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Article title: Yield components and nitrogen use in cereal-pea intercrops in Mediterranean environment.

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ABSTRACT

Intercropping is a cropping practice with the potential to increase the sustainability of the agricultural systems. Field pea has been largely studied in intercrop with wheat and barley in Northern and Central Europe whereas much less research has been carried out in semiarid environments of Southern Europe.

A two-year field experiment was conducted in Southern Italy with the aim to assess yields and yield components and N use of field pea and four different cereals - wheat, barley, oat and triticale – grown in additive and replacement intercrop.

The cereal was the dominant partner in intercrop, strongly outcompeting pea. Cereal height and biomass yield and intercrop density affected yield component parameters of pea causing a severe reduction of number of pods per plant and death of pea plants.

Pea yields were generally lower in intercrop than in sole crop. Consequently, nitrogen accumulation in intercrop and Land Equivalent Ratio (LER) N yields-based values resulted negatively affected. LER values were highest in barley-pea intercrops for both grain and straw nitrogen yields. Cereal grain and straw N content increased in intercrop compared to the sole crop while pea grain N content was reduced.

The results of this study indicate that cereal-pea intercropping can be a way of improving the grain and straw quality of the intercropped cereal and reducing N inputs into agricultural systems compared with the sole cropping. The choice of the companion cereal and the intercrop density should, however, be carefully evaluated in drought-prone environments like those of Southern Europe.

1. INTRODUCTION

Intercropping – described as the simultaneous cultivation of two or more species on the same area of land (Vandermeer 1989) – is a cropping practice with the potential to increase the sustainability of the agricultural systems in temperate areas (Wezel et al. 2014) and to help deliver sustainable and productive agriculture (Lithourgidis et al. 2011). The rationale behind intercropping is that, due to complementarity in plant growth resources use (i.e. light, water and mineral nutrients), the different species intercropped do not compete for the same ecological niches resulting in an improved use of the resources compared to the corresponding sole crops (Willey 1979a).

Field pea (*Pisum sativum*) is largely grown in Europe and is one of the most commonly used grain legumes as an intercrop in wheat or barley cropping systems. When pea was intercropped with wheat or barley, results showed an increase in total intercrop grain yields compared to the sole crops (Bedoussac and Justes 2010a; Jensen 1996), greater land productivity (Bedoussac and Justes 2011; Hauggaard-Nielsen and Jensen 2001; Hauggaard-Nielsen et al. 2006) and reduced weed pressure (Corre-Hellou et al. 2011). In cereal-legume intercrops, advantages of intercropping versus sole cropping are often assumed to arise from the complementary use of N sources by the intercrop components. The cereal is more competitive than the legume for soil inorganic N due to its deeper and faster root system (Hauggaard-Nielsen et al. 2001a) and forces the legume to increase its reliance on symbiotic N_2 fixation (Bedoussac and Justes 2010a; Hauggaard-Nielsen et al. 2009a; Jensen 1996). The complementary use of N sources makes cereal-legume intercrops particularly suited to low-nitrogen-input systems and under those conditions intercropping shows the highest yield increase compared to pure stands (Bedoussac and Justes, 2010a; Ghaley et al. 2005; Naudin et al. 2010). Pelzer et al. (2012) found that yields of wheat-pea intercrops managed with low inputs resulted close to those of conventionally managed cereal crops. Cereal-legume intercropping can also be a way of improving the grain quality of the intercropped cereal compared with the sole cropping. When pea was intercropped with wheat (Bedoussac and Justes 2010a; Gooding et al. 2007; Naudin et al. 2010) or barley (Knudsen et al. 2004) the N content of the intercropped cereal was greater than that of the respective cereal sole crop. Furthermore, Naudin et al.

(2010) demonstrated that no-fertilised wheat-pea intercrops could produce wheat grains with protein content not significantly different from sole cropped fertilised wheat. If the grain quality of the components grown in intercrop has been largely investigated, conversely, the quality of intercrop residues has been an aspect only marginally explored. Neugschwandtner and Kaul (2015) observed a higher N content in the residues of both oat and pea grown in intercrop compared to that of the respective sole crops, however there is still little literature published on this aspect.

Despite its potential benefits, the adoption of the intercropping practice in agricultural systems of developed countries has faced huge competition from large-scale, intensive monocrop farming. The delivery of multiple ecosystem services and products becomes therefore important to increase the interest towards this agronomic practice (Brooker et al. 2014). Cereal-legume intercrop residues, for instance, can find application in the bioethanol industry where cereal straw has gained interest as a low-cost material for the fermentation (Petersson et al. 2007) and the addition of legume biomass can have a positive effect on ethanol yields (Thomsen and Hauggaard-Nielsen 2008). Cereal-legume intercrop residues can have also the potential to improve the synchrony between the rates of N supply to crop and crop demand for N (Hauggaard-Nielsen et al. 2009b).

Wheat- and barley-pea intercrops for grain production have been broadly studied in Northern Europe environments on spring sowing (e.g. Ghaley et al. 2005; Hauggaard-Nielsen et al. 2001b; Hauggaard-Nielsen et al. 2006) and at lower latitudes on winter sowing (e.g. Bedoussac and Justes 2010a and b; Corre-Hellou et al. 2006; Naudin et al. 2010; Pelzer et al. 2012). Limited research, in contrast, is available on wheat– and barley-pea intercrops on winter sowing for grain production in drought-prone Mediterranean environments of Southern Europe where severe terminal drought and heat stress can limit yields of drought sensitive crops, i.e. pea. Furthermore, other small grain winter cereals widely adopted in the agricultural systems of Southern Europe, such as oat and triticale, have been only marginally studied in intercrop with grain legumes. For instance, triticale is well adapted to the Mediterranean environment and modern varieties are considered as a viable alternative to durum wheat (Motzo et al. 2015).

If yield components of cereals and grain legumes have been widely examined in sole cropping conditions, very little research, instead, has been carried out to investigate how the main yield-determining traits of those crops are affected when intercropped. Actually, an understanding of the yield components of crops grown in mixture is a key component in developing intercropping systems (Neugschwandtner and Kaul 2014). If the yield components influenced by the intercropping were closely identified, the findings may assist the selection of appropriate genotypes to be used in intercrops thus contributing to the adoption of this agronomic practice. The aim of this study was to assess four small grain cereal-pea intercrops grown in winter season in a typical Mediterranean environment of Southern Italy with focus on i) yield and its components, ii) grain and straw N content, iii) nitrogen use of cereal–pea intercropping systems as compared to pure stands of both components. The four intercrop combinations were arranged in two intercrop density designs; a 50:50 replacement design and a 100:50 additive design with the cereal sown at full sole crop density and the legume at half of the sole crop density. In the additive design, the cereal was considered as the main crop and the pea was used as a tool to increase the sustainability of continuous cereal monocropping (i.e. biological N fixation and weed control) and to improve the quality of the grain and straw mixture (N content) without affecting cereal yields.

2. MATERIALS AND METHODS

2.1 Experimental site

The experiment was carried out on a clay loam (36% clay, 24% silt and 40% sand) classified as a "Typic Haploxeralfs" (USDA) on the experimental farm of the *University Mediterranea* in Reggio Calabria in Southern Italy (38°10' N, 15°45' E, 232 m a.s.l.) over two growing seasons (2006/07 and 2007/08). The soil (0–30 cm depth) contained 1.95% organic matter, 1.12‰ total N (Kjeldahl), 12.31 ppm P (Olsen), 372.58 ppm K and had a pH of 7.15.

