Cereal/grain legume intercropping in rotation with durum wheat in crop/livestock production systems for Mediterranean farming system

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ABSTRACT

Increasing home-grown production of legumes can contribute to the sustainable development of the agriculture in Europe. The adoption of mixed crop/livestock systems could be a tool to increase the cultivation of legume crops in cereal farming systems, particularly in the smallholder farming systems of Southern Europe. This study proposes cereal-legume intercropping as a sustainable intensification tool specifically suitable for crop/livestock mixed systems under rainfed condition. The cereal/grain legume intercropping is used in this study as an alternative to the sole crops in rotation with durum wheat with the assumption that this agronomic technique can still provide the benefits of legume crops in rotation sustaining the subsequent cereal crop production and overcoming the well-known limitations affecting grain legumes sown as sole crop.

Pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) were respectively intercropped with barley (*Hordeum vulgare* L.) for grain production and grown in rotation with durum wheat (*Triticum turgidum* L. subsp. *durum*) in an area in Southern Italy with typical Mediterranean

climate. The present study has demonstrated that cereal-legume intercropping can be a source of fodder (both pea and faba bean combinations) and still support yields of the following wheat cash crop. Pea-barley combinations (either additive or replacement design) enabled a better yield performance of the succeeding durum wheat as compared to faba bean-based intercropping. Pea-barley intercrops also reached a greater complementarity in the use of N sources and increased the overall sustainability of the rotation.

1. INTRODUCTION

The sharp drop during the 1960s of land cultivated with pure legume for fodder in Europe was the result of the progressive abandon of mixed crop-livestock systems. The specialization in agriculture has resulted in a spatial separation of livestock and feed production between agricultural areas (continents and regions) with a well-known negative impact on the environment. In crop-livestock mixed farming system, grain legume crops offer the opportunity to produce high-protein feed on-farm which is economically convenient (Preissel et al., 2017). The nitrogen coming from the decomposition of manure can also be combined with the nitrogen biologically fixed with the aim to reduce the reliance on synthetic fertilizer and to build soil organic matter within the crop rotation (Watson et al., 2017).

If on a large scale, a return to mixed farming systems is unlikely to happen for logistical, infrastructural and economic reasons, in contrast, a structured feed-livestock production network (short fodder supply chain) is more likely to be built at local level. In such a contest the adoption of mixed crop/livestock systems could be a useful tool to increase the viable use of legumes in cereal farming systems, particularly in the smallholder farming systems of the Mediterranea basin. In drought-prone areas of Southern Italy rainfed agricultural systems mainly depend on winter crops, often durum wheat (*Triticum turgidum* L. subsp. *durum*) along with common wheat (*Triticum aestivum* L.) and the inclusion of grain legumes in rotation with durum wheat, grown as cash crop, is an old traditional practice. The present study proposes the cereal-legume intercropping as an alternative to the legume sole crops in rotation with durum wheat with the assumption that this agronomic practice is able to sustain the yield of the subsequent cereal still providing the well-known benefits of legume crops in rotation.

Intercropping is a farming practice involving two or more crop species, or genotypes, growing together and coexisting for a time (Vandermeer, 1989). Intercropping of cereals and grain legumes has been largely reported as an eco-functional practice that can provide several advantages versus the monocrop system including better land use efficiency, maintenance of soil fertility, reduction of disease and pest incidence, and reduction of N losses from the agro-ecosystem (Bedoussac et al., 2015; Lithourgidis et al., 2011; Bedoussac et al., 2014).

Many limitations affecting grain legumes that grow in sole crop can be overcome by intercropping with cereals and this may facilitate their adoption in crop/livestock mixed systems. A key ecological feature of cereal-legume intercrops is the complementary use of growth resources, especially N (Ofori and Stern, 1987; Jensen, 1996a) and this complementarity can increase the yield stability of the system (Jensen 1996a; Hauggaard-Nielsen et al., 2009a) potentially overcoming low and instability yield issues related with legume sole crops (Bedoussac et al., 2015). The cereal in intercrop can reduce weed pressure (Corre-Hellou et al., 2011) and the C:N ratio of cereal-legume intercrops can lead to a more balanced N mineralization-immobilization turnover compared to that of residues from the respective sole crops (Hauggaard-Nielsen et al., 2003) and this may result in lower N leaching losses and N immobilization (Jensen et al., 2015). This last aspect can be of great importance in cropping systems of Southern Italy (and more broadly in Southern Europe) where high temperatures occurring during the summer promote the metabolic activities of soil microbial community, resulting in enhanced mineralization rates of organic C pools

(Moscatelli et al., 2007) and in late summer/early autumn the recently released mineral N pool can be easily lost through leaching or ammonia volatilization.

Intercrops evaluation should not only be considered in terms of crop management practices but it should also include the pluriannual cropping system. Issues such as integration of intercrops within traditional rotations and their subsequent crop effects, minimum time of return between two intercrops need to be clarified (Bedoussac et al., 2015). By sharing this need, in the present study, pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) were respectively intercropped with barley (*Hordeum vulgare* L.) for grain production (fodder) and grown in rotation with durum wheat (*Triticum turgidum* L. subsp. *durum*) for two consecutive 2–year crop-sequences. Faba bean is largely grown in semiarid and arid arable cropping system in Europe and it is winter-sown in warmer areas (Jensen et al., 2010) and due to its early flowering, pod and seed set, it can escape the incoming drought period typical of Mediterranean-type environments with less than 350 mm of annual average rainfall (Loss and Siddique, 1997). Pea is the most cultivated grain legumes under European temperate climates but its drought sensitivity, lodging and weak competitive ability towards weeds are a major concern for farmers (Hauggaard-Nielsen et al., 2008). Pea was included in the present experiment to assess its suitability as an alternative to faba bean in intercrop in semiarid environments of Southern Italy in light of its remarkable flexibility of utilization (grain and straw, hay or silage).

With the overall aim to assess the suitability of cereal-grain legume intercropping in crop/livestock mixed systems under rainfed condition of Mediterranean climate this study has: (i) evaluated, in two short rotation cycles, the effect on succeeding durum wheat grain yields and quality exerted by pea- and faba bean-intercropping with barley (at two sown arrangements) and by the respective legume sole crop; (ii) assessed the productive performance and N use of pea- and faba bean-intercropping systems as compared to pure stands of both components and their contribution to the overall rotation outcome; (iii) estimated the nitrogen balance for each rotation with a focus on soil N-nitrate dynamics in a Mediterranean contest.

2. MATERIAL AND METHODS

2.1 Experimental site

The experiment was carried out on a sandy-clay-loam soil (55% sand, 24% silt, 21% clay), classified as Fluventic Haploxerepts, coarse silty, mixed, thermic (Soil Survey Staff, 2010), at the agricultural experimental centre of the Regional Agency for Agriculture "ARSSA" located in San Marco Argentano (Italy) (39°38'N, 16°13'E, 100 m above the sea level) over four cropping seasons (2010-2014). Soil properties are as follows: C_{org} 9.81 g kg^{-1}; N_t 0.95 g kg^{-1}; C/N 10.33 ± 0.51; total $CaCO_3$ 18.0 ± 0.5 g kg^{-1}; active $CaCO_3$ 13.5 ± 0.4 g kg^{-1}; $pH_{(KCl)}$ 7.3; CEC 26.2 $cmol_{(+)}$ kg^{-1}; $EC_{1:2}$ 0.21 dS m^{-1}; NH_4^+ - N 17.0 mg kg^{-1}; NO_3^- - N 6.1 mg kg^{-1}; P (Olsen) 12.5 mg kg^{-1}. The annual rainfall in San Marco Argentano is 709 mm and the mean annual air temperature is 16.1°C (1995-2009 period).

