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Niche Separation and Nitrogen Transfer in *Brassica*-Legume Intercrops

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Summary: *Brassica* sp. are interesting late-summer and autumn forages. Since they have high nitrogen requirements, intercropping with legumes may increase yield in low-input systems. However, brassica-legume intercrops still remain poorly documented. Using rhizotrons in a greenhouse, we compared root development of (1) forage rapeseed grown with faba bean to that of forage rapeseed monoculture and (2) fodder cabbage grown with common vetch to that of fodder cabbage monoculture. Legumes were labelled with ^{15}N urea. Seven to eight weeks after sowing, *Brassica* yield and N content were higher under intercropping than in pure stand. Under intercropping, distribution of root ramifications along the taproot differed from that of monoculture, which reduced the effect of competition. In addition, N transfer from legumes to *Brassica* was found to be significant.
Keywords: facilitation, intercropping, N fixation, niche

Introduction

Agriculture is facing new challenges as higher levels of production are expected with less fertiliser. The understanding of the mechanisms involved in agro-ecosystem functioning may help to reach this goal (Altieri 1999). Biological diversity is now recognised as a possible source of productivity. For instance, intercropping has been shown to allow more efficient use of resources than monocultures (Tilman et al. 2002). Interspecific competition may lead to niche separation with more vigorous root systems and more efficient exploration of the soil for nutrients and water, resulting in an increase in productivity (Hauggaard-Nielsen et al. 2001, Hauggaard-Nielsen et al. 2008). Intercropping can improve the use of resources by 10–50% above sole crops grown on the same piece of land, expressed in the Land Equivalent Ratio (LER) (Bulson et al. 1997). LER is the sum of the relative yields of the intercrop components relative to their respective sole crop yield. Most examples of intercrops combine a legume with another crop. In low-input systems, legumes increase the soil N pool throughout their growth, through N symbiotic fixation and rhizodeposition (Jensen 1996a, Fustec et al. 2010). After mineralisation, N can be transferred from the legume to the non-fixing companion crop (Jensen 1996b, Paynel et al. 2008). Most data are based on legume-cereal mixtures. The majority of the time, they reveal a Land Equivalent Ratio higher than 1, indicating that the productivity of legume-cereal intercrops was significantly higher than that of monocultures (de Wit & van den Bergh 1965, Trenbath 1976, Corre-Hellou et al. 2006, Malezieux et al. 2009).

Forage *Brassica* sp. are fast growing, highly productive and digestible crops (Banik et al. 2000). They offer great potential and flexibility for improving stocking rate in late summer and autumn, especially under drought conditions. *Brassica* sp. have relatively high nitrogen requirements, intercropping with legumes may help to increase yield in low-input systems. However, *Brassica*-legume intercropping remains poorly documented. The aims of our study were (1) to compare root development, dry matter and N content in fodder rapeseed and fodder cabbage grown either with legumes or in monoculture, and (2) to investigate the N transfer from legumes to the intercropped *Brassica* sp.

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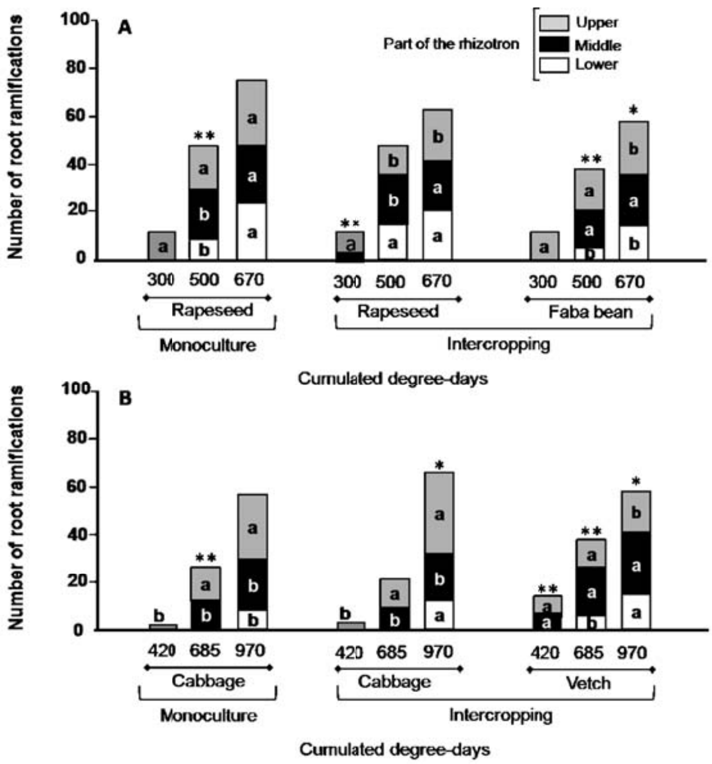

