover (donor plants) accounted for between 3 and 50% of total N in the chicory receiver plant. Wichern et al. (2008) outlined some advantages of the split-root technique. It allows continuous labelling using natural N uptake and assimilation, which should therefore uniformly label all compounds subject to N transfer.

Recent results have demonstrated the benefits of faba bean-rapeseed intercrops (*Vicia faba* L. spp. minor cv. Divine) in terms of dry weight (DW), N content and rapeseed yield, and this is mainly due to the niche complementarity between the two species in sharing soil N resources. Moreover, N transfers from faba bean to rapeseed were detected (about 10%) at the early stages of growth (Cortés-Mora et al., 2010). However, a field study with pea-mustard (*Sinapis alba* L.) intercrops failed to demonstrate significant N transfer from the legume to the non-legume (Waterer et al., 1994). Nevertheless, in a preliminary study, we have shown that rapeseed-legume mixtures maintained the biomass of rapeseed and the N and sulphur (S) contents and that clover in mixture preserved rapeseed leaf chlorophyll content. Moreover, the amount of N in the soil at harvest was significantly higher in rapeseed-lupine and rapeseed-clover mixtures compared to the rapeseed monoculture and rapeseed-vetch mixtures (Génard et al., personal communication).

Using a split-root ^{15}N labelling technique, this study was carried out to determine the effects of three legumes species (Lupine: *Lupinus albus* L., clover: *Trifolium incarnatum* L. and vetch: *Vicia sativa* L.) on rapeseed (*Brassica napus* L.) performances. Thus, it focused on (i) the effect of legume species on rapeseed growth and their ability to grow with rapeseed, (ii) the ability of legume species to take up inorganic N and to translocate N from root to shoot and then shoot to root and (iii) the relative N transfer of these legumes species to rapeseed in order to determine their contribution to rapeseed N nutrition.

2. Materials and methods

2.1. Experimental design and plant growth conditions

Seeds of three legume species (white lupine, *Lupinus albus* L. var. Orus; Italian clover, *Trifolium incarnatum* L. var. Cegalo and common vetch, *Vicia sativa* L. var. Nacre) and rapeseed (*Brassica napus* var. Boheme) were germinated and grown on perlite over demineralized water for 2 weeks. At this time, the legume radicles were trimmed (upper 1 cm left intact) to stimulate the development of lateral roots. Then the seedlings were transferred to perlite for a further 2 weeks to allow the development of lateral roots before transplantation into a split-root design. This design allows separation of the root system of the legume (donor plant) into two equal root parts, each growing in a specific compartment (Fig. 1; Laîné et al., 1994; Jensen, 1996). For this, lateral roots of legumes were separated into two equal parts using a polyethylene Y tube (internal diameter: 8 mm, length of each branch: 40 mm) so as to direct the two parts of the root system. The first part was placed in a "donor compartment" (called DC) containing four litres of a nutrient solution corresponding to: 1 mM NH_4NO_3, 1 mM K_2SO_4, 0.4 mM KH_2PO_4, 0.15 mM K_2HPO_4, 3 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.2 mM EDTA 2NaFe, 14 µM H_3BO_3, 5 µM $MnSO_4$, 3 µM $ZnSO_4$, 0.7 µM $CuSO_4$, 0.7 µM Na_2MoO_4, 0.1 µM $CoCl_2$. Two weeks after transplantation, this nutrient solution was labelled with $^{15}NH_4^{15}NO_3$ (99 atom% ^{15}N). This solution was renewed twice weekly to allow continuous labelling. The second part of the root system was placed in a two litre "receiver compartment" (called RC), in which the roots of the donor plant interacted with roots of rapeseed (receiver) in a silty-clay soil (0.32% total N, 0.10% total S; pH 6.1)/sand (quartz BB 0.8–1.4 mm diameter SIBELCO, Paris, France) mixture (v:v 1/3). Rapeseed plants grown alone in a 2-liters pot filled with a soil/sand mixture were used as control. Both the RC and control plants were watered daily with 60 ml of the nutrient solution described above but deprived of N.
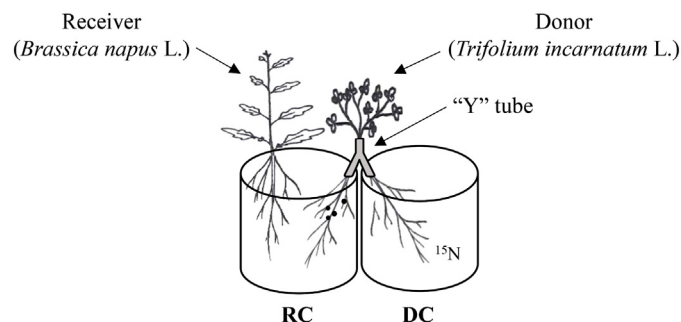
Fig. 1. Experimental unit. DC is the donor compartment; RC is the receiver compartment.