2.2 Experimental set-up and crop management

In 2010/11 cropping season (year 1), six-row barley (B) (*Hordeum vulgare* L.) cv. Aldebaran, faba bean (F) (*Vicia faba* L. subsp. *minor*) cv. Sikelia and a tall, semi-leaf-less, medium early and indeterminate growth pea (P) (*Pisum sativum* L.) cv. Hardy were grown as (1) sole crop (SC), (2) replacement (F50B50, P50B50) and (3) additive (F100B50, P100B50) cereal-legume binary intercrop (IC). Two distinct experimental plots were established for the barley sole crop: one plot preceding unfertilised durum wheat (BSC) and the other one preceding fertilised durum wheat (BSCf). The sole crops were sown with planned density of 90, 40 and 300 seeds m^{-2} for pea, faba bean, and barley respectively. In intercrop each species was sown in alternate rows (16 cm apart) at half of its sole crop densities in the replacement design and at full (legume) and at half (barley) of its relative sole crop density in the additive design. On the individual rows, the actual barley density in both intercrop designs was the same as the sole crop while for the legume in the additive design it was the double of its density in sole crop. Plot size was 30 m^2 (3 x 10 m). The experiment was arranged as a randomized complete block design (RCBD) with three replicates.

The seedling bed was prepared according to conventional tillage practices: ploughing (at a 30-cm depth) in October 2010, chemical fertilization with 84 kg $ha^{-1}$of fertilizer $Ca(H_2PO_4)_2$ (Superphosphate) and disc harrowing (10 cm depth) immediately before seeding. The crops were sown with a precision line seeder on 22nd December 2010. Harvest took place at full maturity stage of barley (24th June 2011) and crop residues were removed from the field. In the following 2011/12 cropping season, durum wheat (*Triticum turgidum* L. subsp. *durum*) cv. Virgilio was sown as a subsequent crop on 30th November 2011 on all the twenty-four field plots at a density of 350 seeds m^{-2}. The durum wheat treatments were named after the preceding intercrop and sole crop treatments (F100B50, P100B50, F50B50, P50B50, PSC, FSC, BSC, BSCf). The seedling bed was prepared following the same tillage practices and fertilisation as the previous cropping season. No N fertilisation was provided to all treatments except to the plot identified as BSCf that received an additional amount of 50 kg N ha^{-1} (as urea) at the tillering stage. Durum wheat was harvested on 19th June 2012. The same experiment was repeated during the cropping seasons 2012/13 (intercrops and sole crops) and 2013/2014 (durum wheat) exactly on the same field plots that had hosted the experiment in the previous two seasons. Crops were sown on the 12th November 2012 and harvested on the 21st June 2013 in the third season and sown on the 9th December 2013 and harvested on the 17th June 2014 in the fourth season. Throughout this article the four growing seasons are often referred to as year 1 (2010/2011), year 2 (2011/2012), year 3 (2012/2013) and year 4 (2013/2014).

2.3 Plant biomass sampling and analysis

At the harvest stage, total aboveground biomass was sampled destructively from an area of 1 m^2 of each plot. Barley and legume plants from IC treatments were carefully separated, whereas plants from sole crop treatments were taken as a whole. Total dry matter production was determined after oven drying at 60°C until a constant weight was reached. The harvested biomass was then separated and threshed into grains and straw and finally ground by using a laboratory mill (1-mm sieve) before further analysis.

Total N content in plant biomass and $\delta^{15}N$ in legumes were determined on 5–10 mg subsamples of finely ground plant material. For total N content the Kjeldahl method was used whereas analysis of $\delta^{15}N$ was carried out at the laboratory of the Department of Chemical and Biochemical Engineering, Ecosystems Programme of the Technical University of Denmark using an ANA-MS (Carlo Erba-Finnigan) system coupled with an isotope ratio mass spectrometer (IRMS).

2.4 Soil sampling and analysis

Soil samples were collected periodically through the year including the main biological stages of the crops. Composite soil samples, each consisting of three randomly collected soil cores per plot pooled together (sub-replicates), were taken at 0-60 cm depth. Field moist samples were stored at 4 °C before being partially air-dried, sieved at < 2 mm particle size, and processed for chemical characterization. NO_3^--N was determined according to Mulvaney (1996) on soil extracted with 2 M KCl (1:10, w/v, soil:solution) under agitation (170 rpm, 1 h) at room temperature and then analysed using a Flow Injection Analysis System (FIAS 400

PerkinElmer, Inc., CT, USA) equipped with Autosampler AS90 (PerkinElmer) and Spectrophotometer UV/Vis Lambda 25 (PerkinElmer). Soil data were expressed as mean values on an oven-dry weight (dw) basis (105°C, 24 h) and referred to a sampling depth of 0-60 cm.

2.5 Calculation and statistics

The efficiency of the intercrop versus the sole crop system was evaluated using the Land Equivalent Ratio (LER). This index is defined as the relative land area under sole crop that is required to produce the same yield achieved by intercropping (Vandermeer, 1989). LER for a cereal-legume intercrop was calculated as the sum of partial LER values for cereal (L_C) and legume (L_L) in accordance with De Wit and Van den Bergh (1965):

$$L_C = \frac{Y_{C-IC}}{Y_{C-SC}}; \; L_L = \frac{Y_{L-IC}}{Y_{L-SC}}; \quad \mathrm{LER} = L_C + L_L$$

$Y_{C\text{-}IC}$ and $Y_{L\text{-}IC}$ are the yield of the cereal and legume component in IC, respectively, and $Y_{C\text{-}SC}$ and $Y_{L\text{-}SC}$ are the yield of the cereal and legume component in SC, respectively.

LER > 1 indicates an advantage from intercropping in terms of the use of environmental resources for plant growth whereas when LER < 1 resources are used more efficiently in sole crop than in intercrop. In the present paper, LER values are based on N accumulated in the aboveground biomass and it is named LER_N.

The percentage of atmospheric N_2 fixed by the legume (%Ndfa) was assessed using the ^{15}N natural abundance technique which is considered a powerful means of assessing N_2 fixation in field experiments (Unkovich et al., 2008). The amount of N fixed (kg ha^{-1}) was calculated as the product of legume biomass, biomass %N content and the percentage of plant N derived from N fixation (%Ndfa) (Hauggaard-Nielsen et al., 2009a). The %Ndfa was calculated using the ^{15}N contents of the legume ($\delta^{15}N_{legume}$) and of a non-fixing reference plant that in this experiment was barley sole crop ($\delta^{15}N_{barley}$) according to Shearer and Kohl (1986):

$$\mathrm{Ndfa}(\%) = 100 \times \frac{\delta^{15}\mathrm{N}\ barley - \delta^{15}\mathrm{N}\ legume}{\delta^{15}\mathrm{N} barley - B}$$

where the correction factor B is the $\delta^{15}N$ of shoots of legumes that are fully dependent upon N_2 fixation and that are grown in pots containing N-free draining coarse sand steam sterilised before use to eliminate resident Rhizobia in a naturally lit, temperature controlled glasshouse (Hauggaard-Nielsen et al., 2009a; Unkovich et al., 2008). In the present study, B values were equal to -0.72 and -0.70‰ for faba bean and pea respectively.

Soil N balances of the different cropping systems were based on measured aboveground biomass N values and calculated as the difference between N inputs and outputs. It was assumed, according to Mayer et al. (2003), that 14.6 and 15.2% of total N accumulation in faba bean and pea respectively was present as belowground plant N. When calculating the amount of fixed N_2 in roots the percentage below ground was corrected for the actual %Ndfa in the shoot. In the present study, simplified N balances were determined to evaluate the net effect on the soil N pool of the different cropping systems. Simplified soil N balances were used in similar studies (i.e Hauggaard-Nielsen et al., 2003; Hauggaard-Nielsen et al., 2009b; Hauggaard-Nielsen et al., 2009c). The results do not provide information on the N remaining quantity in the soil after cropping but give only an indication of the potential N inputs (in particular atmospheric N) and output into/from the system and potential N depletion.

Two-way analysis of variance (ANOVA) for randomized complete block design (RCBD) over years was performed for crop data using the GLM procedure in SAS/STAT Version 9.1 (SAS Institute Inc., Cary, NC, USA). The significance of difference among year, treatments and year x treatment interaction was estimated using Tukey’s HSD test at $P < 0.05$ level of significance. In order to analyse soil nitrate dynamics across the cropping seasons, Repeated Measures ANOVA was performed using the specific PROC GLM procedure in SAS. Sampling date was assumed as the within-subject repeated measures and treatment type was assumed as the between-subject main factor (Norman and Streiner, 2008). Since the Mauchly’s test was significant ($P<.0001$) the null hypothesis of sphericity was rejected and the univariate F test within-subjects effects was adjusted through the Greenhouse and Geisser method (Wolfinger and Chang,1995).