The previous crop on the experimental site was sole crop of barley. At sowing, soil samples (0-60 cm) were taken and soil was sieved to < 4 mm particle size before analysis. NH_4^+-N and NO_3^--N in 2 M KCl soil extracts were determined colorimetrically by using a Flow Injection Analysis System (FIAS 400 PerkinElmer, Inc., CT, USA) equipped with an AS90 Autosampler (PerkinElmer) and linked to a UV/Vis spectrophotometer Lambda 25 (PerkinElmer). Inorganic soil N was 47.3 ±4.0 and 35.3 ±3.13 kg ha^{-1} respectively in the first and second cropping season. 84 kg ha^{-1} of Ca $(H_2PO_4)_2$ (Superphosphate) were incorporated into the soil at sowing. No N fertilization was provided. Crops were sown in the third decade of December in both years. No chemical weed control was undertaken and all treatments were kept free of weeds by hand as much as possible. There was a slight asynchrony among the different species in regards to the full physiological maturity stage. Harvesting was therefore organized according to such a criteria that the crops were harvested at their physiological maturity in sole crop and at full maturity of the last maturing species in intercrop. Harvest took place at pea physiological maturity for pea sole crop (last week of May in both years), and at cereal physiological maturity (Zadoks 92) for both cereal sole crops and intercrops (first week of June in both years).

Air temperature and rainfall during the experimental period are shown in figure 1. Air temperature regimes were similar for the two years and in line with the 20-year mean of the experimental site. February in the second season was colder than in the previous one and the minimum daily air temperature dropped to 4°C. In both seasons, air temperature started increasing constantly from March onwards. The rainfall during the growing seasons (December-June) was 400 mm and 226 mm in the first and second seasons respectively versus the 20-year mean for the same period of 394 mm. The first season was affected by a wet winter (especially March) that was then followed by a sharp increase in the air temperature in April. In the second season, in contrast, the autumn was wet before sowing whereas the crops received much less rainfall from the emergence afterwards compared to the previous year.

2.2 Treatments and experimental design

Durum wheat (W) (*Triticum turgidum* spp. *durum*) cv. Valbelice, Triticale (T) (*Triticum x Secale*) cv. Trica, oat (O) (*Avena sativa*) cv. Argentina, barley (B) (*Hordeum vulgare*) cv. Gotic and a tall, semi-leaf-less, medium early and indeterminate growth pea (P) (*Pisum sativum*) cv. Hardy were grown as (1) sole crop (WSC, TSC, OSC, BSC, PSC), (2) replacement (W50P50, T50P50, O50P50, B50P50) and (3) additive (W100P50, T100P50, O100P50, B100P50) cereal-legume binary intercrop (IC). The sole crops were sown with planned density of 400 seeds m^{-2} for wheat and triticale, 300 seeds m^{-2} for oat and barley and 90 seeds m^{-2} for pea. In intercrop each species was sown in alternate rows at half of its sole crop densities in the replacement design and at full (cereal) and at half (pea) of its sole crop density in the additive design. On the individual rows, the actual pea density in both intercrop designs was the same as the sole crop while for the cereal in the additive design it was the double of its density in sole crop.

The experiment was arranged in a randomized complete block design with three replicates. Plot size was 21 m^2 (7 x 3m). A row spacing of 12.5 cm was adopted for both intercrop and sole crop treatments.

2.3 Data collection and analysis

In each plot above ground plants biomass were manually cut on 1 m^2 sampling area. At full ripeness in the intercrop treatments, cereal and pea were harvested separately. The biomass was dried at 50 °C to constant weight to determine total dry matter production, then separated and threshed into straw and grain. From each sample, number of ears, number of pea plants, one thousand kernels weight (TKW) and one hundred seeds weight of pea (HSW) were obtained. Number of kernels per ear in the cereals and number of pods per plant and number of seed per pod in pea were determined on a subsample of 15 ears and 15 pea plants randomly selected from the 1m^2 sampling area. From the same subsample, the pea production per plant was also calculated by multiplying the number of pods per plant, the number of seeds per pod and the weight of a single seed. Grain and straw total N content was determined on 5–10 mg subsamples of finely ground material (≤1mm) using the Kjeldahl method.

2.4 Calculation and statistics

The efficiency of the intercrop versus the sole crop system was evaluated using the Land Equivalent Ratio (LER). This well-known index is defined as the relative land area under sole crop that is required to produce the same yield achieved by intercropping (Vandermeer 1989). It was calculated in accordance with De Wit and Van den Bergh (1965). In the present paper, LER values are based on N accumulated in the aboveground biomass (grain and straw) and it is named LER_N.

Analysis of variance (ANOVA) for randomized complete block design (RCBD) over two years was performed using GLM procedure in SAS/STAT Version 9.1 (SAS Institute Inc., Cary, NC, USA). The analysis included eight intercrops (combination between cultivar and intercrop design) and five sole crop treatments. Pea sole crop was not included in the statistical analysis only when this was performed on grain and straw yields and pea plant m^{-2}. When a significant year x treatment interaction was found, the analysis for a single year was carried out. If the analysis of variance showed significant treatment effects, the Least Significant Difference (LSD) test was used to compare treatment means ($P \leq 0.05$).

RESULTS

3.1 Yield and yield components of pea

As previously shown in Pellicanò et al. (2015), the cereal was the dominant component in intercrop. The aggressivity of the cereal over the legume showed wide variability between the two seasons determining large differences in pea aboveground biomass yield in intercrop. The average grain yield of intercropped pea in 2006/07 was 22.4 g m^{-2} versus 174 g m^{-2} of pea sole crop with a share on the total intercrop grain yield of 3.7% and 7.2% respectively in the additive and replacement design (tab.1). In 2007/08 the drought at the end of the growing season had a negative impact on the grain yield in pea sole crop (118.7 g m^{-2} on average). In intercrop, in contrast, pea grain yield increased on average to 36 g m^{-2} on reflection of the lower cereal competitiveness compared to the previous season. In the same year, the share of pea grain yield in intercrop went up to 8.7% and 13.5% respectively in the additive and replacement design. There was a tendency of pea grain yield to increase from the additive to the replacement design within the intercrops.

The cropping system strongly influenced the number of pods per plant in pea (fig. 2a) that was always significantly higher in sole crop (4.7) than in the additive (2.1) and replacement intercrop treatments (2.9) (on average of treatments and seasons). The lowest number of pods per plant was observed in pea in additive intercrop with oat (1.7) and triticale (1.5). Within the intercrops, excluding barley-pea intercrop, the additive design had a further depressing effect on the number of pods per plant.

The cropping system affected also HSW (fig. 2b) that resulted, on average, higher in sole crop (13.5 g) than in the additive (12.2 g) and replacement intercrop (12.8 g). HSW in pea sole crop was found to be significantly higher than HSW of pea in W100P50, O100P50, O50P50 and T100P50 only. Within intercrops, no differences for this component emerged comparing additive and replacement design.

No significant effect of the cropping system was observed on number of seeds per pod (fig. 2c), whereas O100P50 showed higher value than O50P50.

On reflection of the lower number of pods per plant and HSW compared to the sole crop, the production per plant in pea was reduced in intercrop, averaging treatments and seasons, by more than half of that of pea sole crop (0.86 vs 1.90 g m^{-2}) (fig. 2d).

In the first season, 28 pea plants m^{-2} were counted (on average of all treatments) at harvest in intercrop, much less than those counted at the emergence that were not far from the planned sowing density of 45 plants m^{-2} (tab. 1). In 2006/07 pea plants m^{-2} were, on average, 23 and 32 respectively in the additive and replacement design and ranged, on average of the two designs, from 22 and 26 plants m^{-2} respectively in triticale and wheat to 32 and 31 plants m^{-2} respectively in barley and oat. In the same season, cereals were significantly taller than they were in the second one and taller in the additive compared to the replacement design (tab. 1). Furthermore, triticale and wheat were taller than oat and barley. Only in 2006/07 pea plant m^{-2} in intercrop was found to be only slightly, though significantly, negatively related to both cereal plant height (n=8; R^2_{adj}=0.48; P=0.035) and cereal biomass m^{-2} (n=8; R^2_{adj}=0.58; P=0.017). The pea plant production was found to be also positively related to pea plants number m^{-2} (n=8; R^2_{adj}=0.58; P=0.0174). The increment of 1 plant m^{-2} increased the pea plant production of 0.023 g.