Following the method of Jensen (1996), the soil/sand mixture was not sterilized (to preserve native soil biota, especially microorganisms involved in N mineralization processes) and the RC was inoculated with suitable strains of symbiotic bacteria (Table 1). Before plant inoculation, each bacterial strain was grown on 100 ml of Bergersen's medium (Bergersen, 1961) modified by the addition of 0.2 g yeast extract l^{-1} and adjusted to pH 6.8 under sterilized conditions and incubated for 48 h at 28 °C. Bacterial cultures were suspended in 100 ml of sterile deionized water and vortexed to obtain homogeneous inoculum suspensions. Each inoculum suspension was applied to the soil of each host legume (5 ml per pot) at the time of seedling transplantation.

This experiment was carried out in a greenhouse under a thermoperiod of 20/17 °C day/night and a photoperiod of 16/8 h. Natural light was supplemented with high pressure sodium lamps (Philips, MASTER Green Power T400W) supplying an average photosynthetically active radiation of 350 μmol photons $m^{-2} s^{-1}$ at canopy height.

2.2. Plant harvest and N transfer assessment

Plants were harvested three months after transplanting and separated into shoots and roots. In RC, the roots of both plants (donor and receiver) were separated. Roots were carefully rinsed with deionized water. Plant samples were weighed and oven dried (60 °C) for 48 h for DW determination and ground to fine powder before total N and $^{15}N/^{14}N$ ratio analysis. The total N and the $^{15}N/^{14}N$ ratio were determined by analysing samples with an isotope ratio mass spectrometer (IRMS) (Horizon, NU Instruments, Wrexham, United Kingdom) linked to a C/N/S analyser (EA3000, Euro Vector, Milan, Italy).

Estimates of total N transfer from donor (legume) to the receiver (rapeseed) were based on the assumption that equal proportions of labelled and non-labelled N were transferred. The percentage of total donor N transferred to the receiver (%$N_{transfer}$) was calculated from the ratio between labelled N in the receiver and total labelled N in both the receiver and the donor plant (Ledgard et al., 1985):

$$\%N_{transfer} = {}^{15}N \text{ content}_{receiver} / ({}^{15}N \text{ content}_{receiver} + {}^{15}N \text{ content}_{donor}) \times 100 \quad (1)$$

where

$$^{15}N \text{ content} = (\text{atom} \% \ ^{15}N \text{ excess} \times \text{ total N}) / \text{atom} \% \ ^{15}N \text{ excess}_{labelled \ N} \quad (2)$$

with: atom % ^{15}N excess$_{labelledN}$ = 99

atom % ^{15}N excess$_{plant}$ = atom % $^{15}N_{plant}$ − atom % $^{15}N_{control}$

Then, total amount of N (mg plant^{-1}) transferred from the donor ($N_{transfer}$) was calculated as:

Table 1. Characteristics (strain, genera, species and plant origin) of symbiotic bacteria used to inoculate different legume species.

Host legume	Strain	Genera and species	Plant origin	Reference
Trifolium incarnatum L.	T354 (MSDJ1056)	*Rhizobium leguminosarum* bv. *trifolii*	*Trifolium pratense* nodule isolate	Mazurier (1989)
Vicia sativa L.	P221 (MSDJ0469)	*Rhizobium leguminosarum* bv. *viciae*	*Pisum sativum* nodule isolate	Laguerre et al. (1992)
Lupinus albus L.	LL13 (MSDJ718)	*Bradyrhizobium* sp.	*Lupinus luteus* nodule isolate	Laguerre et al. (1994)

$$N_{transfer} = \%N_{transfer} \; x \; total \; N_{donor}/100 \tag{3}$$

The percentage of N in the receiver derived from transfer (%Ndft) was calculated as:

$$\%Ndft = N_{transfer} \; x \; 100/total \; N_{receiver} \tag{4}$$

2.3. Data and statistical analysis

The experiment was performed with five replicates except for clover (n = 3). The resulting variations in data are expressed as the means ± S.E. Data were analysed using analysis of variance (ANOVA), after verifying compliance of normality, and significantly different means between treatments were separated with the Tukey's multiple range test (P ≤ 0.05).

