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The effect of intercropping on the efficiency of faba bean – rhizobial symbiosis and durum wheat soil-nitrogen acquisition in a Mediterranean agroecosystem

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ABSTRACT

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The aim of this study was to compare the rhizobial symbiosis and carbon (C) and nitrogen (N) accumulations in soil and plants in intercropping *versus* sole cropping in biennial rotation of a cereal – durum wheat (*Triticum durum* Desf.), and a N_2-fixing legume – faba bean (*Vicia faba* L.) over a three-year period at the INRA (National Institue of Agronomic Research) experimental station in the Mauguio district, south-east of Montpellier, France. Plant growth, nodulation and efficiency in the use of rhizobial symbiosis (EURS) for the legume, nitrogen nutrition index (NNI) for the cereal, and N and C accumulation in the soil were evaluated. Shoot dry weight (SDW) and NNI were significantly higher for intercropped than for the sole cropped wheat whereas there was no significant difference on SDW between the intercropped and sole cropped faba beans. EURS was higher in intercropped than in sole cropped faba bean. Furthermore, by comparison with a weeded fallow, there was a significant increase in soil C and N content over the three-year period of intercropping and sole cropping within the biennial rotation. It is concluded that intercropping increases the N nutrition of wheat by increasing the availability of soil-N for wheat. This increase may be due to a lower interspecific competition between legume and wheat than intra-specific competition between wheat plants, thanks to the compensation that the legume can achieve by fixing the atmospheric nitrogen.

Keywords: carbon storage; grain yield and quality production; legumes; macronutrients; N_2 fixation; plant-soil system

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The world's population increases rapidly and global food production must be adapted to the requirements of human consumption. To increase the cultivated acreage would be very difficult, so it is necessary to promote crop production or the efficient use of existing croplands (Ohyama 2017). In response to this challenge, many farmers turn to a new agriculture called modern agriculture, which produces high yields through the generous use of chemical inputs and non-renewable energy, although these chemical inputs limit the cropping systems sustainability. This modern agriculture is being called into question (Ohyama 2017).

Nutritional benefits and agro-ecological services of legumes may constitute an alternative agronomic practice for a better use of the growth resources, by integrating them into cropping systems either in intercropping or in rotating crops (Scalise et al. 2015). On the one hand, rotation including legumes has replaced fallow since the 18th century in Europe, particularly in the Mediterranean basin (Grigg 1974). Rotation would maintain soil fertility when the cycle includes a legume as food-crop or forage-crop, since it provides nitrogen supporting the accumulation of soil organic matter (Liebman and Dyck 1993). On the other hand, intercropping is defined as a system with two or more crops with complementary growth cycles grown simultaneously in the same field (Vandermeer 1989). This system may increase yields and improve the efficiency of the use of soil resources compared to monocropping through complementarity, facilitation and competition between intercropped legumes and cereals (Hauggaard-Nielsen et al. 2008).

Intercropping may also increase N inputs to the plant-soil system, and the growth and yield of the intercropped cereal, by improving the efficiency of rhizobial symbiosis (EURS) (Betencourt et al. (2012) for wheat-chickpea intercrops and Latati et al. (2016) for maize-common bean intercrops). Under field conditions, Li et al. (2001) reported a significant increase in the yield of wheat when it was grown in intercropping with soybean compared to their respective sole cropping. In a long-term experiment, Cong et al. (2015) showed that both intercropping and rotating wheat and faba bean increased N uptake and below-ground productivity. These authors suggested the importance of interspecific facilitation, with symbiotic N_2 fixation by intercropped faba bean improving N availability in the rhizosphere. However, the impact of the cropping systems on nitrogen-fixing nodules of the rhizobial symbiosis remains poorly understood.

Moreover, the comparative effects on carbon storage of cereal-legumes intercropping and rotations are still poorly known, while it is increasingly established that in intensive agricultural systems, the organic carbon pools in the soil have a negative balance because losses due to root and microbial soil respiration are substantially higher than the gains associated with the decomposition of roots and aerial parts of the plant (Jarecki and Lal 2003). Whereas intensive monocropping systems appear to contribute significantly to the anthropogenic increase of atmospheric CO_2 concentrations (IPCC 2007), species diversity may have a beneficial effect through both functional complementarity and facilitation between plants, by improving productivity while increasing C and N stocks in soil and crop residues (Callaway 2007). Introducing crop management practices such as intercropping of cereals and legumes in agroecosystems can improve soil C stocks (Cong et al. 2015), notably in the rhizosphere of intercropped legumes with low soil organic matter inputs (Tang et al. 2014).