In the second season, plant height significantly dropped in wheat and triticale and differences in cereal plant height between intercrop designs were not significant. Pea plants m^{-2} at harvest was very close to the planned one in all intercrops and none of the above relationships observed in the previous season were found.

In intercrop, number of pods per plant, number of seeds per pod and HSW (and their product the pea plant production) did not largely vary between the two seasons and the marked pea yield increment noticed in 2007/08 in intercrop can be therefore linked to a higher pea plant number m^{-2} compared to the previous season (+62%).

3.2 Yield and yield components of cereals

Overall, grain yield of cereals was higher in sole crop than in intercrop with, however, variable significance depending on cereal species and/or year (tab. 1). In the first season, significant differences were found in oat, between sole crop (374.6 g m^{-2}) and the replacement intercrop (274.1 g m^{-2}), and in wheat, between sole crop (490.4 g m^{-2}) and the two intercrops designs (390.8 g m^{-2} on average). In the second season, barley and triticale sole crop (respectively 466.6 and 406.3 g m^{-2}) over-yielded both intercrops (respectively 372.8 and 283.9 g m^{-2} on average of the two designs) and wheat sole crop produced significantly more grain (354.6 g m^{-2}) only when compared with the replacement intercrop (205.1 g m^{-2}). In both seasons, within the intercrops, no significant differences in cereal grain yield between the two intercrop designs were observed.

Concerning the cereal yield components, on average of all treatments, the cereal showed more kernels per ears in the first compared to the second season (35.6 and 31.6 g respectively) (fig. 2e) and a higher TKW (36.5 and 35.3 g respectively) (fig. 2f).

As expected from the sowing density, number of ears m^{-2}, on average of the two seasons, were lower in the replacement compared to the additive design and to the sole crop; only in barley-pea intercrop, number ears m^{-2} were not significantly different between the two designs (tab.1). The cereal in the replacement design compensated the reduced sowing density by increasing the tillering; barley produced almost 90% of the ears m^{-2} observed in sole crop followed by triticale (83%), wheat (72%) and oat (72%).

Oat produced more kernels per panicle in intercrop than in sole crop (33.7, 34 and 30 respectively in O100P50, O50P50 and OSC) (fig. 2e). The opposite was observed in wheat and barley that showed a number of kernels per ear significantly lower in intercrop than in sole crop. The number of kernels per ear in B100P50 was also lower than in B50P50 (respectively 31.8 vs 35.7). Triticale in T100P50 produced fewer kernels per ear than in T50P50 and TSC (respectively 32.5, 40.9 and 41.2).

TKW generally increased when the cereal sowing density was halved in the replacement design, ranging, on average, from 34.8 and 35.9 g respectively in the additive intercrop and sole crop, to 37.1 g in the replacement design (fig. 2f). However, within the intercrops, no significant differences arose for this component between treatments, except in triticale-pea where TKW was lower in the additive (34.2 g) compared to the replacement design (36.7 g) and sole crop (37.9 g).

3.3 Grain and straw N content

The environmental condition affected the grain N content in both cereals and pea that declined on average respectively from 0.211 to 0.195 g kg^{-1} and from 0.477 to 0.443 g kg^{-1} from the first to second season (table 2). The straw N content did not differ significantly between the two seasons in the cereals (0.0355 g kg^{-1} on average) whereas in pea it grew from 0.136 to 0.148 g kg^{-1} in 2007/08 (table 2). Grain and straw N content in the intercropped cereal was, on average of all treatments, greater than that of the corresponding cereal sole crop with a mean of 0.210 versus 0.188 g kg^{-1} and 0.037 versus 0.032 g kg^{-1} respectively for grain and straw. Differences were found to be significant, however, only in B100P50, B50P50 and T50P50 for grain N content and in B100P50, B50P50 for straw N content. Similarly to the work of Bedoussac and Justes (2010a) and Bedoussac et al. (2015), the grain and straw N content of the cereal in intercrop was plotted as a function of the grain and straw N content of the corresponding cereal sole crop (fig. 3a,b). The bisector $y = x$ indicates an increment of the nitrogen in the grain (A) and straw (B) of the intercropped cereal versus the sole crop equal to zero; the closer the data points to the bisector, the lower the increment of the N content. The intercropped cereal N content versus the respective sole crop increased, on average, by 12% for the grain (fig. 3a) and 17% for the straw (fig. 3b) and this is indicated by the bisector $y=1.12x$ and $y=1.17x$ respectively. The biggest advantage of the intercrop versus the cereal sole crop was observed in barley-pea intercrop (+20% and +50% on average of the two designs for grain and straw respectively).

The grain N content of intercropped pea (0.455 g kg^{-1} on average of all treatments and years) was lower than in sole crop (0.505 g kg^{-1} on average) with the exception of pea in both oat-pea intercrops that showed no significant differences with pea sole crop (tab. 2). Pea reduced the grain N content particularly when intercropped with wheat (-17% compared to pea sole crop). The different density of the cereal in intercrop did not affect pea grain N content. No differences were observed in straw N content, on average, between pea grown in sole crop and intercrop (0.142 g kg^{-1} in both cases).

3.4 Nitrogen accumulation and Land Equivalent Ratio of N yields (LER_N)

The highest total N grain accumulation values in 2006/07 were observed in B50P50 (10.8 g m^{-2}) and T50P50 (10.9 g m^{-2}) that also accumulated more N than pea sole crop (9.12 g m^{-2}). In 2007/08, grain N accumulation generally decreased compared to the previous season due to lower cereal grain yields. B50P50 showed the highest total N accumulation value (9.2 g m^{-2}) (fig. 4a).

Comparing the intercrops with the correspondent cereal sole crop, in the first season, only B50P50 accumulated more N in the grain than its sole crop (+3.3 g m^{-2}). In the second season, due to a greater share of pea on the intercrop grain yield, a greater N grain accumulation compared to the correspondent sole crop was observed in both barley-pea intercrops (+1.6 and +2.5 g m^{-2} respectively) and in O50P50 (+1.8 g m^{-2}).

It was noticed that, in both cropping seasons, N accumulation in the grain of the cereals grown in intercrop was not significantly different from those grown in the correspondent cereal sole crop. The only exceptions was the cereal in T100P50, T50P50 and W50P50 in 2007/08 that accumulated less N in the grain than it did in the correspondent cereal sole crop (-26, -26 and -35% respectively).

N grain accumulation values of intercropped pea were a reflection of its poor grain yield; on average of all treatments, N grain accumulation in pea in intercrop was 12% and 27% of that achieved in sole crop in the first and second season respectively. Very low values were observed in 2006/07 in W100P50 (0.59 g m^{-2}) and T100P50 (0.48 g m^{-2}).

The highest total N straw accumulation values in 2006/07 were observed in O100P50 (5.7 g m^{-2}) and O50P50 (5.3 g m^{-2}) followed by B50P50 (4.2 g m^{-2}). In 2007/08, B100P50 and B50P50 showed the highest total N straw accumulation value (5.5 and 5.2 g m^{-2} respectively) (fig. 4b).

In 2006/07 straw N accumulation in O100P50 and O50P50 was higher than in oat sole crop (respectively +2.6 and +2.3 g m^{-2}) and was not significantly different from pea sole crop (6.5 g m^{-2}). In the same year, B50P50 accumulated more N in the straw than barley sole crop (+2.3 g m^{-2}). In 2007/08 B100P50, B50P50, W100P50, W50P50 and T50P50 accumulated more N in the straw than their correspondent sole crop (respectively +3.9, +3.6, +1.6, +2.3, +1.4 g m^{-2}).

In 2006/07 oat grown in O100P50 accumulated in the straw on its own more N than it did in sole crop (+56%). In 2007/08 barley grown in B100P50 accumulated more N in the straw compared to barley in sole crop (+89%). Straw N accumulation of intercropped pea was greater in the second compared to the first season due to the higher straw yields of the legume in intercrop. The N accumulated in pea straw in intercrop increased from 15% in 2006/07 to 36% in 2007/08 compared to that in pea sole crop.

