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1 **Article title:** Cereal/grain legume intercropping in rotation with durum wheat in
2 crop/livestock production systems for Mediterranean farming system.

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10 **ABSTRACT**

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

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

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

30

31 **1. INTRODUCTION**

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

41 If on a large scale, a return to mixed farming systems is unlikely to happen for logistical,
42 infrastructural and economic reasons, in contrast, a structured feed-livestock production
43 network (short fodder supply chain) is more likely to be built at local level. In such a contest
44 the adoption of mixed crop/livestock systems could be a useful tool to increase the viable use
45 of legumes in cereal farming systems, particularly in the smallholder farming systems of the
46 Mediterranean basin. In drought-prone areas of Southern Italy rainfed agricultural systems
47 mainly depend on winter crops, often durum wheat (*Triticum turgidum* L. subsp. *durum*)
48 along with common wheat (*Triticum aestivum* L.) and the inclusion of grain legumes in

49 rotation with durum wheat, grown as cash crop, is an old traditional practice. The present
50 study proposes the cereal-legume intercropping as an alternative to the legume sole crops in
51 rotation with durum wheat with the assumption that this agronomic practice is able to sustain
52 the yield of the subsequent cereal still providing the well-known benefits of legume crops in
53 rotation.

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

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

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

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

93 With the overall aim to assess the suitability of cereal-grain legume intercropping in
94 crop/livestock mixed systems under rainfed condition of Mediterranean climate this study
95 has: (i) evaluated, in two short rotation cycles, the effect on succeeding durum wheat grain
96 yields and quality exerted by pea- and faba bean-intercropping with barley (at two sown
97 arrangements) and by the respective legume sole crop; (ii) assessed the productive
98 performance and N use of pea- and faba bean-intercropping systems as compared to pure

99 stands of both components and their contribution to the overall rotation outcome; (iii)
100 estimated the nitrogen balance for each rotation with a focus on soil N-nitrate dynamics in a
101 Mediterranean contest.

102

103 **2. MATERIAL AND METHODS**

104 **2.1 Experimental site**

105 The experiment was carried out on a sandy-clay-loam soil (55% sand, 24% silt, 21% clay),
106 classified as Fluventic Haploxerepts, coarse silty, mixed, thermic (Soil Survey Staff, 2010), at
107 the agricultural experimental centre of the Regional Agency for Agriculture “ARSSA”
108 located in San Marco Argentano (Italy) (39°38’N, 16°13’E, 100 m above the sea level) over
109 four cropping seasons (2010-2014). Soil properties are as follows: C_{org} 9.81 g kg⁻¹; N_t 0.95 g
110 kg⁻¹; C/N 10.33 ± 0.51; total CaCO₃ 18.0 ± 0.5 g kg⁻¹; active CaCO₃ 13.5 ± 0.4 g kg⁻¹; pH_(KCl)
111 7.3; CEC 26.2 cmol₍₊₎ kg⁻¹; EC_{1:2} 0.21 dS m⁻¹; NH₄⁺ - N 17.0 mg kg⁻¹; NO₃⁻ - N 6.1 mg kg⁻¹; P
112 (Olsen) 12.5 mg kg⁻¹. The annual rainfall in San Marco Argentano is 709 mm and the mean
113 annual air temperature is 16.1°C (1995-2009 period).

114

115 **2.2 Experimental set-up and crop management**

116 In 2010/11 cropping season (year 1), six-row barley (B) (*Hordeum vulgare* L.) cv. Aldebaran,
117 faba bean (F) (*Vicia faba* L. subsp. *minor*) cv. Sikelia and a tall, semi-leaf-less, medium early
118 and indeterminate growth pea (P) (*Pisum sativum* L.) cv. Hardy were grown as (1) sole crop
119 (SC), (2) replacement (F50B50, P50B50) and (3) additive (F100B50, P100B50) cereal-
120 legume binary intercrop (IC). Two distinct experimental plots were established for the barley
121 sole crop: one plot preceding unfertilised durum wheat (BSC) and the other one preceding
122 fertilised durum wheat (BSCf). The sole crops were sown with planned density of 90, 40 and
123 300 seeds m⁻² for pea, faba bean, and barley respectively. In intercrop each species was sown

124 in alternate rows (16 cm apart) at half of its sole crop densities in the replacement design and
125 at full (legume) and at half (barley) of its relative sole crop density in the additive design. On
126 the individual rows, the actual barley density in both intercrop designs was the same as the
127 sole crop while for the legume in the additive design it was the double of its density in sole
128 crop. Plot size was 30 m² (3 x 10 m). The experiment was arranged as a randomized complete
129 block design (RCBD) with three replicates.

130 The seedling bed was prepared according to conventional tillage practices: ploughing (at a
131 30-cm depth) in October 2010, chemical fertilization with 84 kg ha⁻¹ of fertilizer Ca(H₂PO₄)₂
132 (Superphosphate) and disc harrowing (10 cm depth) immediately before seeding. The crops
133 were sown with a precision line seeder on 22nd December 2010. Harvest took place at full
134 maturity stage of barley (24th June 2011) and crop residues were removed from the field. In
135 the following 2011/12 cropping season, durum wheat (*Triticum turgidum* L. subsp. *durum*)
136 cv. Virgilio was sown as a subsequent crop on 30th November 2011 on all the twenty-four
137 field plots at a density of 350 seeds m⁻². The durum wheat treatments were named after the
138 preceding intercrop and sole crop treatments (F100B50, P100B50, F50B50, P50B50, PSC,
139 FSC, BSC, BSCf). The seedling bed was prepared following the same tillage practices and
140 fertilisation as the previous cropping season. No N fertilisation was provided to all treatments
141 except to the plot identified as BSCf that received an additional amount of 50 kg N ha⁻¹ (as
142 urea) at the tillering stage. Durum wheat was harvested on 19th June 2012. The same
143 experiment was repeated during the cropping seasons 2012/13 (intercrops and sole crops) and
144 2013/2014 (durum wheat) exactly on the same field plots that had hosted the experiment in
145 the previous two seasons. Crops were sown on the 12th November 2012 and harvested on the
146 21st June 2013 in the third season and sown on the 9th December 2013 and harvested on the
147 17th June 2014 in the fourth season. Throughout this article the four growing seasons are