3. RESULTS

3.1 Growing conditions

Climatic conditions during the four-year experiment were typical of the Mediterranean climate with mild and rainy winters and warm and dry summers characterised by an irregular rainfall regime (Fig. 1). Air temperatures were similar among the four growing seasons (period November-June) but the second one (2011/12) when the crops experienced colder temperatures through the winter. In January 2012 – approximately two months after the sowing of durum wheat – monthly mean minimum and maximum daily air temperature were quite low, respectively 0.4 and 12.6°C, with temperatures dropping below 0°C in several occasions. In March 2012, minimum temperatures were below the mean of the period and the daily temperature range was quite large, between 3.4 and 20.9 °C on average. The rainfall was similar among the cropping seasons ranging from 517 mm to 589 mm (period November-June). In the first two cropping seasons, the rainfall was more homogeneously distributed thorough the crop cycle compared to the last two with almost 80 mm of rainfall occurred in May in 2012. In the third cropping season (2012/13) 433 mm rain fell during the period January-March while the spring was warm and very dry – only 42 mm were recorded in total from April to June. In the last cropping season (2013/14), the rainfall was concentrated between November and January (two-third of the total of the cropping season). In particular, in November 2013, 268 mm of rain fell on bare soil after a relatively warm October delaying the sowing of the wheat till the end of December. Similarly to the previous season, May was particularly warm and dry (only 8 mm of rainfall).

3.2 Grain yields of pea, faba bean and barley in intercrop and sole crop

In year 1 combined IC grain yield in P100B50 was the highest observed (5.09 t ha^{-1}) and comparable to grain yields of other intercrops but significantly greater than the sole crops. In year 3 combined IC grain yield were not significantly different than in year 1. Faba bean-intercrops yielded the most (4.21 t ha^{-1} on average) however combined IC grain yields were not significantly higher than any other treatments except than BSCf (2.54 t ha^{-1}) (Table 1).

In year 1 barley yielded significantly more than in year 3 ($P<0.0001$) in all treatments (no significant Y x T interaction). Grain yields of barley in intercrop and sole crop ranged from 2.27 to 4.15 t ha^{-1} and from 1.66 to 2.75 t ha^{-1} respectively in year 1 and 3 (Table 1). Except for F100B50 in year 1 (2.27 t ha^{-1}), barley grain yields in intercrop were never significantly different from the grain yields in sole crop despite the fact that in intercrop the cereal was sown at half of the sole crop density. Barley performed better when intercropped with pea than with faba bean in both year 1 and 3 (respectively +1.00 and +0.73 t ha^{-1} of grain yield on average of intercrop designs). This led to greater barley grain yields in the rotation systems containing pea-barley intercrops compared to those including barley intercropped with faba bean (+1.73 t ha^{-1} on average of intercrop designs over the 4-year period). Barley suffered the coexistence with faba bean especially when it was sown with the legume at full density in F100B50 yielding almost 50% significantly less than in P100B50 (4.15 vs 2.27 t ha^{-1} in year 1). A similar trend for barley was also observed in year 3 even though differences among treatments were not significant. These results are supported by the partial LER values based on barley grain yield (data not shown) that in F100B50 were considerably lower than in all the other treatments.

Contrarily to what observed for barley grain yields, the legumes yielded significantly more in year 3 than in year 1 ($P=0.0007$) and no significant interaction Y x T was observed. The highest legume grain yield of the whole experiment was achieved in faba bean sole crop in year 3 (4.13 t ha^{-1}) while the lowest in P100B50 in year 1 (0.93 t ha^{-1}) (Table 1). Overall, faba bean yielded more than pea and this determined a greater proportion of legume grain in the faba bean-containing systems compared to those containing pea in both first and second rotation cycle (+1.01 and +1.22 t ha^{-1} respectively on average of intercrop and sole crop treatments). Clearly, the full sowing density in the additive intercrop did not bring any yield advantages to the legume having been the yield of pea and faba bean never significantly different between the two designs within intercrops. These results are confirmed by the calculation of the percentage of the legume in the combined intercrop grain yield; the proportion of faba bean in the mixture at harvest was only 16 and 13% more in the additive compared to the replacement design respectively in year 1 and 3. In pea-barley intercrops, the legume proportion was similar between the two designs in year 3 while in year 1 it was 10% lower in the additive compared to the replacement design.

3.3 Nitrogen accumulation and land equivalent ratio of nitrogen yields (LER_N)

On average N accumulation in the legumes was significantly higher in year 3 compared to year 1 ($P=0.0081$), respectively 139.7 kg ha^{-1} and 122 kg ha^{-1}. No significant Y x T interaction was observed. In faba bean sole crop-containing system, legume above-ground biomass N accumulation was >200 kg ha^{-1} in both year 1 and 3 and contributed to achieving the overall 4-year rotation N accumulation of 577.6 kg ha^{-1}, the highest among the cropping systems studied and statistically greater than all the other system, but non statistically different than pea sole crop-containing system (501.6 kg ha^{-1}) (Table 2). The high biomass yields obtained in faba bean sole crop (data not shown) were determinant for reaching high levels of N accumulation as shown by the strong and positive correlation between these two variables in both year 1 and 3 (n=6; R^2_{adj}= 0.96; P= 0.0004 and R^2_{adj}= 0.99; P<0.0001 respectively). Total N accumulation in intercrop-containing systems over the rotation cycles ranged from 419.5 to 474.8 kg ha^{-1}. Competitive interactions between cereal and legume in F100B50 determined almost a 50% reduction in legume N accumulation compared to the respective sole crop (-96.2 and -86.9 kg ha^{-1} respectively in year 1 and 3) despite the same legume sowing density between the two treatments. Similar results were observed also for pea in P100B50 (-66.6 and -103.4 kg ha^{-1} compared to pea sole crop respectively in year 1 and 3).

Barley biomass N accumulation ranged among treatments from 50.7 to 93.0 kg ha^{-1} and from 39.7 to 57.4 kg ha^{-1} respectively in year 1 and 3 (Table 2) and it was significantly higher in year 1 than in year 3 in all treatments (P<0.0001). Due to smaller the biomass yields of barley intercropped with faba bean than with pea, the cereal accumulated more N in pea-barley than in faba bean-barley intercrops. In particular, barley N accumulation was severely reduced in F100B50 (only 50.7 and 39.7 kg ha^{-1} respectively in year 1 and 3).

In Fig. 2 legume N biomass yield-based partial $LER_{N\text{-}LEGUME}$ was plotted as a function of cereal N biomass yield-based partial $LER_{N\text{-}BARLEY}$. LER_N values are all positioned in the area corresponding to $LER_N > 1$ and to $LER_{N\text{-}BARLEY} > LER_{N\text{-}LEGUME}$. This means that N sources were used more efficiently in intercrop than in sole crop up to 68% and 61% respectively in year 1 and 3 indicating an advantage of intercrops compared with sole crops in the use of N sources. Furthermore, all intercrop combinations and designs showed a competitive advantage for N sources use of barley over the legume. The complementary in the use of N sources was more efficient for pea-barley intercrops (LER_N between 1.40 and 1.68) than for faba bean-barley intercrops (LER_N between 1.15 and 1.40) and the reason lies in lower barley partial LER_N when the cereal was intercropped with faba bean than with pea (respectively 0.79 and 1.05 on average of years and designs). In particular, in F100B50 barley partial

LER_N value was the lowest observed (0.60 and 0.77 respectively in year 1 and 3). Legume partial LER_N ranged from 0.41 to 0.69 and from 0.41 to 0.62 respectively in year 1 and 3 with small differences between the two intercrop designs even though the legume/cereal sowing ratio was 1:0.5.

3.4 N_2 fixation of legumes

Atmospheric nitrogen fixed by legume (% Ndfa) was, on average of all treatments, slightly higher in the first than in the third year (respectively 78.5 and 71.8%) (Fig. 3). Averaging over years and treatments, legume reliance on N_2 fixation was lower in faba bean (72%) than in pea (79%) with minimal variation between sole cropping and intercropping. In year 1 the intercropped grain legume increased the proportion of plant N derived from N_2-fixation compared to the respective sole crop by only 5 and 2%, respectively in pea and faba bean, whereas in year 3 the %Ndfa was often lower in intercrop than in sole crop.