Despite the low values of N accumulation observed in pea aboveground biomass, N yield-based LER values (LER_N) showed that N sources were used more efficiently in intercrop than sole crop up to 43% and 74% on average of the two seasons respectively for grain and straw. The complementary in the use of N sources was particularly efficient for pea-barley intercrops that achieved grain and straw LER_N values > 1 in both years. The same was not observed in the other intercrops that in some cases failed to achieve LER_N values > 1.

In fig 5 pea partial LER_N was plotted as a function of cereal partial LER_N as suggested by Bedoussac and Justes (2011). Plotting partial LER values for the first species as a function of the partial LER of the second intercropped species is a way to illustrate the pattern of competitive outcomes in intercrop experiments. Partial LER_N values were always in the area corresponding to a competitive advantage of the cereal over pea and more precisely in the situation where the cereal suppressed the legume ($LER_{N\text{-cereal}}$ > 0.5 and $LER_{N\text{-pea}}$ < 0.5). This was not the case in 2007/08 only for straw LER_N in B50P50 ($LER_{N\text{-barley}}$ and $LER_{N\text{-pea}} \geq 0.5$) and O50P50 ($LER_{N\text{-oat}}$ and $LER_{N\text{-pea}} < 0.5$) (fig. 5d). Because of the low pea N accumulation values, pea partial LER_N was particularly low in 2006/07 (on average 0.12 and 0.16 respectively for grain and straw) and pea intercropped with triticale in the additive design showed the lowest values (0.05 and 0.11 respectively for grain and straw) (fig. 5a,c). Pea partial LER_N substantially increased in the second season (on average 0.27 and 0.37 respectively for grain and straw). The opposite was shown for cereal partial LER_N.

4. DISCUSSION

Hauggaard-Nielsen and Jensen (2001) observed that tall, indeterminate pea cultivars better competed in intercrop with barley than determinate ones and achieved a greater proportion of pea in the total intercrop yield. In our study, a tall indeterminate pea cultivar was purposely chosen to counterbalance the height of the cereals and, in particular, that of wheat and triticale and to allow a balanced canopy architecture in mixture. Nevertheless, the legume was the weaker competitor in intercrop, especially in the first season when the legume achieved just over 10% of the grain yield achieved in sole crop. The share of the legume on the total intercrop grain yield was further lowered in the additive intercrop. Rather disproportional shares of the legume in intercrop in relation to its sowing ratio have been reported in similar experiments. For instance, in Neugschwandtner and Kaul (2014) the grain yields of pea intercropped with oat in a 50:50 replacement design declined by 90% compared to the sole crop. In a 100:50 additive wheat-faba bean intercrop the grain yield of the intercropped legume was around one third of that in sole crop (Agegnehu et al. 2008).

Anderson et al. (1986) and Krarup and Davis (1970) proposed that pea yield could be expressed as the product of number of pods, seeds per pod and seeds weight. Intercropping had a huge, negative impact on number of pods per plant that were reduced by more than 50% and by almost 40% respectively in the additive and replacement design compared to pea sole crop. Such results have been reported in other studies; Ndakidemi and Dakora (2007) reported that cow pea (*Vigna unguiculata*) reduced the number of pods per plant when intercropped with corn (*Zea mays*) and Carruthers et al. (2000) observed in a severely suppressed lupin (*Lupinus albus* L.) intercropped with corn fewer pods per plant compared to the respective sole crop. In Sobkowicz (2006) triticale was a stronger competitor than field beans and the number of pods per plant was significantly reduced in all additive intercrop combinations compared to the pure stand. Shade significantly reduced pods number in pea (Sandaña and Calderini 2012) and in soybean (Jiang and Egli 1993) because of flowers and pods abscission.

Shading of the legume was also reported for reducing seeds weight in legumes both in intercrop and sole crop as seeds filling becomes source limited if photosynthesis is reduced (i.e. see Neugschwandtner and Kaul 2014). This seems to have also been the case in our study where HSW of pea was reduced by 10% and by 5% respectively in the additive and replacement design in comparison to the sole crop. Seeds weight was, however, a less plastic and less responsive yield component than number of pods per plant to changes in cropping system as also observed in Ghaley et al. (2005) and Hauggaard-Nielsen et al. (2006). Significant effects of the cropping system on the number of seeds per pod in pea were not observed in this study. Reason for that, according to Osumi et al. 1998, is that legumes with shorter pods and fewer ovules per pod (such as pea) tend to respond to shading conditions by reducing the fruit set. In contrast, legumes with longer pods and more ovules tend to modify the seeds set.

Our study confirmed the number of pods as the most important component in determining yields and yields variation in pea in intercrop (Rauber et al. 2000). Mainly due to a reduced pods set, pea plant production in intercrop was on average only 40% and 50% of that observed in sole crop respectively in the first and second season. We also noticed that the lower the pea plant number m^{-2}, the lower the pea plant production, a relationship that suggests that the ecological principle according to which interspecific competitions in intercrop are less strong than intraspecific ones (Willey 1979a and 1979b) was weakened.

The aggressivity of the cereal towards pea was discussed in Pellicanò et al. (2015) where the very low pea biomass yield in intercrop in the first season was ascribed to shading of the legume caused by the height of the companion cereal. The study of the yield components in the present study indicated that not only a low number of pods per plant but, in the first season, also a low pea plants number m^{-2} played a key role in lowering the pea biomass yield in intercrop. In the first season, the growth of the cereal during the stem elongation stage was fostered by the rainfall fallen in February and March and by the rise of the temperature afterwards. In the second season, the rain was mostly concentrated in the early autumn before the sowing and the crops received much less water than in the previous season and this translated into shorter cereals and less shading for the legume.

The cereal in intercrop is more competitive than the legume for soil inorganic N (Jensen 1996) that is taken up mainly during the vegetative stages and the vigorous growth may cause shading of the legume (Hauggaard-Nielsen and Jensen 2001). Shading can affect photosynthesis and N_2 symbiotic fixation (Fujita et al. 1992) since N acquisition and light interception are two intimately linked processes (Dreccer et al. 2000). The amount of N_2 fixed per plant in intercrop is largely determined by pea growth and it depends on the competitive strength of the companion cereal for light (Corre-Hellou et al. 2006).

The cropping system and the intercrop design also affected yields components in the cereals although the results observed were not uniform among the species. In barley, the tillering allowed the cereal to fill the initial gap in sowing density and to reach a number of ears m^{-2} that was not significantly different between treatments. A great tillering ability is well known in barley and yields in Mediterranean environments are strongly related to tillering and tiller-survival capabilities of this cereal rather than to changes in kernels weight (Garcia del Moral et al. 2003). Oat showed a higher number of kernels per panicle in intercrop compared to the sole crop. As oat differs from all the other species studied in having a panicle rather than an ear, this may explain the capacity of oat to respond through grain set rather than tillering to favourable growing conditions (Peltonen-Sainio et al. 2007). In cereal-legume intercrops with lower cereal density compared to the cereal pure stand, the cereal can grow fast in the early stage due to better access to growth resources and higher N availability for the individual cereal plant (Kübler et al. 2008). With respect to our study, more favourable growing conditions in the replacement design compared to the additive one allowed cereal grain yields not to be significantly different between the two intercrop designs through an increase of the tillering, number of kernels per ear and TKW. In Musa et al. (2010), analysis of yield components in a 50:50 pea-barley intercrop indicated that higher yields of the cereal than those expected according to the sowing density were the result of a greater number of ears m^{-2} compared to the full stand treatment due to a greater light and N availability. In replacement intercrops of oat and pea, Neugschwandtner and Kaul (2014) ascribed the higher number of panicles per plant and kernels per panicle compared to the full stand to a low competition of the legume towards the cereal that allowed a better use of available light. Number of kernels per ear is reported to be regulated by photosynthate availability (Frederick and Bauer 1999).