148 often referred to as year 1 (2010/2011), year 2 (2011/2012), year 3 (2012/2013) and year 4
149 (2013/2014).

150

151 **2.3 Plant biomass sampling and analysis**

152 At the harvest stage, total aboveground biomass was sampled destructively from an area of 1
153 m² of each plot. Barley and legume plants from IC treatments were carefully separated,
154 whereas plants from sole crop treatments were taken as a whole. Total dry matter production
155 was determined after oven drying at 60°C until a constant weight was reached. The harvested
156 biomass was then separated and threshed into grains and straw and finally ground by using a
157 laboratory mill (1-mm sieve) before further analysis.

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

164

165 **2.4 Soil sampling and analysis**

166 Soil samples were collected periodically through the year including the main biological
167 stages of the crops. Composite soil samples, each consisting of three randomly collected soil
168 cores per plot pooled together (sub-replicates), were taken at 0-60 cm depth. Field moist
169 samples were stored at 4 °C before being partially air-dried, sieved at < 2 mm particle size,
170 and processed for chemical characterization. NO_3^- -N was determined according to Mulvaney
171 (1996) on soil extracted with 2 M KCl (1:10, w/v, soil:solution) under agitation (170 rpm, 1
172 h) at room temperature and then analysed using a Flow Injection Analysis System (FIAS 400

173 PerkinElmer, Inc., CT, USA) equipped with Autosampler AS90 (PerkinElmer) and
174 Spectrophotometer UV/Vis Lambda 25 (PerkinElmer). Soil data were expressed as mean
175 values on an oven-dry weight (dw) basis (105°C, 24 h) and referred to a sampling depth of 0-
176 60 cm.

177

178 **2.5 Calculation and statistics**

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

184

$$185 \quad L_C = \frac{Y_{C-IC}}{Y_{C-SC}}; L_L = \frac{Y_{L-IC}}{Y_{L-SC}}; \quad LER = L_C + L_L$$

186

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

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

193 The percentage of atmospheric N₂ fixed by the legume (%Ndfa) was assessed using the ¹⁵N
194 natural abundance technique which is considered a powerful means of assessing N₂ fixation
195 in field experiments (Unkovich et al., 2008). The amount of N fixed (kg ha⁻¹) was calculated
196 as the product of legume biomass, biomass %N content and the percentage of plant N derived
197 from N fixation (%Ndfa) (Hauggaard-Nielsen et al., 2009a). The %Ndfa was calculated using

198 the ^{15}N contents of the legume ($\delta^{15}\text{N}_{\text{legume}}$) and of a non-fixing reference plant that in this
199 experiment was barley sole crop ($\delta^{15}\text{N}_{\text{barley}}$) according to Shearer and Kohl (1986):

200

$$201 \quad \text{Ndfa}(\%) = 100 \times \frac{\delta^{15}\text{N}_{\text{barley}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{barley}} - B}$$

202

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

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

219 Two-way analysis of variance (ANOVA) for randomized complete block design (RCBD)
220 over years was performed for crop data using the GLM procedure in SAS/STAT Version 9.1
221 (SAS Institute Inc., Cary, NC, USA). The significance of difference among year, treatments

222 and year x treatment interaction was estimated using Tukey's HSD test at $P < 0.05$ level of
223 significance. In order to analyse soil nitrate dynamics across the cropping seasons, Repeated
224 Measures ANOVA was performed using the specific PROC GLM procedure in SAS.
225 Sampling date was assumed as the within-subject repeated measures and treatment type was
226 assumed as the between-subject main factor (Norman and Streiner, 2008). Since the
227 Mauchly's test was significant ($P < .0001$) the null hypothesis of sphericity was rejected and
228 the univariate F test within-subjects effects was adjusted through the Greenhouse and Geisser
229 method (Wolfinger and Chang, 1995).

230

231 **3. RESULTS**

232 **3.1 Growing conditions**

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

252

253

254

255 3.2 Grain yields of pea, faba bean and barley in intercrop and sole crop

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

261 In year 1 barley yielded significantly more than in year 3 ($P < 0.0001$) in all treatments (no
262 significant Y x T interaction). Grain yields of barley in intercrop and sole crop ranged from
263 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
264 for F100B50 in year 1 (2.27 t ha⁻¹), barley grain yields in intercrop were never significantly
265 different from the grain yields in sole crop despite the fact that in intercrop the cereal was
266 sown at half of the sole crop density. Barley performed better when intercropped with pea
267 than with faba bean in both year 1 and 3 (respectively +1.00 and +0.73 t ha⁻¹ of grain yield on
268 average of intercrop designs). This led to greater barley grain yields in the rotation systems
269 containing pea-barley intercrops compared to those including barley intercropped with faba
270 bean (+1.73 t ha⁻¹ on average of intercrop designs over the 4-year period). Barley suffered the
271 coexistence with faba bean especially when it was sown with the legume at full density in
272 F100B50 yielding almost 50% significantly less than in P100B50 (4.15 vs 2.27 t ha⁻¹ in year
273 1). A similar trend for barley was also observed in year 3 even though differences among
274 treatments were not significant. These results are supported by the partial LER values based
275 on barley grain yield (data not shown) that in F100B50 were considerably lower than in all
276 the other treatments.