Total amount of nitrogen fixed by legume in the two rotation cycles, both in IC and SC, ranged between 134.2 and 315.6 kg ha^{-1} with the highest values in faba bean sole crop-wheat system and the lowest in F50B50-wheat one. In IC and SC fixed N_2 yields and legume biomass yields were strongly and positively related (n=6; R^2_{adj} =0.99; P<0.0001 and R^2_{adj} =0.94; P=0.0007 respectively in year 1 and 3). As a result of this relationship, averaging over years, faba bean in sole crop fixed the greatest quantities of N_2 (137.7 kg ha^{-1}) followed by pea sole crop (128.7 kg ha^{-1}) with substantially less N fixed in intercrop (73.9 kg ha^{-1}) (Fig. 3).

3.5 Preceding crop effects on durum wheat

On average wheat grain yields were significantly higher in year 2 than in year 4 (P<0.0001) with a significant Y x T interaction (P=0.0001). There were significant differences in the effects of preceding crops on subsequent winter wheat grain production. In the first rotation, wheat grain yield tended to be higher when the preceding crop was pea in either sole crop or intercrop ranging between 3.5 and 4.3 t ha^{-1} (Table 1). Grain yields values of wheat following F50B50 (2.5 t ha^{-1}) and unfertilised wheat following BSC (2.3 t ha^{-1}) were the lowest observed. In the same year, durum wheat grain yield was found to be slightly and positively correlated to soil NO_3^- content at sowing (n=8; R^2_{adj}=0.64; P=0.011). Experimental plots that had previously hosted pea in either sole crop or additive intercrop (P100B50) had, indeed, soil NO_3^- content of around 80 kg ha^{-1} whereas it significantly dropped to ≤30 kg ha^{-1} after F50B50 and BSC. In year 4, wheat grain yields were reduced compared to year 2 (-0.58 t ha^{-1} on average) and the pre-crop effect was observed after the legume sole crops but not after the intercrops (+0.65 t ha^{-1} on average after PSC and FSC compared to BSC).

Overall, when comparing subsequent winter wheat grain yield, the proportion of the legume in the preceding intercrop did not have any significant effect and only small and not significant differences between the two designs were observed within intercrops (Table 1). Nitrogen fertilization had a substantial effect on wheat productivity in both years as significant higher yields were observed in BSCf compared to BSC (+0.8 t ha^{-1} on average of the two years). Yields of fertilised wheat (BSCf) were, however, never significantly greater than those of unfertilised wheat succeeding to a legume in both intercrop and sole crop.

Averaging over treatments, grain protein content of durum wheat was significantly higher (P=0.021) in year 4 compared to year 2 (respectively 10 and 9.8%) with a significant Y x T interaction (P<0.0001) (Table 2). Differences in grain protein content among succeeding wheat treatments were significant in both seasons (P=0.0033 and P<0.0001 respectively in year 2 and 4) however it was not possible to identify a strong preceding crop effects on this variable. Only in year 4 N accumulation in wheat was found to be positively related to grain protein content (n=8; R_{2adj}=0.7501; P=0.0054).

In year 2, BSCf and PSC showed the highest grain protein content (10.3 and 10.2% respectively) even though differences were significant only when compared to P50B50 (9.4%) that showed the lowest value. In year 4, grain protein content in PSC (11%) was significantly higher than any other treatment but not in FSC (10.7%). N fertilisation increased grain yield and grain protein content in fertilised (BSCf) compared to unfertilised wheat (BSC) in both years, however differences in protein content between the two treatments were never significant. Increase in grain yields might have happened at expenses of protein content however a negative although weak relationship between the two variables was found only in year 4 (n=8; R^2_{adj}=0.65; P=0.01).

N biomass accumulation in wheat was on average slightly higher in year 2 (65.9 kg ha^{-1}) than in year 4 (61.9 kg ha^{-1}) (P=0.0197) and a significant T x Y interaction was observed (P=0.0031). In year 2 of the rotation, durum wheat grown after pea sole crop accumulated significantly more N (88.3 kg ha^{-1}; P<0.0001) than any other treatments due to its relatively high biomass yield and grain N content. Significant differences were observed also between F100B50 (74.5 kg ha^{-1}) and F50B50 (63.2 kg ha^{-1}). N accumulation in subsequent durum wheat after P50B50 (51.4 kg ha^{-1}) and BSC (45.7 kg ha^{-1}) were not significantly different but they were significantly lower when compared to all the other treatments. In year 4, N yields were lower than in year 2 and the only treatments to show an N accumulation value ≥70 kg ha^{-1} were durum wheat succeeding both PSC and FSC. Wheat succeeding BSC needed N fertilisation in both years to reach level of N accumulation of around 70 kg ha^{-1}.

3.6 Soil nitrate content

Repeated Measures ANOVA indicates that the pattern of change in soil nitrate between cropping systems were significantly different over the time (Fig. 4). Highly significant differences between treatments (P<0.001) and over the four cropping seasons (P< 0.001) in NO_3^--N in 0-60 cm soil profile were highlighted. The TREATMENTS x TIME interaction showed significant differences between NO_3^--N variations in soil occurring within and between cropping systems.

In June 2011, at crop harvest, NO_3^--N content in the 0-60 cm soil profile was similar among treatments with the exception of pea sole crop that showed a significantly higher level (36.5 kg ha^{-1}; P<0.001) (Fig. 4). Soil NO_3^--N content increased, as expected, moving towards November 2011 (sowing of durum wheat) due to the mineralization of organic N during the summer and early autumn. At this date, an effect of previous cropping on soil NO_3^--N content was observed (P<0.001): the plots originally cropped with P100B50 and PSC showed the highest levels (82.5 and 77.2 kg ha^{-1} respectively) whereas the lowest levels were found in those plots that had previously hosted the two barley sole crops and F50B50 (<30 kg ha^{-1}).

In the following samplings soil NO_3^--N content was never significantly different among treatments until November 2012 (sowing of barley-legume intercrops and respective sole crops) when the plot hosting the faba bean sole crop-containing rotation showed a significantly (P<0.001) greater soil NO_3^--N content (134.8 kg ha^{-1}) compared to all the other plots (range between 72.8 and 96.1 kg ha^{-1}). Soil NO_3^--N content decreased considerably throughout the crops growth cycle and, at harvest (late June 2013), only small differences were observed among treatments. At this date, the highest NO_3^--N content was observed in FSC that was comparable to all the other treatments but significantly (P<0.01) greater than BSC and BSCf.

With the intensification of the mineralization process throughout the summer, soil NO_3^--N content started to increase again and significant differences among treatments could be appreciated in September 2013 (P<0.001) when the highest NO_3^--N content was found after

FSC and PSC (126 and 107.4 kg ha^{-1}respectively). NO_3^--N after FSC was not significantly different than PSC but significantly higher than any other treatments. Soil NO_3^--N content in the plots following the intercrops ranged from 68.5 to 97.1 kg ha^{-1} whereas it was only 44.5 kg ha^{-1} in those plots previously cropped with barley sole crops (on average of the two fertilisation strategies). At durum wheat sowing (December 2013) differences previously observed among plots were completely offset (P=0.097) probably due to leaching and immobilisation phenomena. Soil NO_3^--N content continued to be low throughout the durum wheat growth cycle with minimal differences among previous crop treatments (in April 2014 soil NO_3^--N content was significantly higher with $P<0.001$ in P50B50 and F50B50 plots compared to the others). Early in September 2014 (last sampling), the highest soil NO_3^--N content was observed in the plot hosting the F100B50-containing rotation (104.7 kg ha^{-1}) which was significantly higher than all other plots. Relatively high was also the NO_3^--N content in the plot of PSC-, P100B50- and FSC-containing rotations (respectively 82.4, 72.1 and 69.1 kg ha^{-1}).