Considering the impact of intensive agriculture on the environment and human health, as well as the many alternatives proposed, such as intercropping legume-cereal, it seems very interesting to know the impact of the intercropping cereal-legume on the symbiotic root nodulation, and to what extent this system would affect the activity of the rhizobial symbiosis in comparison with a legume sole crop.

MATERIAL AND METHODS

Experimental site. Field experiments were carried out over three cropping seasons: 2013, 2014 and 2015. They were located in the INRA (National Institue of Agronomic Research) experimental station in the Mauguio district south-east of Montpellier, France. The centre of the experimental surface was located at 43°37'26.88''N, 3°59'0.34''E.

The region has irregular rainfall, with an annual mean of 609 mm during the period from 2013 to 2015. July was the driest month (mean rainfall of about 14 mm), while the highest rainfall was in autumn (mean of 118 mm in September). The mean annual temperature was about 15°C. The warmest month was July (mean of 25°C), while the coldest month was February (mean of 7°C).

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The soil was approximately 31.4% loam, 21.9% clay, 25.2% fine silt and 21.5% coarse silt. The experimental site was alkaline (pH 8.2), with 17 g/kg $CaCO_3$, and the cation exchange capacity was about 21.8 $cmol_+$/kg. The plots used for the experiment were fallow until 2011 and began to be grown in 2012 using the same cropping systems as in this article.

Cropping systems and plant growth conditions. The field experiment was carried out with one durum wheat cultivar (*Triticum durum* Desf. cv. LA1823) and one faba bean cultivar (*Vicia faba* L. cv. Diva). They were grown as intercrops and as sole crops within biennial rotation between each year. Before crop planting, the first 20 cm of soil were mechanically ploughed using a rotary spading machine, then a passage with a rotary harrow was carried out to prepare seed bed, finally, the seeding was performed at 4 cm depth using an experimental seeder followed by a smooth roller pass to promote the contact between soil and seeds. No chemical or fertilizer treatment was applied to the crops. The experimental design was a split-plot with four blocks. Each block included four sub-plots of 6.2 m × 21 m with a total area of the experimental design of 2083.2 m^2 (4 × 6.2 m × 4 × 21 m). Each sub-plot was the subject of one of the following four cropping systems (1) wheat-faba bean intercropping system; (2) wheat sole cropping system; (3) faba bean sole cropping system; (4) unseeded and weeded fallow surface. For each sub-plot, mechanical weeding and manual complement was made. After each year, rotation was made between sub-plots of wheat and faba bean sole crops. According to the current farming practice, the seeding density was 350 plants per m^2 for durum wheat as a sole crop, 60 plants per m^2 for faba bean as a sole crop and 175 plants for durum wheat and 30 per m^2 for faba bean as intercrops.

Plants and soil sampling and measurements. The soil and plants were sampled at the full flowering stage of the faba beans in 2013–2015 growing seasons. For each sub-plot with a given cropping-system, four sampling points were chosen. On each sampling point, a sample of 3 to 5 plants was collected with soil near the roots. The soil near the roots was carefully removed at 30 cm depth to preserve the roots and soil samples were combined into one sample for each sub-plot. Thereafter, this soil was sieved to less than 4 mm to remove the coarse fraction, and then dried in ambient air at least for 48 h. Finally, the soil obtained after sieving to 4 mm was finely ground using a mechanical grinder and then sieved to 200 µm (Pansu et al. 2001).

In the laboratory, the wheat and faba bean shoots were separated from the roots at the cotyledonary nodes. For each faba bean plant, the nodules were separated from the roots, dried and weighed. The shoots were dried for 48 h at 65°C, and then weighed.

The total yield of both crops was determined following a mechanical harvest using a combine harvester; for each cropping system and sub-plot the crop was collected separately and weighed in the laboratory. In addition, after each harvest the crop residues of each sub-plot were crushed mechanically using the stubble cutter and then incorporated in the soil.

Total N and total C in both plants (shoots, roots, nodules and grain) and soil were determined using an elementary dry combustion analyser (NA2000, Fisons Instruments, Ipswich, UK).