277 Contrarily to what observed for barley grain yields, the legumes yielded significantly more in
278 year 3 than in year 1 ($P = 0.0007$) and no significant interaction Y x T was observed. The

279 highest legume grain yield of the whole experiment was achieved in faba bean sole crop in
280 year 3 (4.13 t ha⁻¹) while the lowest in P100B50 in year 1 (0.93 t ha⁻¹) (Table 1). Overall, faba
281 bean yielded more than pea and this determined a greater proportion of legume grain in the
282 faba bean-containing systems compared to those containing pea in both first and second
283 rotation cycle (+1.01 and +1.22 t ha⁻¹ respectively on average of intercrop and sole crop
284 treatments). Clearly, the full sowing density in the additive intercrop did not bring any yield
285 advantages to the legume having been the yield of pea and faba bean never significantly
286 different between the two designs within intercrops. These results are confirmed by the
287 calculation of the percentage of the legume in the combined intercrop grain yield; the
288 proportion of faba bean in the mixture at harvest was only 16 and 13% more in the additive
289 compared to the replacement design respectively in year 1 and 3. In pea-barley intercrops, the
290 legume proportion was similar between the two designs in year 3 while in year 1 it was 10%
291 lower in the additive compared to the replacement design.

292

293 **3.3 Nitrogen accumulation and land equivalent ratio of nitrogen yields (LER_N)**

294 On average N accumulation in the legumes was significantly higher in year 3 compared to
295 year 1 (P=0.0081), respectively 139.7 kg ha⁻¹ and 122 kg ha⁻¹. No significant Y x T
296 interaction was observed. In faba bean sole crop-containing system, legume above-ground
297 biomass N accumulation was >200 kg ha⁻¹ in both year 1 and 3 and contributed to achieving
298 the overall 4-year rotation N accumulation of 577.6 kg ha⁻¹, the highest among the cropping
299 systems studied and statistically greater than all the other system, but non statistically
300 different than pea sole crop-containing system (501.6 kg ha⁻¹) (Table 2). The high biomass
301 yields obtained in faba bean sole crop (data not shown) were determinant for reaching high
302 levels of N accumulation as shown by the strong and positive correlation between these two

303 variables in both year 1 and 3 ($n=6$; $R^2_{adj}= 0.96$; $P= 0.0004$ and $R^2_{adj}= 0.99$; $P<0.0001$
304 respectively). Total N accumulation in intercrop-containing systems over the rotation cycles
305 ranged from 419.5 to 474.8 kg ha⁻¹. Competitive interactions between cereal and legume in
306 F100B50 determined almost a 50% reduction in legume N accumulation compared to the
307 respective sole crop (-96.2 and -86.9 kg ha⁻¹ respectively in year 1 and 3) despite the same
308 legume sowing density between the two treatments. Similar results were observed also for
309 pea in P100B50 (-66.6 and -103.4 kg ha⁻¹ compared to pea sole crop respectively in year 1
310 and 3).

311 Barley biomass N accumulation ranged among treatments from 50.7 to 93.0 kg ha⁻¹ and from
312 39.7 to 57.4 kg ha⁻¹ respectively in year 1 and 3 (Table 2) and it was significantly higher in
313 year 1 than in year 3 in all treatments ($P<0.0001$). Due to smaller the biomass yields of barley
314 intercropped with faba bean than with pea, the cereal accumulated more N in pea-barley than
315 in faba bean-barley intercrops. In particular, barley N accumulation was severely reduced in
316 F100B50 (only 50.7 and 39.7 kg ha⁻¹ respectively in year 1 and 3).

317 In Fig. 2 legume N biomass yield-based partial $LER_{N-LEGUME}$ was plotted as a function of
318 cereal N biomass yield-based partial $LER_{N-BARLEY}$. LER_N values are all positioned in the area
319 corresponding to $LER_N > 1$ and to $LER_{N-BARLEY} > LER_{N-LEGUME}$. This means that N sources
320 were used more efficiently in intercrop than in sole crop up to 68% and 61% respectively in
321 year 1 and 3 indicating an advantage of intercrops compared with sole crops in the use of N
322 sources. Furthermore, all intercrop combinations and designs showed a competitive
323 advantage for N sources use of barley over the legume. The complementary in the use of N
324 sources was more efficient for pea-barley intercrops (LER_N between 1.40 and 1.68) than for
325 faba bean-barley intercrops (LER_N between 1.15 and 1.40) and the reason lies in lower barley
326 partial LER_N when the cereal was intercropped with faba bean than with pea (respectively
327 0.79 and 1.05 on average of years and designs). In particular, in F100B50 barley partial

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

332 **3.4 N₂ fixation of legumes**

333 Atmospheric nitrogen fixed by legume (% Ndfa) was, on average of all treatments, slightly
334 higher in the first than in the third year (respectively 78.5 and 71.8%) (Fig. 3). Averaging
335 over years and treatments, legume reliance on N₂ fixation was lower in faba bean (72%) than
336 in pea (79%) with minimal variation between sole cropping and intercropping. In year 1 the
337 intercropped grain legume increased the proportion of plant N derived from N₂-fixation
338 compared to the respective sole crop by only 5 and 2%, respectively in pea and faba bean,
339 whereas in year 3 the %Ndfa was often lower in intercrop than in sole crop.