3.7 Nitrogen balance in different legume-based rotation

The overall N balances in the 4-year period (from November 2010 to June 2014) were negative in all rotation systems (as expected due to the removal of the residues and the zero/low N fertilisation) indicating depletion of nitrogen independent of preceding crop and subsequent wheat cropping strategy (fertilised/unfertilised) (Fig. 5). The least negative N deficit was observed in the barley-fertilised wheat rotation (-150.5 kg ha^{-1}) that was comparable to those of pea and faba bean sole crop-containing rotations (respectively -180 and -201 kg ha^{-1}). Nitrogen depletion >200 kg ha^{-1} was found in the systems including the intercrops that ranged from 235.5 to 261.6 kg ha^{-1}. N balance of P50B50-containing system was the least negative among the intercrops-containing systems.

In year 1 pea and faba bean sole crops led to slightly negative or close to zero annual N balances compared to the intercrops and barley sole crops giving a total depletion after the first 2-year rotation cycle of only 89.8 (PSC-containing system) and 93.2 kg ha^{-1} (FSC-containing system) and comparable with BSCf-containing system (87.9 kg ha^{-1}). Compared to the previous cycle, a slightly lower N depletion was observed after the second 2-year rotation cycle, however treatments showed a similar trend.

N depletion after durum wheat in year 2 and year 4 was a reflection of its biomass yields and this led to a bigger N depletion in those systems with high wheat yields. N depletion was significantly reduced compared to any other treatments in both year 2 and 4 when 50 kg ha^{-1} of N were supplied to wheat (BSCf).

4. DISCUSSION

4.1 Yield performance of subsequent wheat

The durum wheat cv. *Virgilio* grown in this study achieved an average grain yield of around 4 t ha^{-1} in previous field trials carried out in several locations in Southern Italy between 2004 and 2008 (Quaranta et al., 2013). Based on those figures, in the present study, unfertilised durum wheat succeeding to pea in both intercrops and to faba bean in the additive intercrop can be considered to have reached satisfactory yield levels in year 2 - between 3.20 and 3.80 t ha^{-1} - also upon consideration that no N fertilisation was provided. This can be argued for all treatments studied except for wheat succeeding F50B50 that in year 2 achieved only 2.50 t ha^{-1} of grain, a poor performance that could be ascribed to the relatively low amount of residual NO_3^--N measured in the soil profile at crop establishment. A similar positive effect of the intercrop on succeeding wheat yields was not confirmed in year 4 during which the cereal crop was affected by several factors: a low NO_3^--N concentration at crop establishment (December 2013) compared to the previous sampling (September 2013) that can be related to immobilization phenomena and leaching phenomena thorough the autumn; extreme rainfall events thorough the autumn in 2013 that also delayed the sowing of the crop; mild air temperatures during the winter that reduced the tillering of the cereal and rather warm and dry climatic conditions in May that affected the grain filling. All these factors might have contributed to an overall contraction of wheat grain yields in year 4 and levelled out potential effects of the legume in sole crop and intercrop on the succeeding cereal. Yields instability of durum wheat in Mediterranean regions including Southern Italy is, indeed, known as a frequent issue (Campiglia et al., 2015). However, despite the variability of wheat grain yield between year 2 and 4 observed in this study it can be concluded that, practically speaking, cereal-legume intercrops can be a strategy for supporting wheat yields with potential N savings (50 kg ha^{-1} in the present study).

Unlike other similar experiments (for instance Hauggaard-Nielsen et al. 2009b), this study indicates a positive response to the preceding crop on subsequent wheat grain yields not only when the legume was grown in sole crop but also when it was intercropped with barley. Our results show indeed a wheat grain yield increase after pea-barley intercrop that was irrespective of intercrop design. Yet, this study shows that pea-based combinations (either in additive or replacement design) were more beneficial in a short crop rotation than faba bean-based combinations to the succeeding cereal presumably due to a release of more readily available soil N to the crop. Interestingly, our study suggests that despite crop residues were removed at harvest, stubble of previous legume crops may still explain a relevant part of the variation in subsequent wheat grain yield (Asseng et al., 1998) since roots and rhizodeposits are rich in N (Fustec et al., 2010; Cong et al., 2015; Latati et al., 2017). The N-enriched compounds entering the soil are prone to mineralization and stimulate the microbial activity (Hauggaard-Nielsen et al., 2008; Latati et al., 2017; Lalati et al., 2016) and this, in our experiment, seems to have affected the post-harvest soil NO_3^--N content with additional nitrate available to wheat following legumes in both sole crop and intercrop (respectively up to approximately +130 and +60 kg N ha−1 compared with wheat after barley). The discrepancy observed in the two legumes ability to increase soil N could be explained by the different quality and quantity of easily mineralizable substrates (rhizodeposits and crop residues) released from the legumes root systems (Mayer et al., 2003; Mayer et al., 2004), the root growth pattern (Knudsen et al., 2004), as well as from their different N harvest index (Senaratne and Hardarson, 1988). The increased N availability occurring in soil at the beginning of the wheat growing season may also explain the N aboveground biomass accumulation levels found in wheat succeeding legume sole crops and intercrops: they were significantly higher than those obtained in wheat after barley but only when no additional N-fertilizer was supplied (approximately +50 and +30% respectively). However, how much of this N was derived from the preceding legume could not be verified and generalisation from similar studies cannot be done as results are very context-specific and not univocal (for instance see studies of Sawatsky and Soper, 1991 and Danso and Papastylianou, 1992).

Residual N in the soil can have a marked effect on protein content in wheat grain (Olson et al., 1976) and the two variables can be found significantly and positively correlated (Evans et al., 2003). Despite the fact that wheat succeeding legume crops it is reported to have increased its grain protein content in Mediterranean conditions (Lopez-Bellido et al., 1998) and semiarid environments (Gan et al., 2003), in our study we did not observe a clear and strong pre-crop effect on this variable. Only in year 2 in wheat succeeding F50B50 the low residual soil NO_3^--N at crop establishment seemed to have negatively affected both grain yield and protein content. It seems, however, that increase in protein content in wheat after a legume can be strongly appreciated when N is a limiting factor for crop growth (Casagrande et al. 2009). Furthermore, assessing the pre-crop effect on succeeding wheat grain protein content is often not straightforward as it is largely dependent not only on residual soil N content but also on genotype (Johnson et al., 1985; Stoddard and Marshall, 1990) and on environment and cropping practices (Erekul and Köhn, 2006; Rao et al., 1993). In particular, the well-known negative correlation between grain yield and grain protein content (Fowler, 2003) has to be taken into account.

4.2 Productive performance of the intercrops

The grain yield contraction observed in the intercropped legume in the replacement design compared to the respective sole crop was, overall, in line with the reduced sowing density. Both pea and faba bean in the replacement design did not result largely outcompeted by the cereal as contrarily observed in similar experiments where the legume was clearly the weaker competitor in intercrop and showed a reduction in grain yields compared to the sole crop, up to 90% (i.e. Hauggaard-Nielsen and Jensen 2001; Monti et al., 2016; Neugschwandtner and Kaul, 2014). In the additive design, in contrast, the full sowing density seems not having provided any advantage in terms of grain production. There was only approximately 2–16% difference of legume share in the harvested grain mixture between the two designs even though the sown proportions differed with 50%. Hauggaard-Nielsen et al. (2009b) ascribed the small difference between the two designs to strong inter-specific interactions occurring in the additive design with the legume sown at full density. In light of the sowing scheme adopted in this experiment - the actual legume density on the individual row in the additive design was the double of its density in sole crop - we speculate that the legume overpopulation on the row could have been also a significant source of intra-specific competition able to shape the final proportion of the legume in the intercrop at harvest. Barley in both intercrop designs recovered from the reduced density through an increased tillering as also observed in pea-barley intercrop by Monti et al. (2016) and in pea-oat by Baxevanos et al. (2017). As a result, yields of barley in intercrop were never significantly different than those in sole crop with the only exception being F100B50 where the complementarity in the use of growth resources did not work as well as it did in the other intercrops as suggested by the low grain yield-based LER observed for this treatment (data not shown).

In pea-barley intercrops the presence of barley supported the growth in height of pea and the establishment of a dense canopy with this resulting in more effective weed suppression than faba bean-based especially when compared to F50B50 (data not shown). On the other hand, intercrops containing faba bean provided a higher proportion of legume grain in the mixture than those containing pea due to an overall greater yielding capacity of faba bean compared to pea and to overall lower yields of the cereal intercropped with faba bean rather than with pea. Once again faba bean showed to be well adapted to the Mediterranean environment (Siddique et al., 1993) and due to its earlier flowering compared to pea it could better escape the terminal drought.