Figure 1. Distribution of brassica and legume roots in the upper, middle and lower layers of the rhizotrons: A – Rapeseed in monoculture or sown with faba bean, B – Cabbage in monoculture or sown with vetch.
*, **, *** indicate statistical differences between depth sections at the same date. (a, b) indicate significant differences between brassicas and legumes for a given depth at a given date

Slika 1. Raspored korena kupusnjača i mahunarki u gornjim, srednjim i donjim slojevima rizotrona: A – repica u čistom ili združenom usevu sa bobom, B – kupus u čistom ili združenom usevu sa grahoricom.
*, **, *** ukazuju na statističke razlike između dubinskih slojeva istog datuma. (a, b) ukazuju na statističke razlike između kupusnjača i mahunarki za datu dubinu i dati datum

Materials and Methods

Experimental Design

In June 2009, seeds were sown in rhizotrons made of Plexiglas® (inner parts: 47 cm length × 19 cm width × 4.6 cm depth) filled with a clay and sandy-rich soil sieved to a 3 mm particle size. We studied four modalities with two plants per rhizotron (N=10): (1) monospecific forage rapeseed (*Brassica napus* L. cv. 'Licapo'), (2) forage rapeseed with faba bean (*Vicia faba* L. ssp. *minor* cv. 'Gloria'), (3) monospecific fodder cabbage (*B. oleracea* L. cv. 'Proteor'), and (4) fodder cabbage with common vetch (*Vicia sativa* L. cv. 'Pepite'). The rhizotrons were randomly distributed along culture tables in a greenhouse, placed with an inclination of 50 degrees to the horizontal plane, and protected from light with black plastic bags. Soil was kept at field capacity with an automatic watering system.

Root Development

Root progression was drawn every two days on the lowest side of the rhizotrons, using different colours at each date. At the end of the experiment, the root length, the number of ramifications and their distribution in the upper, middle and lowest parts of the rhizotrons were recorded.

Isotopic Methods

In five rhizotrons of each *Brassica*-legume intercropping, the legume was continuously labelled with 0.2% v/w urea 0.99% atom ^{15}N until harvest (Mahieu et al. 2009). Twenty-five and 28 days after sowing, faba beans and vetches respectively were labelled by cotton wick stem-feeding (Mahieu et al. 2007). Unlabelled rhizotrons were kept as controls (N=5). In all modalities, the aboveground parts were harvested 45 days after sowing, separately dried (70°C), weighed and ground before preparation for ^{15}N:^{14}N mass spectrometer measurements. Transfer of N from legume to *Brassica* (%Ntrans) was estimated based on equation (1) (Høgh-Jensen & Schjoerring 2000).

(1)%Ntrans = [N brassica × (B_1-B_0)] / [N legume (L_1-A_0) + N brassica × (B_1-0.3663)] × 100

where N brassica and N legume denote the aboveground N content of *Brassica* and legume respectively; B_1 is the enrichment (atom%) of labelled *Brassica*; B_0 is the average natural abundance (atom%) of *Brassica* growing in unlabelled conditions; L_1 is the enrichment of labelled *Brassica* and 0.3663 is the natural abundance of air. Estimation of the legume N derived from atmosphere was calculated as described by the natural abundance method (Hansen & Vinther 2001), and *Brassica* monocultures were used as reference. Data were compared with Kruskal-Wallis and Mann-Whitney tests based on median equality (GraphPad Prism 5 Software 2007).

Results

Niche Separation in the Rhizotrons

In the *Brassica* monocultures, the distribution of root ramifications in the rhizotron parts did not differ between the two sown plants ($P > 0.05$, data not shown). At 670 degree-days (harvest), rapeseed roots colonised the three compartments similarly (Fig. 1A). In cabbage monocultures, there was no root ramification in the lowest part of the rhizotron (Fig. 1B).

When intercropped with faba bean, the proportion of the roots of rapeseed located in the upper part of the rhizotron was significantly lower than in monoculture ($P < 0.001$, Fig. 1A).

Table 1. Dry matter weight and N content in Brassica grown either with a legume or in a monospecific rhizotron – mean (s.e.)
Tabela 1. Prosečne vrednosti (i njihove standardne greške) prinosa suve materije i sadržaja azota kupusnjača gajenih sa mahunarkama ili u čistom rizotronu

	Dry matter weight (g plant^{-1}) Prinos suve materije		N content (g plant^{-1}) Sadržaj azota	
Companion Komponenta Brassica	Cabbage Kupus	Rapeseed Repica	Cabbage Kupus	Rapeseed Repica
Monoculture Čist usev	1.77 (0.13) b	4.37 (0.30) b	2.69 (0.22) b	4.99 (0.46) b
Common vetch Obična grahorica	2.39 (0.22) a	-	3.77 (0.33) a	
Faba bean Bob	-	6.91 (0.80) a	-	8.67 (0.94) a
P	*	**	*	**