340 Total amount of nitrogen fixed by legume in the two rotation cycles, both in IC and SC,
341 ranged between 134.2 and 315.6 kg ha⁻¹ with the highest values in faba bean sole crop-wheat
342 system and the lowest in F50B50-wheat one. In IC and SC fixed N₂ yields and legume
343 biomass yields were strongly and positively related ($n=6$; $R^2_{adj}=0.99$; $P<0.0001$ and R^2_{adj}
344 $=0.94$; $P=0.0007$ respectively in year 1 and 3). As a result of this relationship, averaging over
345 years, faba bean in sole crop fixed the greatest quantities of N₂ (137.7 kg ha⁻¹) followed by
346 pea sole crop (128.7 kg ha⁻¹) with substantially less N fixed in intercrop (73.9 kg ha⁻¹) (Fig.
347 3).

348 **3.5 Preceding crop effects on durum wheat**

349 On average wheat grain yields were significantly higher in year 2 than in year 4 ($P<0.0001$)
350 with a significant Y x T interaction ($P=0.0001$). There were significant differences in the

351 effects of preceding crops on subsequent winter wheat grain production. In the first rotation,
352 wheat grain yield tended to be higher when the preceding crop was pea in either sole crop or
353 intercrop ranging between 3.5 and 4.3 t ha⁻¹ (Table 1). Grain yields values of wheat following
354 F50B50 (2.5 t ha⁻¹) and unfertilised wheat following BSC (2.3 t ha⁻¹) were the lowest
355 observed. In the same year, durum wheat grain yield was found to be slightly and positively
356 correlated to soil NO₃⁻ content at sowing ($n=8$; $R^2_{adj}=0.64$; $P=0.011$). Experimental plots that
357 had previously hosted pea in either sole crop or additive intercrop (P100B50) had, indeed,
358 soil NO₃⁻ content of around 80 kg ha⁻¹ whereas it significantly dropped to ≤ 30 kg ha⁻¹ after
359 F50B50 and BSC. In year 4, wheat grain yields were reduced compared to year 2 (-0.58 t ha⁻¹
360 on average) and the pre-crop effect was observed after the legume sole crops but not after the
361 intercrops (+0.65 t ha⁻¹ on average after PSC and FSC compared to BSC).

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

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

376 In year 2, BSCf and PSC showed the highest grain protein content (10.3 and 10.2%
377 respectively) even though differences were significant only when compared to P50B50
378 (9.4%) that showed the lowest value. In year 4, grain protein content in PSC (11%) was
379 significantly higher than any other treatment but not in FSC (10.7%). N fertilisation increased
380 grain yield and grain protein content in fertilised (BSCf) compared to unfertilised wheat
381 (BSC) in both years, however differences in protein content between the two treatments were
382 never significant. Increase in grain yields might have happened at expenses of protein content
383 however a negative although weak relationship between the two variables was found only in
384 year 4 ($n=8$; $R^2_{adj}=0.65$; $P=0.01$).

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

396

397 **3.6 Soil nitrate content**

398 Repeated Measures ANOVA indicates that the pattern of change in soil nitrate between
399 cropping systems were significantly different over the time (Fig. 4). Highly significant

400 differences between treatments ($P < 0.001$) and over the four cropping seasons ($P < 0.001$) in
401 NO_3^- -N in 0-60 cm soil profile were highlighted. The TREATMENTS x TIME interaction
402 showed significant differences between NO_3^- -N variations in soil occurring within and
403 between cropping systems.

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

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

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

424 FSC and PSC (126 and 107.4 kg ha⁻¹ respectively). NO₃⁻-N after FSC was not significantly
425 different than PSC but significantly higher than any other treatments. Soil NO₃⁻-N content in
426 the plots following the intercrops ranged from 68.5 to 97.1 kg ha⁻¹ whereas it was only 44.5
427 kg ha⁻¹ in those plots previously cropped with barley sole crops (on average of the two
428 fertilisation strategies). At durum wheat sowing (December 2013) differences previously
429 observed among plots were completely offset (P=0.097) probably due to leaching and
430 immobilisation phenomena. Soil NO₃⁻-N content continued to be low throughout the durum
431 wheat growth cycle with minimal differences among previous crop treatments (in April 2014
432 soil NO₃⁻-N content was significantly higher with P<0.001 in P50B50 and F50B50 plots
433 compared to the others). Early in September 2014 (last sampling), the highest soil NO₃⁻-N
434 content was observed in the plot hosting the F100B50-containing rotation (104.7 kg ha⁻¹)
435 which was significantly higher than all other plots. Relatively high was also the NO₃⁻-N
436 content in the plot of PSC-, P100B50- and FSC-containing rotations (respectively 82.4, 72.1
437 and 69.1 kg ha⁻¹).