4.3 Nitrogen use and biological fixation

Our results agree with other similar study that observed differences in aboveground biomass N accumulation ranked in the order: legume sole crop > intercrops > cereal sole crop (Bedoussac and Justes, 2010; Chapagain and Riseman, 2014; Hauggaard-Nielsen et al., 2009a) and no difference in total N accumulation comparing additive and replacement designs within the intercrops (Hauggaard-Nielsen et al., 2009a). Results for N accumulation indicate the importance of the legume for high N yields in intercrop. Despite the fact that in most cases the intercrops fixed significantly less N than the respective legume sole crop, they still played a key role in the overall N economy of the cropping system through inputs of biologically fixed N_2. This confirms that pea- and faba bean-barley intercrops can represent a valuable strategy to increase the input of fixed N_2 into agro-ecosystems without compromising yield level and stability, as argued by several authors (Anil et al., 1998; Jensen, 1996b; Lithourgidis et al., 2007; Tosti and Guiducci, 2010).

N biomass yield-based LER (LER_N) > 1 obtained in our experiment recalled the results of the experiment of Hauggaard-Nielsen et al. (2009a) carried out in the same location as the present study and confirmed the greater efficiency of intercropping compared to sole cropping for N sources due to the complementary use of mineral soil N and atmospheric N_2 between the two companion species (Bedoussac and Justes, 2010; Jensen, 1996a; Naudin et al., 2010; Ofori and Stern, 1987). Partial LER_N always higher for barley than for pea and faba bean highlighted that the legume had suffered the most in terms of N uptake. The complementary use of N sources by intercrop components was particularly efficient for pea-barley intercrops whereas faba bean intercropped with barley had more of an effect on barley (especially in the additive design) determining lower barley partial LER_N values. In regard to pea-based intercropping, our results agree with Baxevanos et al. (2017) who observed high land-use efficiency a highly competitive ability of pea intercropped with oat in a soil characterized by low NO_3^--N concentration.

In crop rotations, N_2-fixing ability of grain legumes is the main factor for N contribution in succeeding non-legumes and it can reduce the mineral N fertilizer demand of the cropping system (Mayer et al., 2003). Many studies, i.e. Bedoussac and Justes (2010), Bedoussac et al. (2015), Chapagain and Riseman (2014), Hauggaard-Nielsen et al. (2008), Hauggaard-Nielsen et al. (2009a), Jensen (1996a), Knudsen et al. (2004), observed an increase of N_2 fixed by pea and faba bean in intercrop of 10-20% when compared to the respective sole crop which was instead not observed in our study. Apparently, in our experiment, the intercropped barley did not stimulate, through depletion of available soil N (Jensen, 1996a, Hauggaard-Nielsen et al., 2001), the legume into higher N_2-fixation rates compared to when it grew in sole crop. When available soil N is large enough to meet the N demand of the crop, the plant fixes little N_2 (van Kessel and Hartley, 2000; Unkovich and Pate, 2000) and this might have been the case in our study as also observed by Hauggaard-Nielsen et al. (2009a), Papastylianou (1988) and Bedoussac and Justes (2010). In this regards the present study highlights an interesting aspect that can be extended to a broader Mediterranean contest: despite the fact that the experiment was conducted on a soil relatively poor in N, the temperature-moisture regimes typical of a Mediterranean dryland area stimulated the mineralization process of plant-derived C-substrates, determining temporary high soil NO_3^--N levels at the establishment and at the early stages of the crop and potentially suppressing microbial N_2-fixation activity. Moreover, this observation justifies the lower nitrogen fixation by legumes in the third year compared to the first year.

As a general principle, cultivation of grain legumes may increase total soil N when the amount of fixed N not removed from the system is greater than the amount of soil N removed in the grain and crop residue (van Kessel and Hartley, 2000). In our experiment, the amount of N that was removed from the intercrops at harvest was bigger than that fixed by the legume and N balances showed depletion of N independent of preceding crop and cropping strategy.

In this regard, our results agree with other field experiments that reported depletion of soil N after pea-barley additive and replacement intercrops (Hauggaard-Nielsen et al., 2009b; Jensen, 1996b). However, simplified soil N balances as calculated in this study can give only an overall idea of N depletion into a system as they do not take into account the N contribution to the soil coming from relevant N sources i.e. mineralisation of soil organic matter, root exudates or decomposition of nodules (Unkovich et al., 1997).

5. CONCLUSIONS

In the context of a crop/livestock production system, the present study has demonstrated that cereal-legume intercropping can be a source of fodder (both pea and faba bean combinations with barley) and still support yields of the following wheat cash crop. Cereal-legume intercrops with similar ripening times are easy to harvest simultaneously using traditional on-farm equipment, however the difficulty to obtain commercially suitable grains because of lack of effective post-harvest technology to separate the grains remains the main obstacle to the diffusion of cereal grain legume. Using the intercrop for on-farm animal feed production could be, in contrast, a feasible way to use the harvested mixture of grain and, if needed, to adjust the proportion of the two species. (Bedoussac et al., 2015).

Pea-barley combinations enabled a better yield performance of the succeeding durum wheat as compared to faba bean-based intercropping due to a larger release of mineral N. Pea-barley intercrops also reached a greater complementarity in the use of N sources and increased the overall sustainability of the rotation. In light of the result of this study, the use of faba bean in intercrop with barley – especially in the additive design – has to be reconsidered.

Finally, from this study emerged that grain legumes sown also in intercrop can enhance post-harvest soil N levels even if residues are removed at harvest. Nevertheless, in those arable soils where the soil fertility level is a limiting factor for crop growth the N supply for the succeeding cereal might not persist over a longer period and crop residues removal might led to a chronical soil resources impoverishment.

Beneficial effects of legumes in cereal rotations may not always be immediate (Campbell et al., 1992) and longer-term experiments can better understand the N dynamics of such systems and the positive, cumulative effect of grain legumes on the succeeding phases of the rotation. Moreover, findings of this study should be interpreted within a context of low inputs managed cropping systems as the magnitude of legume crop benefits diminishes as higher rates of N fertiliser are applied (Kirkegaard et al., 2008).

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LIST OF HIGHLIGHTS

- Pre-crop benefits on grain yields in wheat succeeding pea-barley intercrops were appreciated.
- Pea-based intercrops were more beneficial than faba bean-based combinations to the succeeding wheat.
- The legume in intercrop highly contributed to the N economy of the rotation through inputs of fixed N_2.
- Intercropping in Southern Mediterranean can sustain wheat production in livestock/cereal agroecosystem

TABLES AND FIGURES CAPTIONS

Table 1. Grain yields (t ha^{-1}) of barley (B), pea (P) and faba bean (F) grown in intercrop and sole crop (SC) and proportion (%) of legume grains in the harvested mixture in year 1 and 3, and grain yields (t ha^{-1}) of succeeding durum wheat in year 2 and 4 of the rotation. A 4-year total grain yield is also shown. Values are the mean (*n*=4). Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop. BSCf = barley sole crop preceding fertilised durum wheat.

Using Tukey's Studentized Range (HSD) Test values marked with the same letter are not significantly different.

NS not significant; **significant at P ≤ 0.01; ***significant at P ≤ 0.001.

Table 2. Nitrogen accumulation (kg ha^{-1}) at harvest in above-ground biomass of barley (B), pea (P) and faba bean (F) grown in intercrop and sole crop (SC) in year 1 and 3 and nitrogen accumulation (kg ha^{-1}) in above-ground biomass of succeeding durum wheat in year 2 and 4 of the rotation. For succeeding durum wheat grain protein content (%) is also shown. Values are the mean (*n*=4). Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop. BSCf = barley sole crop-fertilised durum wheat system.

Using Tukey's Studentized Range (HSD) Test values marked with the same letter are not significantly different.

NS not significant; *significant at P ≤ 0.05; **significant at P ≤ 0.01; ***significant at P ≤ 0.001.

Figure 1. Monthly total rainfall (mm), monthly mean maximum and minimum temperatures (°C) during the experiment.