3. Results

3.1. Biomass, total N amount and ^{15}N excess of legumes and rapeseed

Clover had a higher total dry weight than lupine and vetch (Table 2). Shoot, total root and RC root DW of clover followed the same trend. The DC root DW of lupine and clover were similar and significantly higher than vetch (Table 2).

The N amounts in whole plants, shoots and DC roots were not significantly different for lupine and clover and significantly higher than those of vetch (for example, about 14, 15 and 7 fold higher in clover than in vetch, respectively). Moreover, compared to vetch, clover showed the highest values for N amount in RC (18 fold) and total root (12 fold). The lack of difference in the N amounts observed between clover and lupine could be explained by the higher N content of lupine (around 3 *versus* 2%), which offset its lower dry weight (Table 2).

Table 2. Dry weight and N amount in donor plant (lupine, clover or vetch) parts (root in receiver compartment (RC), root in donor compartment (DC), total root or shoot) or in the whole plant at harvest time. Each value represents mean ±S.E. for n = 3 (clover) or n = 5 (lupine and vetch). For each parameter, different letters (a, b and c) indicate significant differences between species at p < 0.05 (Anova followed by Tuckey test).

Legumes	Dry weight (g plant^{-1})					N amount (mg N plant^{-1})				
	RC root	DC root	Total root	Shoot	Whole plant	RC root	DC root	Total root	Shoot	Whole plant
Lupine	0.64 a (±0.16)	1.29 b (±0.31)	1.94 a (±0.38)	6.33 a (±1.13)	8.27 a (±1.47)	17.22 ab (±4.40)	41.66 b (±12.04)	58.87 ab (±14.63)	272.99 b (±44.37)	331.86 b (±58.50)
Clover	4.52 b (±2.12)	1.45 b (±0.24)	5.97 b (±2.24)	22.47 b (±6.10)	28.44 b (±8.08)	65.05 b (±30.35)	49.62 b (±10.89)	114.67 b (±28.86)	466.93 b (±110.55)	581.60 b (±137.72)
Vetch	0.37 a (±0.22)	0.18 a (±0.04)	0.48 a (±0.20)	1.47 a (±0.55)	1.94 a (±0.60)	3.56 a (±2.29)	6.73 a (±1.44)	9.58 a (±3.05)	31.21 a (±8.19)	40.79 a (±10.69)

The atom%^{15}N excess in whole plants, shoots, total roots and DC roots of clover and vetch was significantly higher (around 60%, except for clover total root: 35%) than in lupine (around 15%). The lowest values were measured in RC roots for which clover showed higher values than lupine and vetch (Table 3).

Total DW, shoot and root DW and the total N amounts in rapeseed grown in mixtures (rapeseed-lupine, rapeseed-clover and rapeseed-vetch) was similar to the DW and total N amounts of rapeseed grown alone (Control) (Table 4). The atom% ^{15}N excess of rapeseed grown with clover was significantly higher than in rapeseed grown with lupine or vetch, the latter showing a similar value to rapeseed grown alone (Table 4). These values of ^{15}N enrichment found in shoots and roots of

Table 3. ^{15}Nitrogen labeling (expressed in atom% ^{15}N excess) in donor plants (lupine, clover or vetch) at harvest time. RC root and DC root correspond to legume roots in the receiver compartment (RC) and donor compartment (DC), respectively. Each value represents mean ±S.E. for n = 3 (clover) or n = 5 (lupine and vetch). For each parameter, different letters (a, b and c) indicate significant differences between species at p < 0.05 (Anova followed by Tuckey test).