438

439 **3.7 Nitrogen balance in different legume-based rotation**

440 The overall N balances in the 4-year period (from November 2010 to June 2014) were
441 negative in all rotation systems (as expected due to the removal of the residues and the
442 zero/low N fertilisation) indicating depletion of nitrogen independent of preceding crop and
443 subsequent wheat cropping strategy (fertilised/unfertilised) (Fig. 5). The least negative N
444 deficit was observed in the barley-fertilised wheat rotation (-150.5 kg ha⁻¹) that was
445 comparable to those of pea and faba bean sole crop-containing rotations (respectively -180
446 and -201 kg ha⁻¹). Nitrogen depletion >200 kg ha⁻¹ was found in the systems including the

447 intercrops that ranged from 235.5 to 261.6 kg ha⁻¹. N balance of P50B50-containing system
448 was the least negative among the intercrops-containing systems.

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

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

459

460 **4. DISCUSSION**

461 **4.1 Yield performance of subsequent wheat**

462 The durum wheat cv. *Virgilio* grown in this study achieved an average grain yield of around 4
463 t ha⁻¹ in previous field trials carried out in several locations in Southern Italy between 2004
464 and 2008 (Quaranta et al., 2013). Based on those figures, in the present study, unfertilised
465 durum wheat succeeding to pea in both intercrops and to faba bean in the additive intercrop
466 can be considered to have reached satisfactory yield levels in year 2 - between 3.20 and 3.80 t
467 ha⁻¹ - also upon consideration that no N fertilisation was provided. This can be argued for all
468 treatments studied except for wheat succeeding F50B50 that in year 2 achieved only 2.50 t ha⁻¹
469 of grain, a poor performance that could be ascribed to the relatively low amount of residual
470 NO₃⁻-N measured in the soil profile at crop establishment. A similar positive effect of the

471 intercrop on succeeding wheat yields was not confirmed in year 4 during which the cereal
472 crop was affected by several factors: a low $\text{NO}_3\text{-N}$ concentration at crop establishment
473 (December 2013) compared to the previous sampling (September 2013) that can be related to
474 immobilization phenomena and leaching phenomena thorough the autumn; extreme rainfall
475 events thorough the autumn in 2013 that also delayed the sowing of the crop; mild air
476 temperatures during the winter that reduced the tillering of the cereal and rather warm and dry
477 climatic conditions in May that affected the grain filling. All these factors might have
478 contributed to an overall contraction of wheat grain yields in year 4 and levelled out potential
479 effects of the legume in sole crop and intercrop on the succeeding cereal. Yields instability of
480 durum wheat in Mediterranean regions including Southern Italy is, indeed, known as a
481 frequent issue (Campiglia et al., 2015). However, despite the variability of wheat grain yield
482 between year 2 and 4 observed in this study it can be concluded that, practically speaking,
483 cereal-legume intercrops can be a strategy for supporting wheat yields with potential N
484 savings (50 kg ha^{-1} in the present study).

485 Unlike other similar experiments (for instance Hauggaard-Nielsen et al. 2009b), this study
486 indicates a positive response to the preceding crop on subsequent wheat grain yields not only
487 when the legume was grown in sole crop but also when it was intercropped with barley. Our
488 results show indeed a wheat grain yield increase after pea-barley intercrop that was
489 irrespective of intercrop design. Yet, this study shows that pea-based combinations (either in
490 additive or replacement design) were more beneficial in a short crop rotation than faba bean-
491 based combinations to the succeeding cereal presumably due to a release of more readily
492 available soil N to the crop. Interestingly, our study suggests that despite crop residues were
493 removed at harvest, stubble of previous legume crops may still explain a relevant part of the
494 variation in subsequent wheat grain yield (Asseng et al., 1998) since roots and rhizodeposits
495 are rich in N (Fustec et al., 2010; Cong et al., 2015; Latati et al., 2017). The N-enriched

496 compounds entering the soil are prone to mineralization and stimulate the microbial activity
497 (Hauggaard-Nielsen et al., 2008; Latati et al., 2017; Lalati et al., 2016) and this, in our
498 experiment, seems to have affected the post-harvest soil NO₃⁻-N content with additional
499 nitrate available to wheat following legumes in both sole crop and intercrop (respectively up
500 to approximately +130 and +60 kg N ha⁻¹ compared with wheat after barley). The
501 discrepancy observed in the two legumes ability to increase soil N could be explained by the
502 different quality and quantity of easily mineralizable substrates (rhizodeposits and crop
503 residues) released from the legumes root systems (Mayer et al., 2003; Mayer et al., 2004), the
504 root growth pattern (Knudsen et al., 2004), as well as from their different N harvest index
505 (Senaratne and Hardarson, 1988). The increased N availability occurring in soil at the
506 beginning of the wheat growing season may also explain the N aboveground biomass
507 accumulation levels found in wheat succeeding legume sole crops and intercrops: they were
508 significantly higher than those obtained in wheat after barley but only when no additional N-
509 fertilizer was supplied (approximately +50 and +30% respectively). However, how much of
510 this N was derived from the preceding legume could not be verified and generalisation from
511 similar studies cannot be done as results are very context-specific and not univocal (for
512 instance see studies of Sawatsky and Soper, 1991 and Danso and Papastylianou, 1992).