Efficiency in the use of rhizobial symbiosis. For legumes, the relationship between changes in the biomass of nitrogen-fixing symbiotic nodules and changes in the plant biomass may be an estimator of the efficiency in utilization of the rhizobial symbiosis (Drevon et al. 2011). This simple relationship can also be considered as an indicator of the ability of symbiotic nodules to fix atmospheric nitrogen. In addition, in practice, shoot biomass is often used instead of the total plant biomass to avoid possible underestimation of root biomass during field sampling.

Nitrogen nutrition index. Lemaire et al. (2008) defined the nitrogen nutrition index (NNI) as an indicator for crop nitrogen nutrition. It is defined as the ratio between the actual crop N uptake (N_a) and the critical N uptake (N_c) corresponding to the minimal N uptake without deficiency to allow the maximal growth rate at any time of plant growth, so:

$$NNI = \%N_a/\%N_c \quad (1)$$

$\%N_c$ is determined with empirical dilution curve such as:

$$\%N_c = ac \times W^{-b} \quad (2)$$

Where: W – actual crop mass (t/ha); *ac* – critical plant N concentration for 1 t W/ha, and *b* – constant (Lemaire et al. 2008). For wheat, the values of *ac* and *b* are 5.3% and 0.44 (Lemaire et al. 2008).

Statistical analysis and calculations. One- and two-way analyses of variance (ANOVA) were per-

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Table 1. *P*-values of two-way ANOVAs with factors year and cropping system on plants and soil variables of this study

	Shoot dry weight (g/m^2)		Nodule dry weight (g/m^2)	Nodules number (nod/pl)	Nitrogen nutrition index		Soil C	Soil N
	wheat	faba bean	faba bean		wheat	faba bean	(%)	
Growing season (= year)	0.23^{ns}	$1.7 \times 10^{-5***}$	$< 2 \times 10^{-16***}$	0.5^{ns}	$3.3 \times 10^{-07***}$	$58 \times 10^{-5***}$	$4 \times 10^{-4***}$	$2.6 \times 10^{-7***}$
Cropping system	$12 \times 10^{-5***}$	0.94^{ns}	$< 2 \times 10^{-16***}$	0.4^{ns}	$1.6 \times 10^{-09***}$	0.88^{ns}	0.05^{*}	$7.5 \times 10^{-4***}$
Interaction between growing-season and cropping system	0.51^{ns}	0.15^{ns}	0.57^{ns}	0.8^{ns}	$66 \times 10^{-5***}$	0.21^{ns}	0.48^{ns}	$3.7 \times 10^{-4***}$

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{ns}not significant ($P > 0.05$)

formed using the R software (R Core team 2016). The means were subsequently prioritized using the Tukey's multiple comparison tests at $P = 0.05$. For the cropping systems with faba bean and for each year, a relationship between shoot (SDW) and nodule (NDW) dry weights was determined by a linear regression.

In order to compare the biomass stocks per equivalent area units (g/m^2), SDW and, for faba bean, NDW per plant (g/plant) were converted into stocks as follows:

$$\text{SDW (or NDW) stocks} = \text{SDW (or NDW)} \times SD_{corr} \quad (3)$$

Where: SD_{corr} – sowing density (SD) of a given species corrected by the area actually occupied by this species. Thus, SD_{corr} = SD for sole crops, and $SD_{corr} = SD/0.5 = SD \times 2$ in intercropping since the area was halved in intercropping for each species.

Furthermore, to compare the grain protein content, grain N content was converted into protein content as follows:

$$\text{Protein (\%)} = \text{N(\%)} \times k \quad (4)$$

Where: k – conversion factor, with wheat $k = 5.7$ and faba bean $k = 6.25$ (Rharrabti et al. 2001, Mohamed Osman Ali et al. 2014).

RESULTS AND DISCUSSION

Nodulation. As shown in Table 1, nodule dry weight of faba bean was very significantly affected by years and cropping system in both cases as compared to nodule number per plant (NN). Figure 1 shows that the NDW per equivalent unit area (NDW g/m^2) was significantly higher in sole cropping than in intercropping (+ 54%) in 2013, but not in 2014 and 2015. In contrast, no significant effect of the cropping system or years was observed on nodules number per plant.

Figure 2 shows significant and positive correlations between NN and NDW in intercropping and in sole cropping within biennial rotation for faba bean. In 2014, the individual nodule-mass remained between 3 and 4 mg whatever the cropping system. However, it was 3.8 mg in intercropping *vs.* 5.2 mg in sole cropping in 2015.