	Atom% ^{15}N excess				
	RC root	DC root	Total root	Shoot	Whole plant
Lupine	2.432 a (±0.267)	21.084 a (±4.191)	14.678 a (±3.306)	15.773 a (±2.558)	15.584 a (±2.657)
Clover	15.497 b (±2.363)	62.492 b (±8.735)	35.194 ab (±3.028)	69.247 b (±7.401)	62.349 b (±6.129)
Vetch	0.688 a (±0.124)	74.448 b (±6.648)	58.444 b (±8.069)	64.076 b (±9.177)	62.647 b (±7.257)

Table 4. Total dry weight, N amount and atom % ^{15}N excess in rapeseed grown alone (control) and rapeseed (R, receiver) grown in mixture with different donor plants (lupine, L; clover, C or vetch, V) at harvest time. Each value represents mean ±S.E. for n = 3 (RC) or n = 5 (RL and RV). For each parameter, different letters (a, b and c) indicate significant differences between mixtures at p < 0.05 (Anova followed by Tuckey test).

	Dry weight (g plant^{-1})	N amount (mg N plant^{-1})	Atom% ^{15}N excess
control	17.45 a (±0.93)	143.79 a (±7.53)	0.371 a (±0.007)
RL	23.53 a (±1.76)	189.36 a (±15.51)	0.457 b (±0.024)
RC	18.70 a (±2.54)	186.91 a (±28.08)	1.310 c (±0.366)
RV	18.94 a (±1.68)	150.81 a (±13.07)	0.408 ab (±0.018)

rapeseed indicate that N had been transferred from the nutrient solution to legumes and then from legumes to rapeseed. From these data, it could be underlined that the ^{15}N enrichment is maintained from DC roots to shoots but the enrichment from shoots to RC roots is less effective, especially in vetch (94 fold lower).

3.2. N transfer from clover and lupine to rapeseed

No significant N transfer occurred from vetch to rapeseed. The percentages of N transferred (%N$_{transfer}$) from lupine or clover to rapeseed were not significantly different (p = 0.208), which was also reflected in similar amounts of N being transferred to rapeseed from lupine and clover, respectively (p = 0.252). Finally, in rapeseed the N derived from transfer (%Ndft) accounted for a slightly higher percentage (p = 0.07) of total N in rapeseed-lupine than in rapeseed-clover (Table 5).

Table 5. Percentage (N transfer %) and amount (mg N plant^{-1}) of N transferred from donor (Lupin (L) or clover (C)) to receiver plants (Rapeseed (R)); contribution (Ndft %) to total N of the receiver plant.

	N transfer (%)	N transferred (mg N plant^{-1})	Ndft (%)
RL	2.17 (±0.64)	6.04 (±0.96)	3.14 (±0.32)
RC	0.88 (±0.44)	4.01 (±1.31)	2.02 (±0.40)

4. Discussion

Facilitation of N resources between legumes and their associated companion crops has been widely described, especially for cereals (Vandermeer, 1989; Loreau and Hector, 2001) and constitutes a promising tool for agro-ecology. Such interactions have been attributed to two potentially interconnected processes: (i) niche segregation between species that can force one partner to exploit resources neglected in a single species system (for example, deeper soil nutrient pools by trees in agroforestry) and (ii) N transfer from an N_2 fixing legume to the companion crop. To satisfy these processes, legume species need to ensure proper growth in intercrops without limiting the companion species and then they must release and transfer significant amounts of N to the commercial crop.

Our results show that in comparison to control plants, which were grown alone, growth at the rosette stage of rapeseed was not limited by clover, lupine or vetch, even though rapeseed had to share the same soil volume and nutrient pools. This contrasts with decrease of *Brassica* performance measured in mustard − pea or − lentil mixtures by Banik et al. (2000) or Jamont et al. (2013) who noticed a 30% growth increase of rapeseed when associated with faba bean. Taken together, these results suggest that the choice of the legume is crucial for the growth performance of *Brassica* species. In our experiment, lupine and especially clover grew well with rapeseed, whereas vetch produced a very low dry weight and then appeared to be less compatible.