513 Residual N in the soil can have a marked effect on protein content in wheat grain (Olson et
514 al., 1976) and the two variables can be found significantly and positively correlated (Evans et
515 al., 2003). Despite the fact that wheat succeeding legume crops it is reported to have increased
516 its grain protein content in Mediterranean conditions (Lopez-Bellido et al., 1998) and semiarid
517 environments (Gan et al., 2003), in our study we did not observe a clear and strong pre-crop
518 effect on this variable. Only in year 2 in wheat succeeding F50B50 the low residual soil NO₃⁻-
519 N at crop establishment seemed to have negatively affected both grain yield and protein
520 content. It seems, however, that increase in protein content in wheat after a legume can be

521 strongly appreciated when N is a limiting factor for crop growth (Casagrande et al. 2009).
522 Furthermore, assessing the pre-crop effect on succeeding wheat grain protein content is often
523 not straightforward as it is largely dependent not only on residual soil N content but also on
524 genotype (Johnson et al., 1985; Stoddard and Marshall, 1990) and on environment and
525 cropping practices (Erekul and Köhn, 2006; Rao et al., 1993). In particular, the well-known
526 negative correlation between grain yield and grain protein content (Fowler, 2003) has to be
527 taken into account.

528 **4.2 Productive performance of the intercrops**

529 The grain yield contraction observed in the intercropped legume in the replacement design
530 compared to the respective sole crop was, overall, in line with the reduced sowing density.
531 Both pea and faba bean in the replacement design did not result largely outcompeted by the
532 cereal as contrarily observed in similar experiments where the legume was clearly the weaker
533 competitor in intercrop and showed a reduction in grain yields compared to the sole crop, up
534 to 90% (i.e. Hauggaard-Nielsen and Jensen 2001; Monti et al., 2016; Neugschwandtner and
535 Kaul, 2014). In the additive design, in contrast, the full sowing density seems not having
536 provided any advantage in terms of grain production. There was only approximately 2–16%
537 difference of legume share in the harvested grain mixture between the two designs even
538 though the sown proportions differed with 50%. Hauggaard-Nielsen et al. (2009b) ascribed
539 the small difference between the two designs to strong inter-specific interactions occurring in
540 the additive design with the legume sown at full density. In light of the sowing scheme
541 adopted in this experiment - the actual legume density on the individual row in the additive
542 design was the double of its density in sole crop - we speculate that the legume
543 overpopulation on the row could have been also a significant source of intra-specific
544 competition able to shape the final proportion of the legume in the intercrop at harvest. Barley
545 in both intercrop designs recovered from the reduced density through an increased tillering as

546 also observed in pea-barley intercrop by Monti et al. (2016) and in pea-oat by Baxevanos et
547 al. (2017). As a result, yields of barley in intercrop were never significantly different than
548 those in sole crop with the only exception being F100B50 where the complementarity in the
549 use of growth resources did not work as well as it did in the other intercrops as suggested by
550 the low grain yield-based LER observed for this treatment (data not shown).

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

560

561 **4.3 Nitrogen use and biological fixation**

562 Our results agree with other similar study that observed differences in aboveground biomass
563 N accumulation ranked in the order: legume sole crop > intercrops > cereal sole crop
564 (Bedoussac and Justes, 2010; Chapagain and Riseman, 2014; Hauggaard-Nielsen et al.,
565 2009a) and no difference in total N accumulation comparing additive and replacement designs
566 within the intercrops (Hauggaard-Nielsen et al., 2009a). Results for N accumulation indicate
567 the importance of the legume for high N yields in intercrop. Despite the fact that in most cases
568 the intercrops fixed significantly less N than the respective legume sole crop, they still played
569 a key role in the overall N economy of the cropping system through inputs of biologically

570 fixed N₂. This confirms that pea- and faba bean-barley intercrops can represent a valuable
571 strategy to increase the input of fixed N₂ into agro-ecosystems without compromising yield
572 level and stability, as argued by several authors (Anil et al., 1998; Jensen, 1996b; Lithourgidis
573 et al., 2007; Tosti and Guiducci, 2010).

574 N biomass yield-based LER (LER_N) > 1 obtained in our experiment recalled the results of the
575 experiment of Hauggaard-Nielsen et al. (2009a) carried out in the same location as the present
576 study and confirmed the greater efficiency of intercropping compared to sole cropping for N
577 sources due to the complementary use of mineral soil N and atmospheric N₂ between the two
578 companion species (Bedoussac and Justes, 2010; Jensen, 1996a; Naudin et al., 2010; Ofori
579 and Stern, 1987). Partial LER_N always higher for barley than for pea and faba bean
580 highlighted that the legume had suffered the most in terms of N uptake. The complementary
581 use of N sources by intercrop components was particularly efficient for pea-barley intercrops
582 whereas faba bean intercropped with barley had more of an effect on barley (especially in the
583 additive design) determining lower barley partial LER_N values. In regard to pea-based
584 intercropping, our results agree with Baxevanos et al. (2017) who observed high land-use
585 efficiency a highly competitive ability of pea intercropped with oat in a soil characterized by
586 low NO₃⁻-N concentration.

587 In crop rotations, N₂-fixing ability of grain legumes is the main factor for N contribution in
588 succeeding non-legumes and it can reduce the mineral N fertilizer demand of the cropping
589 system (Mayer et al., 2003). Many studies, i.e. Bedoussac and Justes (2010), Bedoussac et al.
590 (2015), Chapagain and Riseman (2014), Hauggaard-Nielsen et al. (2008), Hauggaard-Nielsen
591 et al. (2009a), Jensen (1996a), Knudsen et al. (2004), observed an increase of N₂ fixed by pea
592 and faba bean in intercrop of 10-20% when compared to the respective sole crop which was
593 instead not observed in our study. Apparently, in our experiment, the intercropped barley did
594 not stimulate, through depletion of available soil N (Jensen, 1996a, Hauggaard-Nielsen et al.,