Several other field studies have addressed the effect of intercropping in various legume-cereal systems on nodule growth in the Mediterranean

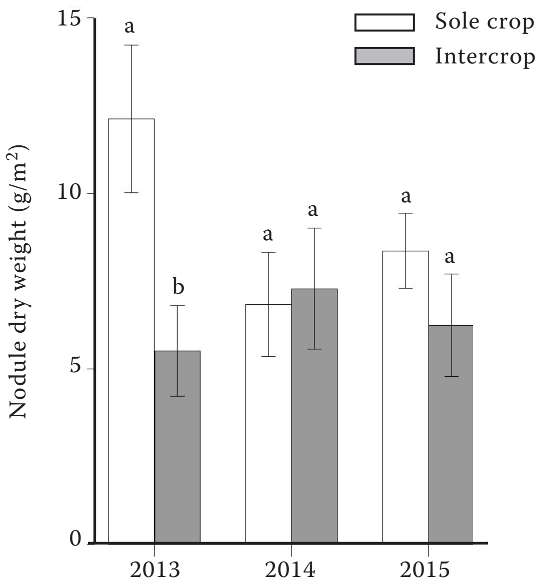

Figure 1. Nodule dry weight of faba bean for each year in sole cropping versus intercropping. Data are means and standard errors of 16 replicates harvested at 130 days after sowing. Within a given year, mean values labelled with different letters are significantly different at $P < 0.05$

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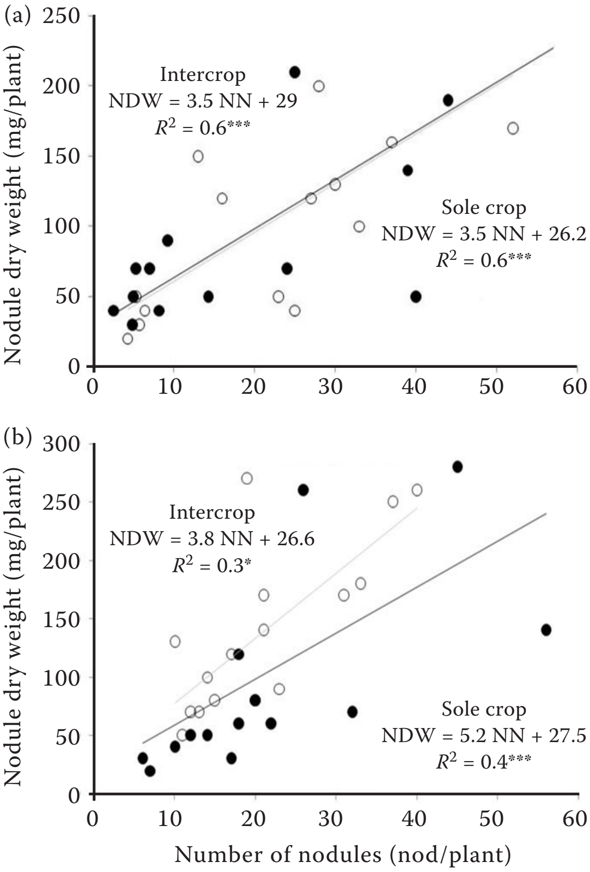

Figure 2. Nodule dry weight as a function of number of nodules for each plant for sole cropped faba beans (white dots) or intercropping (black dots) for (a) 2014 and (b) 2015. The equations on the charts are the regressions for sole crops (light grey text) and intercrops (dark grey text). All regressions were established from sixteen replicates (sixteen plants). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

region (Betencourt et al. 2012, Latati et al. 2016a) and in greenhouse experiments (Li et al. 2016).

Efficiency in the use of the rhizobial symbiosis. Figure 3 shows significant relationships between nodule dry weight and shoot dry weight. The EURS was significantly higher for intercropping than for sole cropping in 2014 (115 *vs*. 49 g SDW/g NDW) and in 2015 (54 *vs*. 12 g SDW/g NDW), but not in 2013 (17 *vs*. 11 g SDW/g NDW). However, Table 1 shows a very significant effect of years on shoot dry weight of faba bean but no significant effect of the cropping system.