The atom $\%^{15}N$ excesses we measured were significantly higher in both clover and vetch than in lupine (around 60% against 15%). This shows that these two species derived the most part of their N nutrition from the donor compartment (DC), not the receiver compartment (RC), even though their root biomass was 2 to 3 fold greater in the latter. Thus, a clear segregation between rapeseed and these two legumes was revealed, the companion crops exploiting two different N pools. For these two legumes, N_2 fixation therefore seemed to be limited compared to root uptake. Such limitations in N fixation have been described by numerous authors for clover (Macduff et al., 1996; Soussana et al., 2002) and attributed to a decrease in nodule number and dry weight due to high nitrate availability. Indeed, in our experimental design, nitrate was highly available in the DC thanks to a nutrient solution renewed twice weekly, and very few nodules were observed when roots were collected. The atom $\%^{15}N$ excess of lupine was much lower, indicating that the ^{15}N nutrient solution did not constitute its main N resource, whereas unlike both clover and vetch the root biomass of lupine was slightly higher in the DC (p < 0.1). At harvest, we observed numerous large nodules on the root systems from the two compartments suggesting that the nodulation process was effective. Some authors such as Luciñski et al. (2002) have noted that *Lupinus albus* growth is not affected by mineral N when NH_4NO_3 is provided. Serrano & Chamber (1990)

showed that some *Bradyrhizobium* strains are able to infect roots sufficiently at a relatively high nitrate concentration (12 mM). Moreover, Goergen et al. (2009) found that lupine may rely mostly on fixed N_2 when N nutrient solutions up to 5 mM are provided. Together with our observations on nodule formation and the low root biomass in RC, this led us to conclude that in our system, the N nutrition of lupine relied mainly on N_2 fixation and to a lesser extent on NH_4NO_3 uptake from the DC. Therefore, species niche segregation for N nutrition also occurred between this legume and rapeseed.

In our study, a split root system was used for the first time to study N-transfer from three legume species to rapeseed. This method allows continuous ^{15}N labelling using a natural pathway of N uptake and assimilation, which should uniformly label all organs and compounds subject to N transfer from the legume root system in the DC to the shoot then the roots in the RC, and finally to the companion plant (rapeseed). This allows a more complete and realistic monitoring than shoot-labelling methods (Chalk et al., 2014; Wichern et al., 2008). Overall, the ^{15}N enrichment of the legumes was high and equivalent in both DC roots and shoots, indicating a high translocation level in the xylem. The ^{15}N enrichment of legume RC roots was much weaker, being reduced 94 fold (vetch), 6 fold (lupine) and 4 fold (clover) compared to shoots (Table 3). Considering that legume root biomass was statistically similar in both the RC and DC, the ^{15}N amount in RC roots was low and suggested a low phloem translocation of N compounds from shoot to roots, especially for vetch. This explained the lack of ^{15}N enrichment in rapeseed grown with vetch compared to rapeseed grown alone (Table 4). In contrast, significant ^{15}N enrichment had been found in the shoot and roots of rapeseed grown with lupine and clover, with the latter having a significantly higher atom% ^{15}N excess (Table 4). However, in the RC, where roots of lupine were less ^{15}N labelled than roots of clover, both species transferred statistically similar amounts ($p = 0.252$) of N (6.04 ± 0.96 and 4.01 ± 1.31, respectively). Taking into account the amount of N in rapeseed, the Ndft % was even slightly higher ($p = 0.07$) when mixed with lupine ($3.14 \pm 0.32\%$) than when mixed with clover ($2.02 \pm 0.40\%$). Despite possible variation according to species, plant age, ^{15}N addition, harvest, duration of ^{15}N labelling and the number of ^{15}N applications, the results obtained in our study are consistent with values previously monitored using a split root design and reported in the review of Chalk et al. (2014) ($<10\%$ and often $<1\%$). For example, Ndft values of 0.3% when intercropping barley with pea and between 0.7 and 1.4% when intercropping maize with soybean were obtained by Johansen and Jensen (1996) and Van Kessel et al. (1985), respectively. For the three rapeseed-legume mixtures studied, our results revealed a ten fold higher N transfer from legume to rapeseed than reported by Jamont et al. (2013) who considered faba bean associated with rapeseed at a similar stage of development, biomass and rapeseed N content as in the current work.

5. Conclusion

From this study, it could be concluded that rapeseed growth was not altered by the presence of legumes. In contrast to vetch, lupine and clover may be considered as suitable candidates for rapeseed legume mixtures because both species have shown significant capacity for N transfer that is available for rapeseed nutrition. Moreover, lupine may be particularly interesting since its N nutrition relies mainly on N_2 fixation, which promises a good niche segregation from the main crop and high N inputs into the crop system.

Declarations

Author contribution statement

Thaïs Génard, Philippe Etienne, Philippe Laîné, Jean-Claude Yvin, Sylvain Diquélou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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