595 2001), the legume into higher N₂-fixation rates compared to when it grew in sole crop. When
596 available soil N is large enough to meet the N demand of the crop, the plant fixes little N₂ (van
597 Kessel and Hartley, 2000; Unkovich and Pate, 2000) and this might have been the case in our
598 study as also observed by Hauggaard-Nielsen et al. (2009a), Papastylianou (1988) and
599 Bedoussac and Justes (2010). In this regards the present study highlights an interesting aspect
600 that can be extended to a broader Mediterranean contest: despite the fact that the experiment
601 was conducted on a soil relatively poor in N, the temperature-moisture regimes typical of a
602 Mediterranean dryland area stimulated the mineralization process of plant-derived C-
603 substrates, determining temporary high soil NO₃⁻-N levels at the establishment and at the
604 early stages of the crop and potentially suppressing microbial N₂-fixation activity. Moreover,
605 this observation justifies the lower nitrogen fixation by legumes in the third year compared to
606 the first year.

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

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

618

619 **5. CONCLUSIONS**

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

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

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

639 Beneficial effects of legumes in cereal rotations may not always be immediate (Campbell et
640 al., 1992) and longer-term experiments can better understand the N dynamics of such systems
641 and the positive, cumulative effect of grain legumes on the succeeding phases of the rotation.
642 Moreover, findings of this study should be interpreted within a context of low inputs managed

643 cropping systems as the magnitude of legume crop benefits diminishes as higher rates of N
644 fertiliser are applied (Kirkegaard et al., 2008).

645

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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 N₂.
- Intercropping in Southern Mediterranean can sustain wheat production in livestock/cereal agroecosystem

881 **TABLES AND FIGURES CAPTIONS**

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

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

890 NS not significant; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$.

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

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

901 NS not significant; *significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$.

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

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

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

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

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

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

925

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

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

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

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

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

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

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

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955 **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	**		***						

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959 **Table 2**

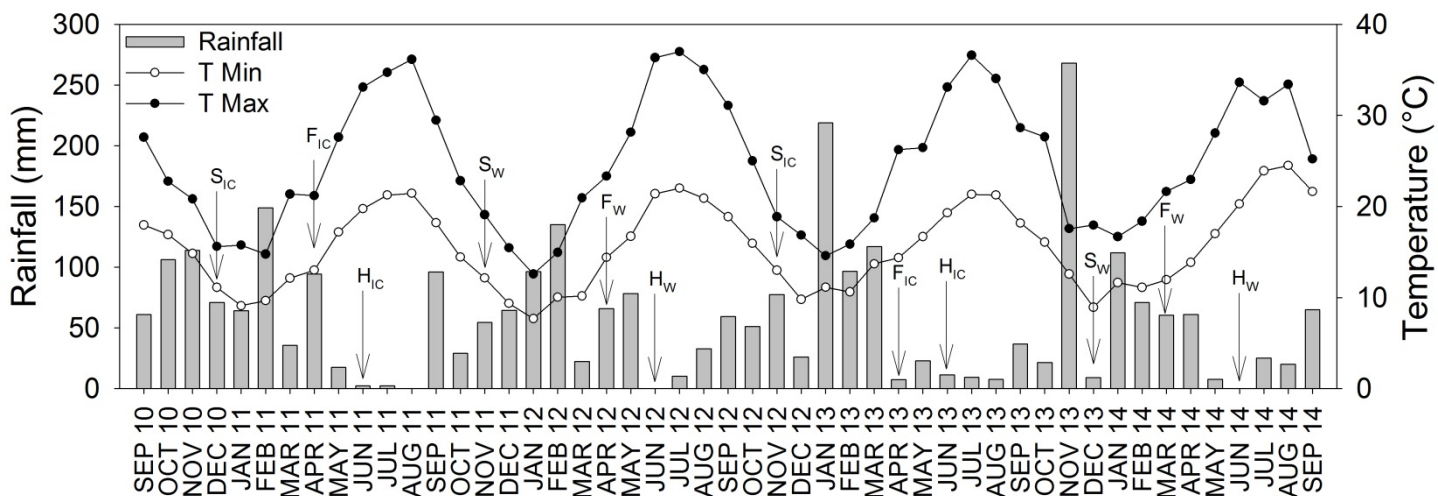
Preceding crop	Year 1			Year 2 Wheat		Year 3		Year 4 Wheat	Total rotation	Wheat protein content (%)	
	Barley kg ha ⁻¹	Legume kg ha ⁻¹	Total Intercrop kg ha ⁻¹	kg ha ⁻¹	Barley kg ha ⁻¹	Legume kg ha ⁻¹	Total Intercrop kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	Year 2	Year 4
P100B50	93.0 a	79.5 c	172.5 ab	64.1 bc	57.4 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	***	**						***	

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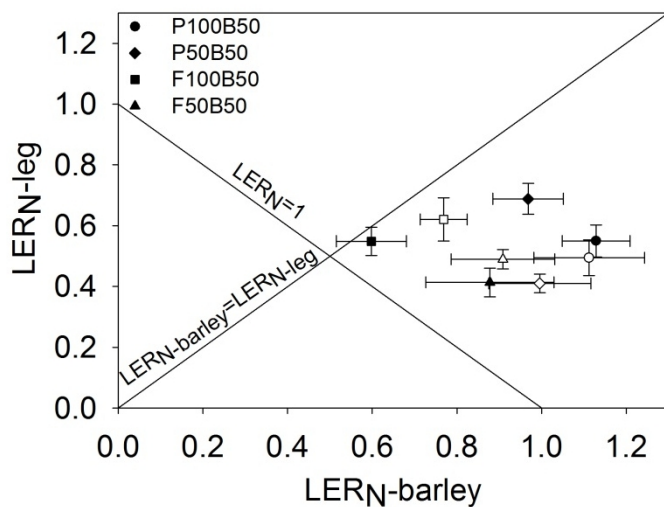
963 **Figure 1**