This increase in EURS under the intercropping system has also been reported by several other recent studies on Mediterranean agroecosystems (Drevon et al. 2011, Latati et al. 2016). However, for intercropping wheat and faba bean, our results show that NDW (g/m^2) was lower for intercropping in 2013 while for 2014 and 2015, there were no significant differences in the number of nodules and NDW between the two cropping systems. Therefore, as the estimated EURS was higher for intercropping, it would seem that the symbiotic fixation of atmospheric nitrogen does not depend only on nodule biomass. Latati et al. (2014) also reported decoupling between the estimated EURS and the nodular biomass with a

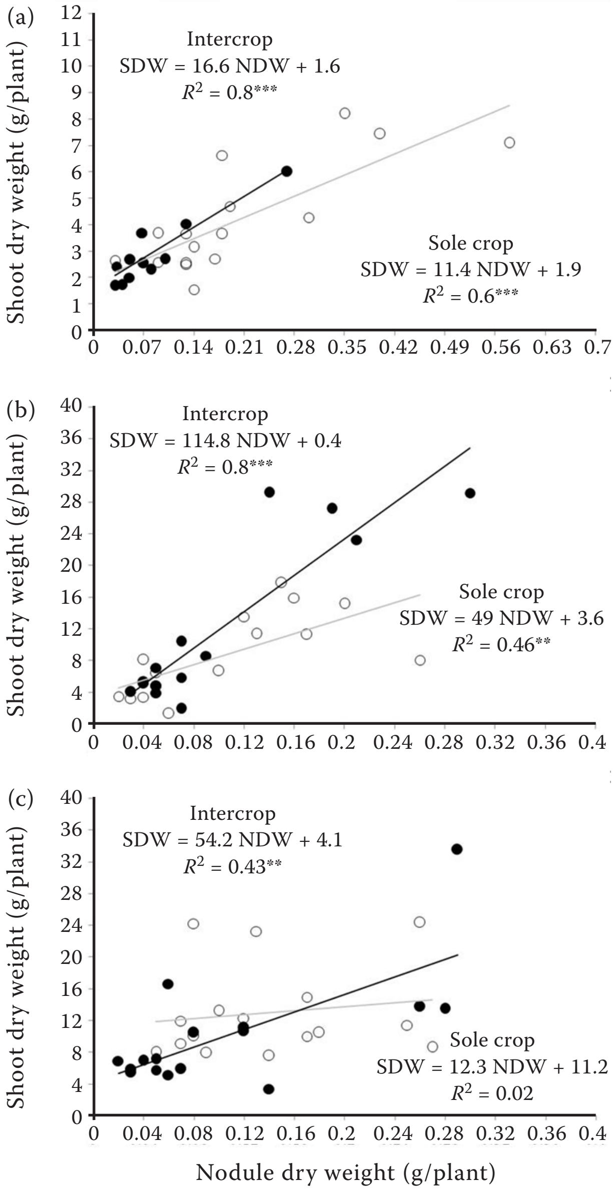

Figure 3. Efficiency in use of the rhizobial symbiosis for faba bean in sole cropping (white dots) *versus* intercropping (black dots) for (a) 2013; (b) 2014 and (c) 2015. The equations on the charts are the regressions for sole crops (light grey text) and intercrops (dark grey text). All regressions were established from sixteen replicates (sixteen plants). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

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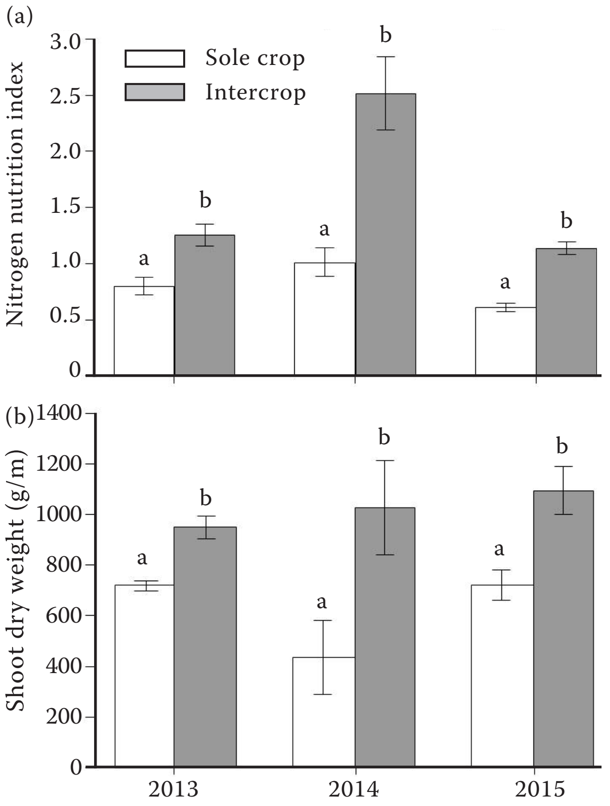