S_{IC}= intercrop sowing, F_{IC}= intercrop flowering (legume), H_{IC}= intercrop harvest, S_W= wheat sowing, F_W= wheat flowering, H_W= wheat harvest

Figure 2. Partial Land Equivalent Ratio calculated from biomass nitrogen yield (LER_N) of faba bean (F) and pea (P) as a function of Partial Land Equivalent Ratio of barley (B) in year 1 (closed symbols) and 3 (open symbols). Values are the mean (n = 4) ±SE. Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop.

The bisector corresponding to $LER_{N\text{-}BARLEY} = LER_{N\text{-}LEGUME}$ separates the areas in which the legume has a competitive advantage over durum wheat for grain yield production and vice versa. The other diagonal corresponding to $LER_N = 1$ separates the areas where sole crops are more efficient than the intercrop for grain yield and vice versa.

Figure 3. Amount of N_2-fixation (kg ha^{-1}), N soil uptake and percentage of total N at maturity derived from N_2-fixation (%Ndfa) in faba bean (F) and pea (P) aboveground biomass grown in intercrop and sole crop (SC) in year 1 (left) and 3 (right). Values are the mean (n = 4) ±SE. Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop.

Using Tukey's Studentized Range (HSD) Test values marked with the same letter are not significantly different.

Figure 4. NO_3^--N content (kg ha^{-1}) in 0–60 soil depths measured thorough the 4-year experiment in the plots that hosted faba bean (F), pea (P) and barley (B) grown in intercrop (IC) and sole crop (SC) (year 1 and 3) and succeeding durum wheat (year 2 and 4) . Values are the mean (n = 4) ±SE.

Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop. BSCf = barley sole crop preceding fertilised durum wheat.

***significant at $P \leq 0.001$ and **significant at $P \leq 0.01$ using Tukey's Studentized Range (HSD) Test.

S= crop sowing, HW= crop harvest, H= heading cereal, NC= No crop.

Figure 5. Soil N balances (kg ha^{-1}) after the two 2-year rotation cycles and after the all 4-year period. Rotation cycles are named after the preceding sole crop (SC) or intercrop of faba bean (F), pea (P) and barley (B). N balances were calculated as N inputs (N_2-fixation + N seeds + N fertilisation) – N outputs (N grain + N straw). Values are the mean (n = 4) ±SE.

Additive intercrop (i.e., P100B50) = 100% of the sole crop density for the legume and 50% of sole crop density for barley. Replacement intercrop (i.e., P50B50) = 50% of the sole crop density for each crop. BSCf = barley sole crop preceding fertilised durum wheat.

Using Tukey's Studentized Range (HSD) Test values marked with the same letter are not significantly different. LSD value for 1st rotation = 34.3; LSD value for 2nd rotation = 55

Table 1

Preceding crop	Year 1				Year 2 Wheat	Year 3				Year 4 Wheat	4-year total
	Barley t ha^{-1}	Legume t ha^{-1}	Total Intercrop t ha^{-1}	Legume %	t ha^{-1}	Barley t ha^{-1}	Legume t ha^{-1}	Total Intercrop t ha^{-1}	Legume %	t ha^{-1}	t ha^{-1}
P100B50	4.15 a	0.93 c	5.09 a	18.4	3.82 ab	2.67 a	1.26 c	3.93 ab	32.0	2.54 bc	15.4
P50B50	3.40 ab	1.35 bc	4.76 ab	28.4	3.49 ab	2.65 a	1.15 c	3.80 ab	30.2	2.62 ac	14.7
F100B50	2.27 b	2.01 b	4.28 ac	47.0	3.18 bd	1.66 a	2.55 b	4.22 a	60.6	2.74 ac	14.4
F50B50	3.27 ab	1.45 bc	4.72 ab	30.7	2.53 cd	2.21 a	2.00 bc	4.20 a	47.5	2.60 ac	14.1
PSC	-	1.58 bc	1.58 d	100.0	4.27 a	-	2.65 b	2.65 ab	100.0	3.03 ab	11.5
FSC	-	3.44 a	3.44 c	100.0	3.64 ab	-	4.13 a	4.13 ab	100.0	3.04 a	14.3
BSC	3.89 a	-	3.89 ac	-	2.30 d	2.75 a	-	2.75 ab	-	2.39 c	11.3
BSCf	3.56 a	-	3.56 bc	-	3.32 ac	2.54 a	-	2.54 b	-	2.96 ab	12.4
Mean	3.43	1.80	3.92		3.32	2.41	2.29	3.53		2.74	13.5
Anova											
Year (Y)	***	***	NS		***						
Treatments (T)	***	***	***		***						
Y x T	NS	NS	**		***						

Table 2

Preceding crop	Year 1			Year 2 Wheat	Year 3			Year 4 Wheat	Total rotation	Wheat protein content (%)	
	Barley kg ha^{-1}	Legume kg ha^{-1}	Total Intercrop kg ha^{-1}	kg ha^{-1}	Barley kg ha^{-1}	Legume kg ha^{-1}	Total Intercrop kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	Year 2	Year 4
P100B50	93.0 a	79.5 c	172.5 ab	64.1 bc	57.4 a	93.7 b	151.0 bc	56.4 cd	444.1 bc	10.1 ac	9.8 c
P50B50	78.5 ab	99.7 c	178.2 ab	51.4 d	50.7 a	80.6 b	131.3 c	58.5 bc	419.5 c	9.4 c	9.8 c
F100B50	50.7 b	113.0 bc	163.7 b	74.5 b	39.7 a	136.3 b	176.0 ac	60.6 bc	474.8 bc	9.6 ac	10.0 bc
F50B50	70.3 ab	84.6 c	154.8 b	63.2 c	45.4 a	107.6 b	153.0 bc	58.4 bc	429.4 bc	9.6 ac	10.1 bc
PSC	-	146.1 b	146.1 b	88.3 a	-	197.1 a	197.1 ab	70.1 a	501.6 ab	10.2 ab	11.0 a
FSC	-	209.2 a	209.2 a	69.6 bc	-	223.2 a	223.2 a	75.6 a	577.6 a	9.5 bc	10.7 ab
BSC	83.4 ab	-	83.4 c	45.7 d	52.2 a	-	52.2 d	48.4d	229.6 d	9.9 ac	9.4 c
BSCf	73.6 ab	-	73.6 c	70.8 bc	52.3 a	-	52.3 d	66.8 ab	263.6 d	10.3 a	9.9 bc
Mean	74.9	122.0	147.7	65.9	49.6	139.7	142.0	61.9	417.5	9.8	10.0
Anova											
Year (Y)	***	**	NS	*						*	
Treatments (T)	**	***	***	***						***	
Y x T	NS	NS	***	**						***	