* $P < 0.05$; ** $P < 0.001$; – letters a and b indicate significant differences between lines within a column; N = 10 - Mann-Whitney and Kruskal-Wallis tests
* $P < 0.05$; ** $P < 0.001$; – slova a i b ukazuju na značajne razlike između vrednosti unutar kolone; N = 10 – Man-Vitnijev i Kraskel-Volisovi testovi

Table 2. Estimation of the part of the legume N derived from fixation (% Ndfa) and of the Brassica N derived from the legume in Brassica-legume intercrops (% Ntrans) – mean (s.e.).
Tabela 2. Prosečne vrednosti (i njihove standardne greške) procene učešća azota mahunarki iz fiksacije (% Ndfa) i azota kupusnjača iz združenog useva kupusnjača i mahunarki (% Ntrans)

	% Ndfa	% Ntrans
Rapeseed - faba bean Repica - bob	75.0 (5.7)	12.0 (2.9)
Cabbage – common vetch Kupus –obična grahorica	66.3 (1.9)	7.8 (0.4)

The proportion of the roots located in the lowest part of the rhizotron was higher in rapeseed than in faba bean ($P < 0.001$ at 670 degree-days). Conversely, vetch produced a markedly higher amount of ramifications than cabbage in the upper and the middle part of the rhizotron at 420 and 685 degree-days ($P < 0.001$, Fig. 1B). At 970 degree-days, most of the root ramifications in the cabbage cultivar were located in the upper section, while there were more vetch roots in the middle section ($P < 0.001$, Fig. 1B).

Dry Matter Weight and Nitrogen Measurements

Yield and N content of *Brassica* cultivars were significantly higher when they were grown with a legume than in monospecific rhizotrons (Tab. 1).

In *Brassica*-legume rhizotrons, the part of the legume N derived from symbiotic fixation was higher than 65% (Tab. 2). About 10% of the *Brassica* N was derived from the companion legume.

Discussion and Conclusions

In agreement with previous studies of belowground competition (Gersani et al. 2001), in a monoculture of *Brassica,* roots competed in all soil layers. However, as demonstrated in pea-barley mixtures by Corre-Hellou et al. (2007), spatial niche separation of roots may be positively involved in yield and N content differences between monoculture and intercropped *Brassica* sp. From the beginning of biological N fixation, intercropped *Brassica* and legume cultivars used different N sources, which enhanced the niche separation. Corre-Hellou et al. (2006) showed that because of such complementarity effects, barley meets its N requirement more easily in pea-barley intercrops than in monoculture.

In the early stages of root growth, only 20 days of continuous ^{15}N urea labelling were sufficient to reveal substantial N transfer between legume and *Brassica.* Based on data reported in previous literature, N transfer may represent up to 30-50% of N rhizodeposition of vetch and faba bean (Wichern et al. 2008, Fustec et al. 2010). Jensen (1996b) showed that N transfer between pea and barley may be enhanced by root intermingling. As a consequence, in *Brassica*-legume intercropping, too different distribution of root ramifications between the plants would not be efficient to combine the effects of niche separation with those of N transfer on the *Brassica* yield and N content.

In conclusion, our results suggest that LER values higher than one can be obtained by cocultivating a legume with a *Brassica* forage. Further experiments should be undertaken in greenhouse conditions and in field conditions to better characterise the plant-to-plant belowground interactions. A better management of these complex biotic interactions would be useful for increasing yield by optimising the balance between root competition, niche complementarity and N transfer. Root traits of associated genotypes are crucial points for maximising N flux in *Brassica*-legume mixtures.

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Nišno razdvajanje i prenos azota u združenim usevima kupusnjača i mahunarki

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Izvod: Kupusnjače (*Brassica* sp.) predstavljaju zanimljive kasnoletnje i jesenje krmne biljke. Pošto se odlikuju izraženim zahtevima za azotom, združeni usev sa mahunarkama može da dovede do povećanja prinosa uz mala ulaganja. Međutim, združeni usevi kupusnjača i mahunarki i dalje ostaju nedovoljno proučeni. Korišćenjem rizotrona u stakleniku, uporedili smo razviće korena (1) krmne repice u združenom usevu sa bobom u odnosu na čist usev krmne repice i (2) krmni kupus u združenoj setvi sa običnom grahoricom u odnosu na čist usev krmnog kupusa. Mahunarke su bile obeležene ^{15}N ureom. Između sedam i osam nedelja nakon setve, prinos kupusnjača i sadržaj azota bili su veći u združenom u odnosu na čist usev. U združenoj setvi, raspored grananja korena duž glavnog korena razlikovao se u odnosu na čist usev, što je umanjilo uticaj kompeticije. Takođe, prenos azota iz mahunarki u kupusnjače bio je značajan.

Ključne reči: azotofiksacija, nišno razdvajanje, omogućavanje, prenos azota, prinos, združeni usev