Figure 4. Durum wheat nitrogen nutrition index (a) and shoot dry weight (b) for sole crops and intercrops. Means and standard error for sixteen replicates harvested at 130 days after sowing. For each year, mean values labelled with the same letters are not significantly different at $P < 0.05$

significantly lower nodule biomass but a higher estimated EURS for intercropping.

Cereal nitrogen nutrition index and growth. Table 1 indicates that with a two-way ANOVA the NNI was very significantly affected by years and by cropping systems. There was also a significant interaction between these two factors, but, as indicated by Figure 4a, this interaction was not a cross interaction, and it was only the fact that wheat NNI was particularly high under intercropping system in 2014 growing season. Furthermore, Figure 4a shows that NNI for intercropping was greater than 1, while it was less than 1 or equal to 1 for sole cropping. NNI for intercropping was 56, 148 and 85% higher than for sole cropping in 2013, 2014 and 2015, respectively.

As shown in Table 1 wheat SDW was very significantly affected by the cropping system but not by the year. The wheat SDW was significantly higher in intercropping than in sole cropping within biennial rotation: by 32% in 2013, 136% in 2014 and 52% in 2015 (Figure 4b).

The nitrogen nutrition index also indicated that nitrogen nutrition was higher for the intercropped wheat compared to the sole cropped wheat within biennial rotation. This is similar to the results of Latati et al. (2016) who found that the NNI of maize was higher when intercropped with common bean than when monocropped. Furthermore, the shoot dry weight of wheat was significantly higher for intercropping than for sole cropping within biennial rotation over the three- year period of the study. This is in agreement with another study at the same experimental station that showed a higher shoot dry weight of wheat when intercropped with cowpea (Betencourt et al. 2012).

Soil nitrogen and carbon content. Soil organic nitrogen and carbon contents (%) were calculated

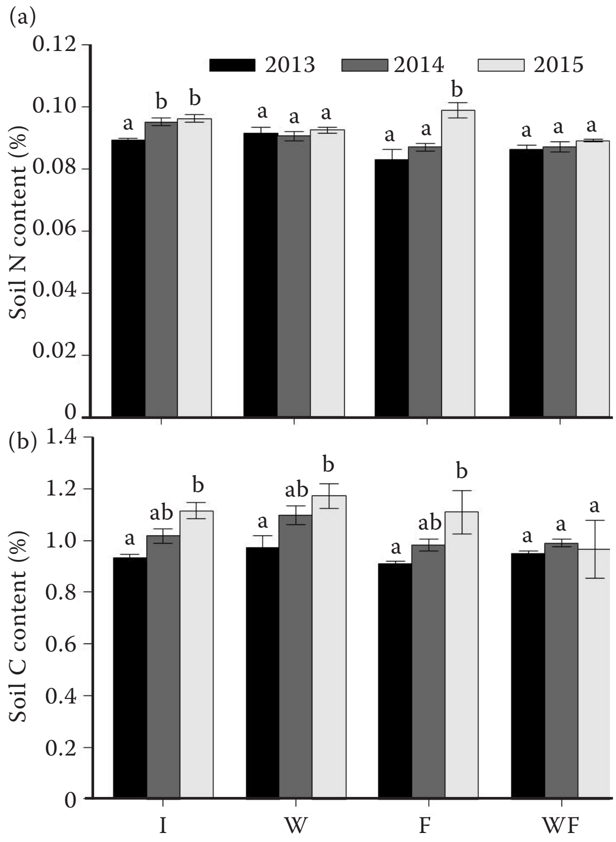

Figure 5. Soil nitrogen (a) and carbon (b) contents in sole cropping versus intercropping *versus* fallow for 2013, 2014 and 2015. Means and standard error of four replicates for intercropping and unseeded weeded control and eight replicates for rotation. For each cropping system, mean values labelled with the same letters are not significantly different at $P < 0.05$. Where I – intercrops; W – wheat sole crop; F – faba bean sole crop; WF – weeded fallow