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966 **Figure 2**



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983 **Figure 3**

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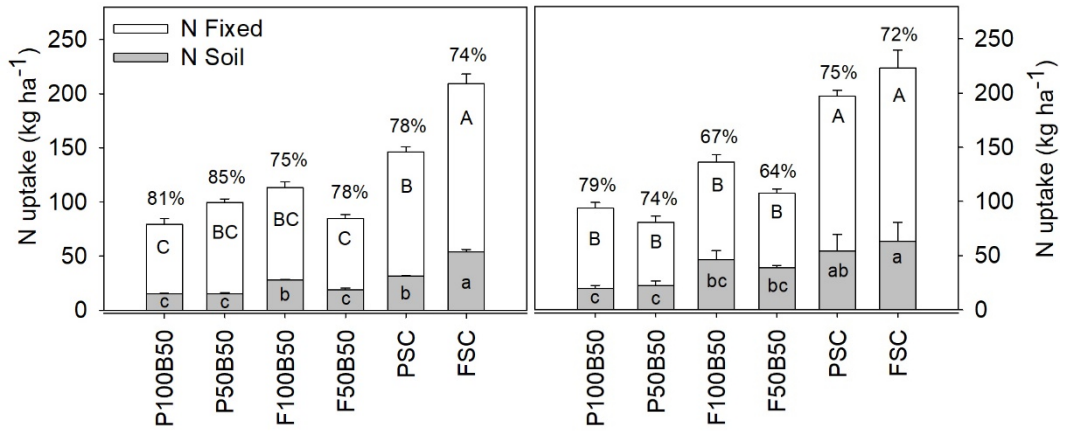
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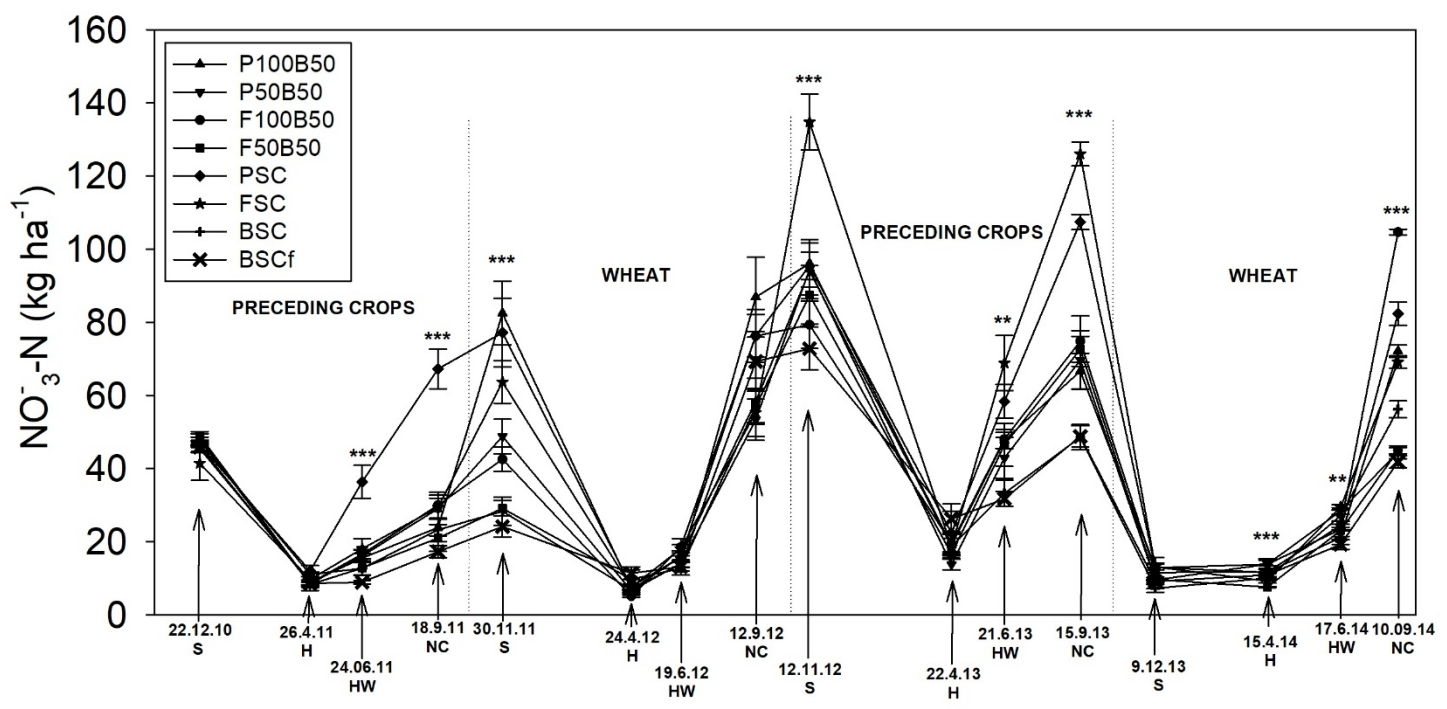
994 **Figure 4**

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1000 **Figure 5**

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