Figure 1

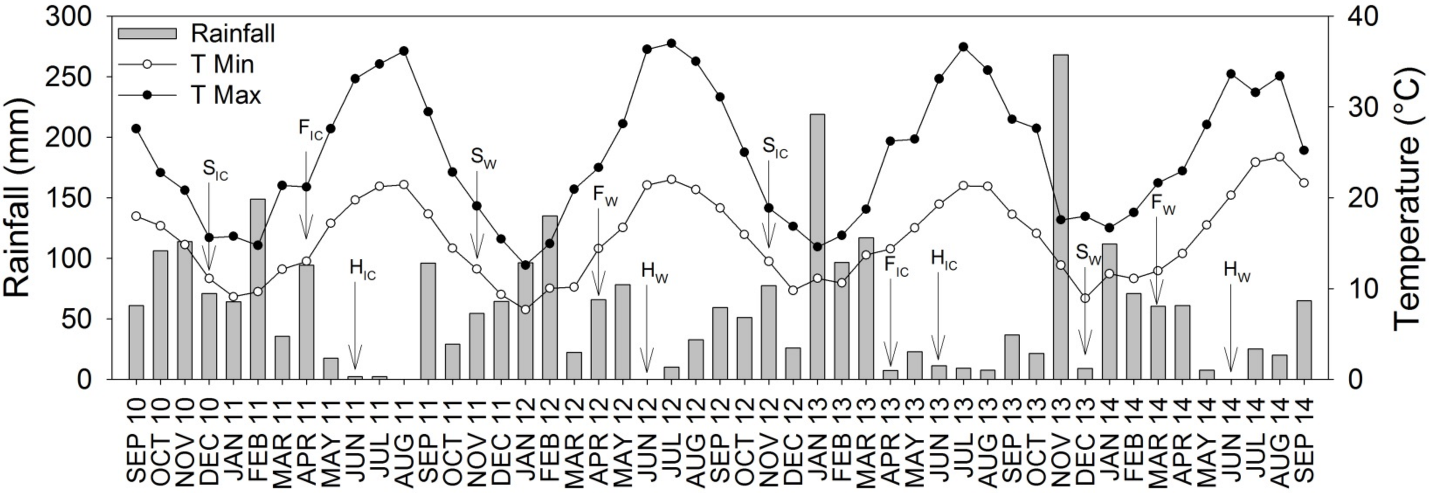


Figure 2

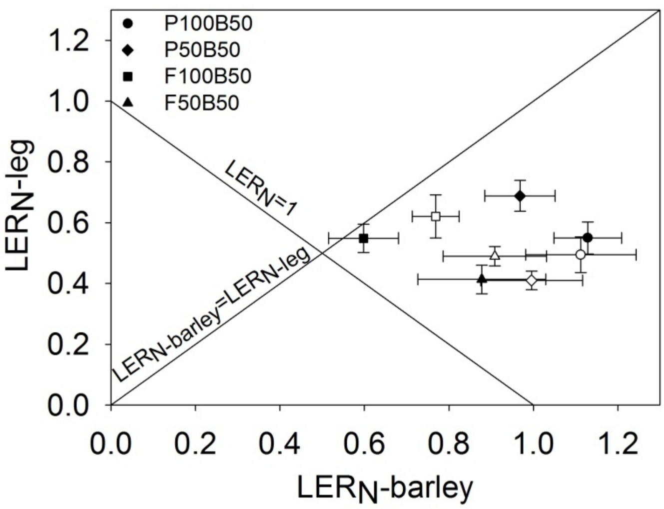


Figure 3

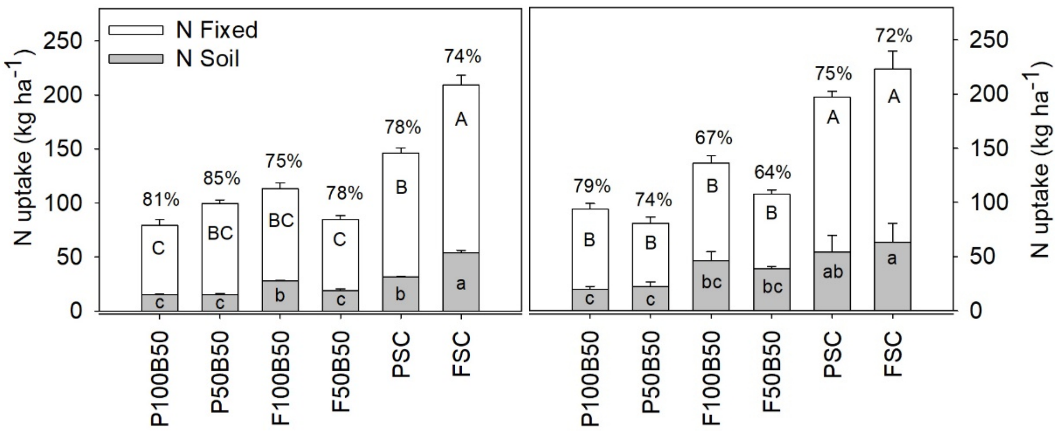


Figure 4

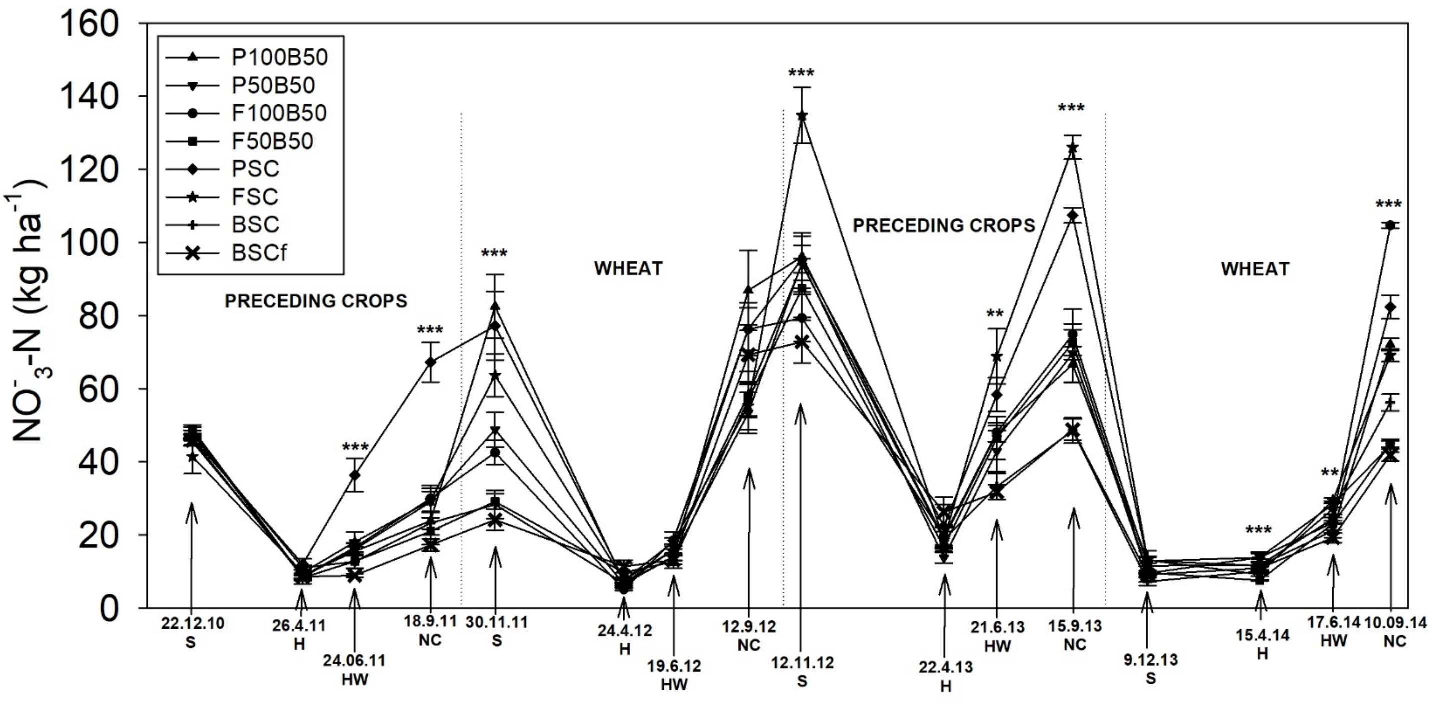
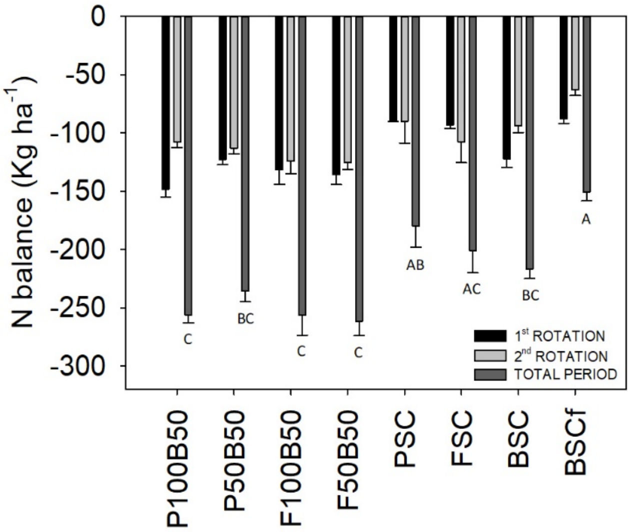


Figure 5



N balance (Kg ha-1)
0
-50
-100
-150
-200
-250
-300
C
BC
C
C
AB
AC
BC
A
1st ROTATION
2nd ROTATION
TOTAL PERIOD
P100B50
P50B50
F100B50
F50B50
PSC
FSC
BSC
BSCf