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Table 2. *P*-values of two-way ANOVAs with factors year and cropping system on grain yield and grain protein content variables of this study

	Grain yield (t/ha)		Grain protein (%)	
	durum wheat	faba bean	durum wheat	faba bean
Growing season (= year)	0.62^{ns}	0.61^{ns}	$3.94 \times 10^{-12***}$	0.2^{ns}
Cropping system	$7.93 \times 10^{-5***}$	0.86^{ns}	$4.73 \times 10^{-14***}$	$5.26 \times 10^{-6***}$
Interaction between growing-season and cropping system	0.48^{ns}	0.41^{ns}	$3.53 \times 10^{-6***}$	0.4^{ns}

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{ns}not significant ($P > 0.05$)

for the 0–30 cm soil layer for intercropping, for sole crop within biennial rotation, and for unseeded weeded fallow for three years. Table 1 shows a significant effect of years and the cropping system on soil organic nitrogen and carbon content.

As shown in Figure 5a, soil organic N content increased significantly over the three growing seasons when faba bean was present in the cropping system, but no significant increase of N content was observed for wheat sole crop within the biennial rotation and the unseeded-weeded fallow plots. The increase was 8% and 14% for intercropping and faba bean sole crop within biennial rotation, respectively. This is in accordance with the results obtained by Cong et al. (2015), who showed that soil nitrogen storage in a long-term experiment was 11 ± 1% higher in intercropping faba bean with maize and wheat than in cereal monocropping, or by Hauggaard-Nielsen et al. (2008) who reported that the soil N concentration was higher under pea, faba bean and lupin intercropped with barley, in comparison with barley sole crop. In our study, the soil N content increase in the presence of faba bean may be due to the symbiotic fixation of atmospheric nitrogen by legume which is able to reduce the soil N consumption, and then to increase the soil N availability. On the other hand, for wheat sole crop within biennial rotation, our study suggests that the additional nitrogen brought by legumes the previous year is consumed during the growing season.

Furthermore, Figure 5b shows an increase of soil C content over the three years under intercropping, faba bean and wheat sole crops within biennial rotation (by 19.3% C, 22.2% C and 20.6% C, respectively), but no significant increase was observed under the unseeded-weeded fallow plots. An increase of C content over time in systems including legumes has already been observed in different parts of the world (e.g. Barthes et al. (2006) in tropical zone for maize and cassava intercropped with beans and peanuts, Dyer et al. (2012) for intercropped maize and soybean; Chapagain and Riseman (2014) for intercropped barley-pea, Scalise et al. (2015) in a south Italian agroecosystem for intercropped barley and faba bean). However, an increase in C content

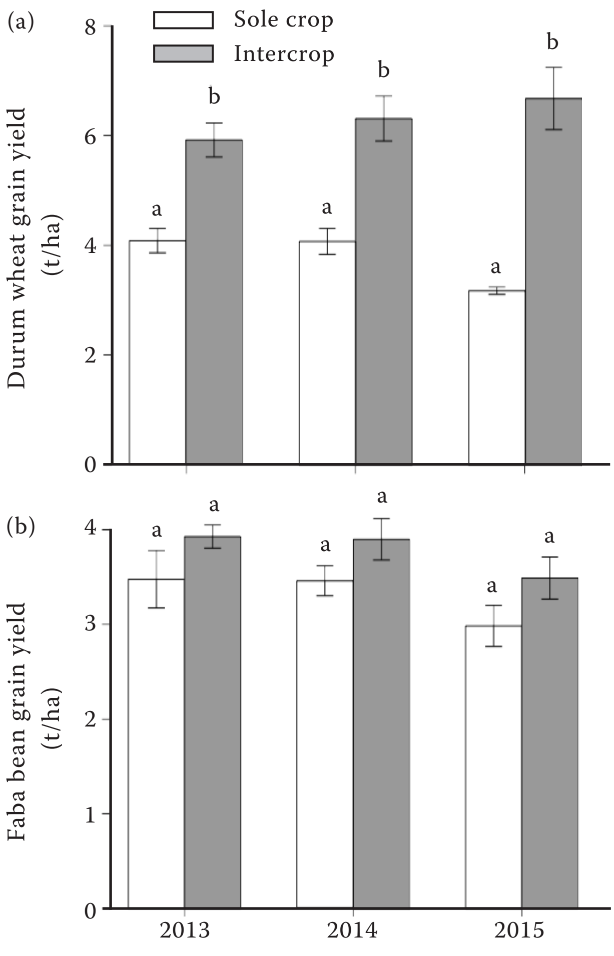

Figure 6. Durum wheat (a) and faba bean (b) grain yield for sole crops and intercrops. Means and standard error for sixteen replicates harvested at total maturity. For each year, mean values labelled with the same letters are not significantly different at $P < 0.05$

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under wheat sole crop was also observed in this study. This shows that the incorporation of cereal crop residues into soil quite poor in organic matter can also have a significant effect in terms of soil carbon storage.