The seed protein content depends on the genotype (Mosse and Baudet 1983) but it can also be highly influenced by the environmental conditions and agronomy practices (Erekul and Köhn 2006). The differences in grain N content of both cereals and legume between the two years observed in our experiment can be associated to the different rainfall and temperature occurred during the crop season. Similar effects were observed by Gooding et al. (2003), Igbasan et al. (1996), Rao et al. (1993).

The grain and straw N content of the cereal increased through intercropping whereas a reverse trend was noticed for pea grain N content. Other studies showed that grain N content of non-legumes increased when intercropped with legumes. Some authors (i.e. Bedoussac and Justes 2010a; Gooding et al. 2007; Neugschwandtner and Kaul 2014) associated the increment of grain N content of cereals intercropped with pea with a higher soil N availability for the cereal on a per plant or per grain basis compared to the sole crop. This can be considered a reflection of the low competitiveness of the legume versus the cereal for soil inorganic N or/and the decline of cereal grain yields due to competitiveness for growth resources (other than N) between the two partners. In our study, the reduction in cereal yields from sole cropping to intercropping can explain only in part the increment in grain and straw N content observed. Although the circumstances under which N is transferred from legume to non-legume plants have not been fully explored, legumes can contribute up to 15% of the N in an intercropped cereal (Brooker et al., 2014). If this had also been the case in our study it could not be proved, however, it can be speculated that the higher pea biomass share on the intercrop total in barley-pea compared to the other intercrops might have contributed towards the great increment in cereal grain and straw N content observed in this crops combination. With respect to this matter, Gooding et al. (2007) found that intercropping increased the grain N concentration in wheat most when it had the least deleterious effect on partial LER of faba bean.

The reduction in grain weight and grain N content of suppressed pea in intercrop compared to the sole crop suggests that interspecific competition affected the legume through the entire crop season including the final stages of the crop, when in Mediterranean environment intra- and inter-competition can be worsened by unfavourable growing condition. Legume in intercrop can fulfil its nitrogen requirements by N_2 fixation and showing no differences in grain N content with pea sole crop (Bedoussac et al. 2015). In this study, in contrast, as observed by Patriquin et al. (1988) for faba beans, our results could be ascribed to a reduced accumulation of nitrogen by the legume due to poor nitrogen fixation.

Our results are consistent with other studies showing values of $LER_N > 1$ for cereal-legume intercrops (i.e. Bedoussac and Justes 2010a; Hauggaard-Nielsen et al. 2009a). Our findings confirmed the advantage of the intercrop versus the sole crop system for N acquisition more than for biomass production as reported by Bedoussac and Justes (2010b) (for biomass-based LER values see Pellicanò et al. 2015). The results can be explained by the fact that the greater efficiency generally observed in legume-cereal intercrops compared to sole crop systems is mainly due to the complementary use of mineral soil N and atmospheric N_2 between the two companion species (Bedoussac and Justes 2010a; Jensen 1996; Naudin et al. 2010; Ofori and Stern 1987). Since the early growth, the cereal is more competitive than the legume for soil mineral N and this forces the legume to mainly rely on N_2-fixation and to increase the percentage of N derived from air compared with the sole crop.

Despite the fact that in the first season the majority of LER_N values for grain and straw were above the unity, the plotting in fig. 5 clearly shows that this was achieved mainly through high values of partial cereal LER_N that were a result of the cereal largely outcompeting the legume. Lower pea N yields in intercrop and a smaller pea partial LER_N compared to the expected one according to the sowing density were also observed in the experiment of Hauggaard-Nielsen et al. (2001b) for a strongly outcompeted pea in barley-pea replacement (50:50) intercrop. In contrast, when higher degrees of complementary between the two companion species are achieved, partial LER_N values of the legume close to 0.50 are more likely to be observed, i.e. in the experiment of Bedoussac and Justes (2010a) and Hauggaard-Nielsen et al. (2009a) for barley-pea replacement (50:50) intercrops.

In the present study, the complementary in the use of N resources worked better for some intercrops (i.e. barley-pea) than for others (i.e. wheat-pea) and better for N straw yields than for grain N yields. A greater share of pea biomass in the mixture compared to the other intercrops together with a high increase of the grain and straw N content of the cereal grown in intercrop versus the correspondent sole crop, can explain the high N use complementarity observed in barley-pea intercrop. High straw LER_N values – as pointed out by Neugschwandtner and Kaul (2015) – reinforce the ecological role of intercropping (through the use of legume) as a sustainable way of introducing nitrogen into low input agro-systems.

CONCLUSION

One of the aims of the present study was to gain an understanding of how the agronomic practice of the intercropping would affect the yield determining components of small grain cereals and pea in a

Mediterranean environment. Our results, confirming the findings of similar studies, indicated that the intercropping system affected the pod setting stages in pea causing a large reduction in number of pods per plant and, consequently, in pea grain and N yields. In addition, the results highlighted a severe interspecific-competition in mixture that resulted in the death of pea plants in intercrop. From this study emerged that, compared to the additive design, the 50:50 cereal-pea intercrop allows a higher share of the legume on the total intercrop grain yield and therefore can provide a well-balanced mixture in drought prone environments. In contrast, the additive design, with the cereal sown at the full sole crop density and the pea at half of the sole crop, appeared to be too challenging for the legume in this environment. In such an overcrowded density design, not only the legume was highly outcompeted by the companion cereal but also the cereal failed to achieve in several cases similar yields as in the respective sole crop. Moreover, the adoption of four cereal species intercropped with pea enabled us to appreciate the different response of the combinations studied in terms of N yield, N use and grain and straw N content of the two crops. It was noticed that i) intercropping reduced pea grain N content compared to pea sole crop in almost all the intercrops; ii) N resources use worked out better for barley-pea intercrops compared to the other combinations; iii) barley was the only cereal that increased grain and straw N content when intercropped with pea.

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TABLES AND FIGURES CAPTIONS

Table 1. Grain and straw yields of cereal and pea, ear m^{-2}, cereal plant height, pea plant m^{-2} in sole crop (SC) and intercrop during the two cropping seasons. Values are the mean (*n*=3). W= wheat, B= barley, O= oat, T= triticale, P= pea. Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. Pea sole crop was not included in the statistical analysis.

NS not significant, *significant at $P \leq 0.05$, *** significant at $P \leq 0.001$.

Table 2. Nitrogen content (g kg^{-1}) in grain and straw of cereal and pea grown in intercrop and sole crop (SC). Values are the mean of two seasons (*n*=6). For each column, different letters (LSD) indicate significant differences among treatments ($P \leq 0.05$).

Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50 % of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. W= wheat, B= barley, O= oat, T= triticale, P= pea.

NS not significant, *significant at $P \leq 0.05$, *** significant at $P \leq 0.001$.

Figure 1. Monthly total rainfall (mm), monthly mean maximum and minimum temperatures (°C) during the growth seasons. S= sowing, F=flowering, H= harvest.

Figure 2. Pods per plant, pea Hundred Seeds Weight (HSW), seeds per pod, pea production per plant, kernels per ear and cereal Thousand Seed Weight (TKW) in intercrop and sole crop (SC). Values are the mean of two seasons (*n*=6). Different letters (LSD) indicate significant differences at $P \leq 0.05$ between treatments.

W= wheat, B= barley, O= oat, T= triticale, P= pea. Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50 % of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. Y= Year, T= Treatments.

NS not significant, *Significant at $P \leq 0.05$, *** Significant at $P \leq 0.001$.

Figure 3. Nitrogen content (g kg^{-1}) of the cereal in grain (A) and straw (B) in intercrop (IC) as a function of that in sole crop (SC). Values are the mean of two seasons (n=6) ±*SE*. The solid line represents the relationship. The bisector y = x (lower dotted line) and the regression y = 1.12x (upper dotted line) in A and B, and y = 1.17x in B are indicated in order to illustrate the increased range of nitrogen content in the grain and straw of the cereal in intercrop compared with the respective sole crop.

Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. W= wheat, B= barley, O= oat, T= triticale, P= pea.

*Significant at $P \leq 0.05$, *** Significant at $P \leq 0.001$

Figure 4. Nitrogen (N) accumulation (g m^{-2}) in grain (A) and straw (B) of pea and cereals grown intercrop and sole crop (SC) during the two cropping seasons. Values are the mean (n=3)±SE.

Additive intercrop (i.e.,W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e.,W50P50) = 50% of the sole crop density for each crop. W= wheat, B= barley, O= oat, T= triticale, P= pea.

Figure 5. Partial Land Equivalent Ratio calculated from nitrogen yield (LER_N) of pea as a function of Partial Land Equivalent Ratio of the cereal for grain (A and B) and straw (C and D) during the two cropping seasons. Values are the mean (n=3) ±*SE*.

Additive intercrop (i.e.,W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e.,W50P50) = 50 % of the sole crop density for each crop. W= wheat, B= barley, O= oat, T= triticale, P= pea.

TABLES AND FIGURES

Table 1 Grain and straw yields of cereal and pea, ear m^{-2}, cereal plant height, pea plant m^{-2} in sole crop (SC) and intercrop during the two cropping seasons. Values are the mean (n = 3). W = wheat, B = barley, O = oat, T = triticale, P = pea. Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. Pea sole crop was not included in the statistical analysis.

	2006/07							2007/08						
Treatments	Cereal				Pea			Cereal				Pea		
	Ears	Plant height	Grain	Straw	Plants	Grain	Straw	Ears	Plant height	Grain	Straw	Plants	Grain	Straw
	n m^{-2}	cm	g m^{-2}	g m^{-2}	n m^{-2}	g m^{-2}	g m^{-2}	n m^{-2}	cm	g m^{-2}	g m^{-2}	n m^{-2}	g m^{-2}	g m^{-2}
W100P50	355	112.0	389.2	903.3	24	14.5	57.5	287	84.7	278.4	550.7	40	30.4	136.9
W50P50	281	111.0	392.4	759.2	28	24.2	74.7	222	82.7	205.1	534.3	48	44.8	196.4
WSC	379	108.7	490.4	926.8	-	-	-	311	86.7	354.6	762.9	-	-	-
B100P50	373	78.3	435.2	631.4	25	19.4	56.9	400	63.3	363.7	527.2	46	42.0	165.2
B50P50	360	69.3	469.5	556.0	39	39.3	106.9	301	60.3	381.8	431.5	50	54.1	214.7
BSC	360	78.7	459.2	618.2	-	-	-	381	66.3	466.6	608.6	-	-	-
O100P50	316	87.7	315.8	798.2	25	17.1	67.8	322	75.7	267.8	671.6	43	22.6	141.0
O50P50	254	75.3	274.1	692.6	37	33.4	105.3	262	74.3	252.4	511.3	48	32.2	184.8
OSC	397	84.3	374.6	734.4	-	-	-	312	76.3	261.5	738.7	-	-	-
T100P50	348	116.3	406.5	935.4	19	10.2	46.8	305	83.7	297.1	757.4	44	23.3	128.2
T50P50	230	107.0	447.8	854.7	26	21.1	71.4	287	86.3	270.7	652.9	45	38.8	193.9
TSC	291	113.0	475.9	953.0	-	-	-	335	86.3	406.3	976.7	-	-	-
$LSD_{0.05}$	*74*	*5.3*	*86.7*	*92.7*	*8.3*	*5.1*	*12.3*	*84*	*8.6*	*80.0*	*123.3*	*5.0*	*13.1*	*40.1*
Mean	*329*	*95.1*	*410.9*	*780.3*	*27.9*	*22.4*	*73.4*	*310*	*77.2*	*317.2*	*643.7*	*45.5*	*36.0*	*170.1*
PSC	-	-	-	-	83	174.0	471.4	-	-	-	-	84	118.7	487.4
Anova														
Year (Y)	*NS*	***	***	***	***	***	***							
Treatments (T)	***	***	***	***	***	***	***							
Y x T	*NS*	***	*NS*	***	*NS*	*	*NS*							

NS not significant.

*significant at P ≤ 0.05.

***significant at P ≤ 0.001.

Table 2 - Nitrogen content (g kg^{-1}) in grain and straw of cereal and pea grown in intercrop and sole crop (SC). Values are the mean of two seasons (n = 6). For each column, different letters (LSD) indicate significant differences among treatments (P ≤ 0.05).

	Cereal		Pea	
Treatments	Grain	Straw	Grain	Straw
W100P50	0.219 ab	0.0279 c	0.419 d	0.141 ab
W50P50	0.218 ab	0.0265 c	0.421 d	0.134 b
WSC	0.203 ac	0.0257 c	-	-
B100P50	0.183 c	0.0461 a	0.456 bc	0.137 ab
B50P50	0.186 c	0.0458 a	0.456 bc	0.145 ab
BSC	0.154 d	0.0308 bc	-	-
O100P50	0.220 ab	0.0457 a	0.488 ab	0.149 a
O50P50	0.214 ab	0.0419 ab	0.502 a	0.147 ab
OSC	0.197 bc	0.0414 ab	-	-
T100P50	0.216 ab	0.0318 bc	0.448 cd	0.142 ab
T50P50	0.225 a	0.0321 bc	0.446 cd	0.142 ab
TSC	0.199 bc	0.0300 bc	-	-
PSC	-	-	0.505 a	0.142 ab
Mean	*0.203*	*0.0355*	*0.460*	*0.142*
Mean 06/07	*0.211*	*0.037*	*0.477*	*0.136*
Mean 07/08	*0.195*	*0.034*	*0.433*	*0.148*
Anova				
Year (Y)	***	*NS*	***	***
Treatments (T)	***	***	***	*NS*
Y x T	*NS*	*	*NS*	*NS*

Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. W = wheat, B = barley, O = oat, T = triticale, P = pea.

NS not significant.

*significant at P ≤ 0.05

***significant at P ≤ 0.001.

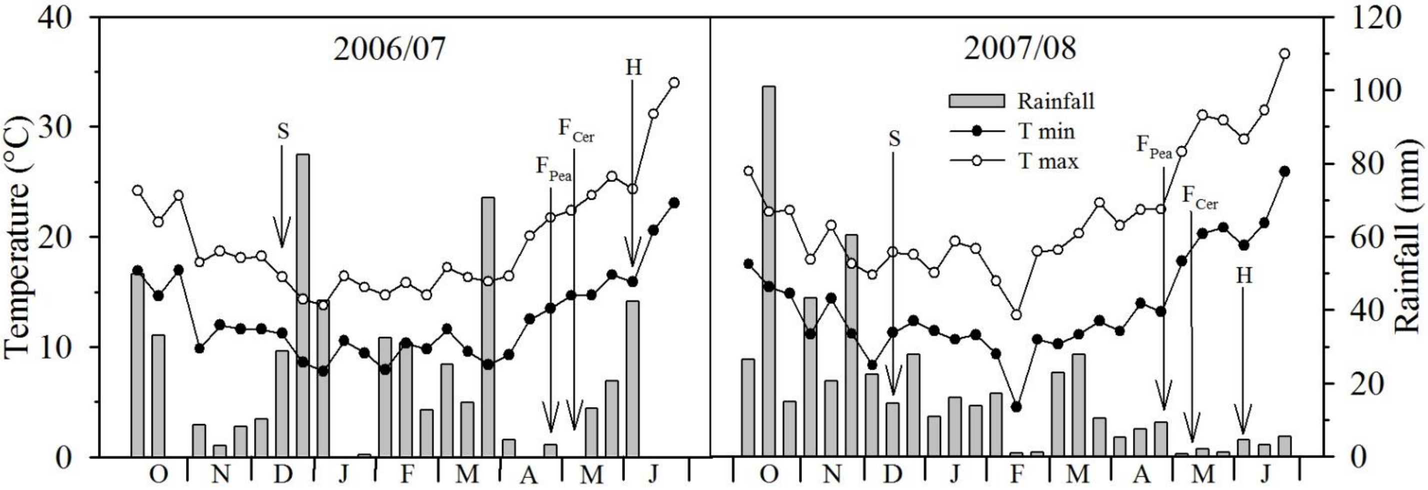

Figure 1 - Fig. 1. Monthly total rainfall (mm), monthly mean maximum and minimum temperatures (◦C) during the growth seasons. S = sowing, F = flowering, H = harvest.