Grain yield and grain protein content. Table 2 indicates that the grain yield of durum wheat was significantly affected by the cropping systems and not by the year (Figure 6a); however, faba bean grain yield was not affected by the cropping systems and the year (Figure 6b).

As shown in Figure 6a, the wheat grain yield was significantly higher in intercropping than in sole cropping within the biennial rotation – by 27% in 2013, 58% in 2014 and 50% in 2015.

Furthermore, Table 2 also indicates that the protein content in wheat and faba bean grain was significantly affected by cropping systems. Figure 7a shows

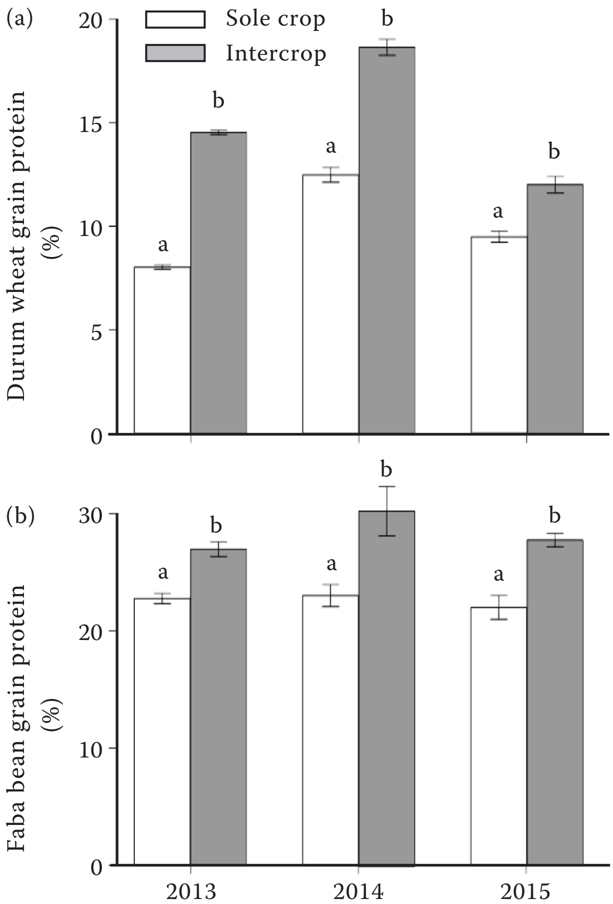

Figure 7. Durum wheat (a) and faba bean (b) grain protein content of sole crops and intercrops. Means and standard error for sixteen replicates harvested at total maturity. For each year, mean values labelled with the same letters are not significantly different at $P < 0.05$

an increase in the protein content in wheat grain intercropped compared to sole crop within biennial rotation by 45% in 2013, 55% in 2014 and 110% in 2015. Similarly, faba bean grain protein content for intercropping was 18, 32 and 26% higher than for sole cropping within biennial rotation in 2013, 2014 and 2015, respectively (Figure 7b).

This increase in wheat productivity in intercropping has also been reported by Huňady and Hochman (2014) who found that grain yield of wheat was higher in intercropping with faba bean than in monocropping. It was also reported in several studies with either maize (Li et al. 2005, Dahmardeh et al. 2010) or durum wheat (Zhang and Li 2003) intercropped with cowpea, faba bean and soybean. Furthermore, the protein content in wheat and faba bean grain was significantly higher for intercropping than for sole cropping in the biennial rotation over the three-year period of the study. This is consistent with another study that found an increase in the protein grain content of wheat and pea concentrations in intercrop compared to the two monocrops (Bedoussac and Justes 2010).

This study provides further evidence that cereal-legume intercropping is a promising alternative to intensive agricultural systems without legumes. Also, the ecological services provided by the legume seem to be able to reduce the need for N inputs, with higher land productivity, acquisition of N and sequestration of C in biomass and soil.

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