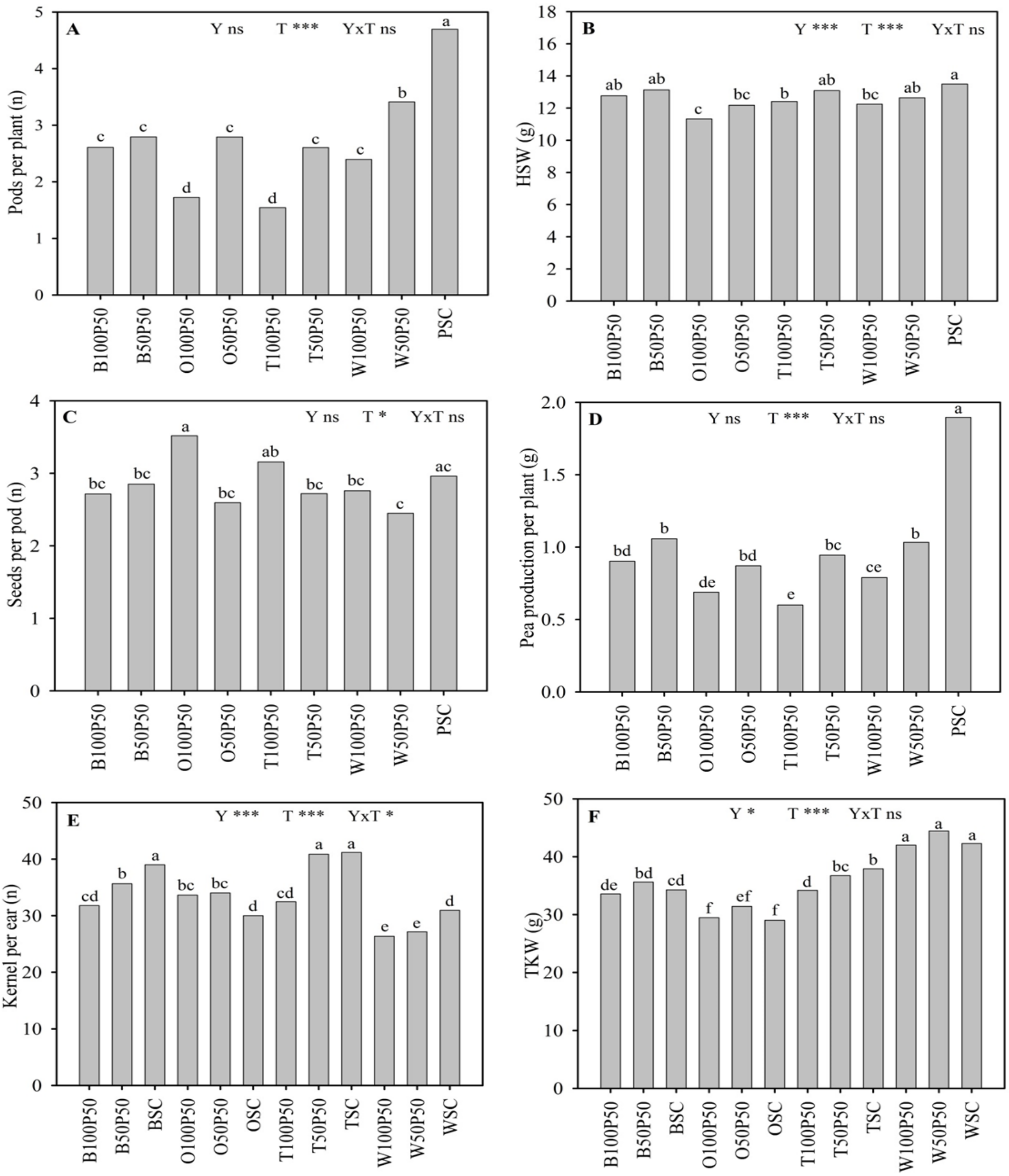

Figure 2 - Pods per plant, pea Hundred Seeds Weight (HSW), seeds per pod, pea production per plant, kernels per ear and cereal Thousand Seed Weight (TKW) in intercrop and sole crop (SC). Values are the mean of two seasons (n = 6). Different letters (LSD) indicate significant differences at P ≤ 0.05 between treatments. W = wheat, B = barley, O = oat, T = triticale, P = pea. Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. Y = Year, T = Treatments. NS not significant, *Significant at P ≤ 0.05, *** Significant at P ≤ 0.001.

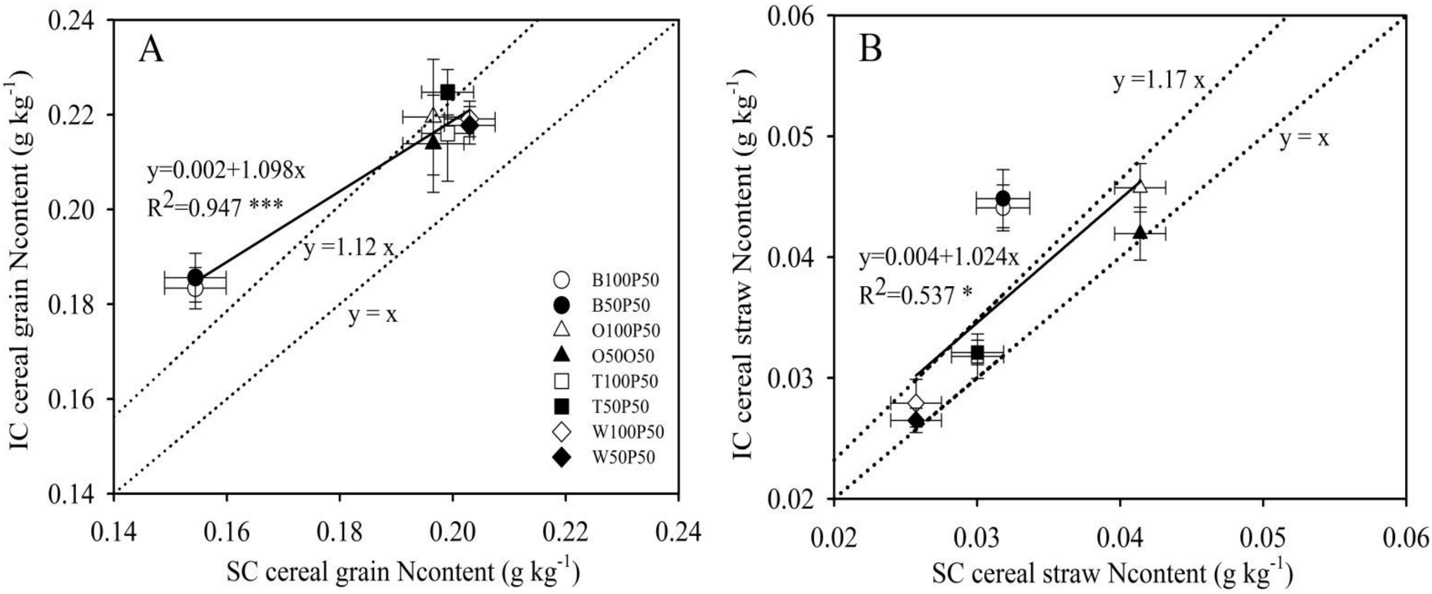

Figure 3 - Nitrogen content (g kg−1) of the cereal in grain (A) and straw (B) in intercrop (IC) as a function of that in sole crop (SC). Values are the mean of two seasons (n = 6) ±SE. The solid line represents the relationship. The bisector y = x (lower dotted line) and the regression y = 1.12 x (upper dotted line) in A and B, and y = 1.17 x in B are indicated in order to illustrate the increased range of nitrogen content in the grain and straw of the cereal in intercrop compared with the respective sole crop. Additive intercrop (i.e., W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e., W50P50) = 50% of the sole crop density for each crop. W = wheat, B = barley, O = oat, T = triticale, P = pea.*Significant at $P \leq 0.05$, *** Significant at $P \leq 0.001$.

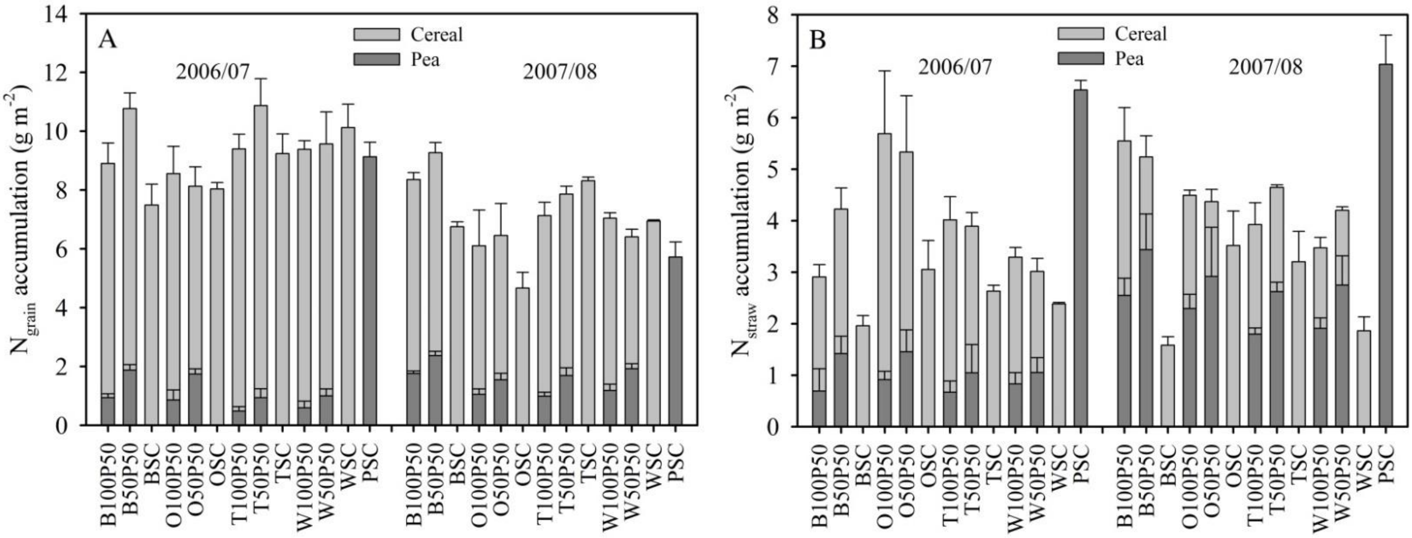

Figure 4 - Nitrogen (N) accumulation (g m−2) in grain (A) and straw (B) of pea and cereals grown intercrop and sole crop (SC) during the two cropping seasons. Values are the mean (n = 3) ± SE. Additive intercrop (i.e.,W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e.,W50P50) = 50% of the sole crop density for each crop. W = wheat, B = barley, O = oat, T = triticale, P = pea.

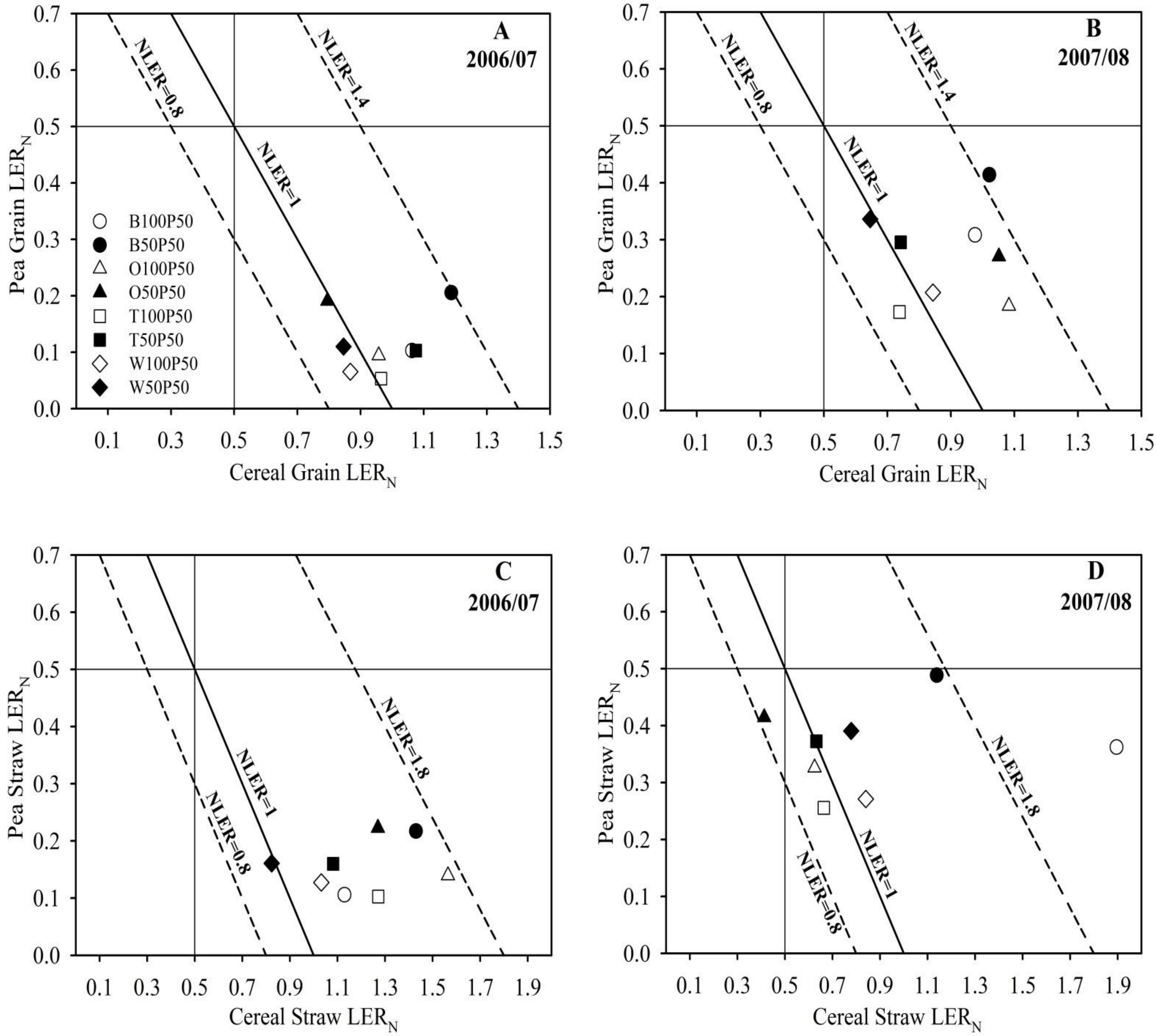

Figure 5 - Partial Land Equivalent Ratio calculated from nitrogen yield (LER$_N$) of pea as a function of Partial Land Equivalent Ratio of the cereal for grain (A and B) and straw (C and D) during the two cropping seasons. Values are the mean (n = 3) ±SE. Additive intercrop (i.e.,W100P50) = 100% of the sole crop density for the cereal and 50% of sole crop density for the legume. Replacement intercrop (i.e.,W50P50) = 50% of the sole crop density for each crop. W = wheat, B = barley, O = oat, T = triticale, P = pea.