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The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth

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Abstract

Grain protein concentration of durum wheat is often too low, particularly in low-N-input systems. The aim of our study was to test whether a durum wheat - winter pea intercrop can improve relative yield and durum wheat grain protein concentration in low-N-input systems. A 2-year field experiment was carried out in SW France with different fertilizer-N levels to compare wheat (*Triticum turgidum* L., cv. Nefer) and pea (winter pea, *Pisum sativum* L., cv. Lucy) grown as sole crops or intercrops in a row-substitutive design. Without N fertilization or when N was applied late (N available until pea flowering less than about 120 kg N ha^{-1}), intercrops were up to 19% more efficient than sole crops for yield and up to 32% for accumulated N, but were less efficient with large fertilizer N applications. Wheat grain protein concentration was significantly higher in intercrops than in sole crops (14% on average) because more N was remobilized into wheat grain due to: i) fewer ears per square metre in intercrops and ii) a similar amount of available soil N as in sole crops due to the high pea N_2 fixation rate in intercrops (88% compared to 58% in sole crops).

Keywords

Complementary resource use, grain protein concentration, land equivalent ratio (LER), nitrogen acquisition, nitrogen fixation, plant competition

Introduction

The intensification of agriculture during the last 50 years has contributed, in some areas, to the appearance of problems such as soil erosion, environmental contamination by fertilizer and pesticides and also selection of diseases, pests, and weeds resistant to chemical treatments (Jackson and Piper 1989; Vandermeer et al. 1998; Griffon 2006). Consequently, the efficiency of agricultural systems needs to be improved and diversification of agro-systems has been proposed as one of several solutions for future agriculture (Altieri 1999; Griffon 2006). Intercropping (IC) - the simultaneous growing of two or more species in the same field for a significant period but without necessarily being sown and harvested at the same time (Willey 1979a) - could be one way to increase the number of species cultivated (Vandermeer et al. 1998; Malézieux et al. 2008). Grass-legume intercrops are common in natural ecosystems, but they are now rarely used in European countries, except in a few cropping systems for animal feeds (Anil et al. 1998). For these reasons there has been a renewed interest in intercropping (Anil et al. 1998; Malézieux et al. 2008) and particularly grain legume-cereal intercrops, which use available resources more efficiently than the corresponding sole crops (Willey 1979ab; Ofori and Stern 1987; Vandermeer 1989; Willey 1990; Fukai and Trenbath 1993). The advantage of such systems can be explained by the fact that the two intercropped species do not compete for exactly the same resource niche and thereby tend to use resources in a complementary way (Snaydon and Satorre 1989; Hauggaard-Nielsen et al. 2001ab). Cereals in particular seem to be more competitive for soil inorganic N (Jensen 1996) compared to grain legumes such as peas, due to faster and deeper root growth and the higher N demand of the cereal (Fujita et al. 1992; Corre-Hellou 2005; Hauggaard-Nielsen et al. 2003; Corre-Hellou and Crozat 2005). Consequently, the grain legume increases its reliance on symbiotic N_2 fixation (Li et al. 2008). Furthermore, growing a grain legume-cereal intercrop at various N levels shows that the grain legume has a higher interspecific competitive ability at lower soil N levels, whereas that of the cereal is lower (Hauggaard-Nielsen and Jensen 2001; Ghaley et al. 2005). The complementary use of N sources between species could be of particular interest in low-N-input cropping systems and organic farming, particularly for cereals with high N requirements such as durum wheat.

In 2007, in southern France durum wheat represented 19% of the cereal area and peas 76% of the legume area (AGRESTE 2008). Fulfilling the N demand of durum wheat is crucial to obtaining maximum yield and grain protein concentration (Garrido-Lestache et al. 2004). Consequently, durum wheat is generally fertilized with high levels of N in conventional cropping systems, which can lead to nitrate leaching during the following winter when drainage normally occurs (Abad et al. 2004). In low-N-input systems and organic farming, where N is often a limiting resource, it is difficult to reach the grain protein concentration threshold needed to avoid kernel vitreousness (Garrido-Lestache et al. 2004), which makes it unsuitable for high-quality pasta (semolina) production (Samaan et al. 2006) and hence for human consumption.

The advantages of legume-cereal intercrops are often assumed to arise from the complementary use of N sources by the components of the intercrop (Ofori and Stern 1987; Jensen 1996). Thus, when intercropped, the cereal should have access to a greater proportion of soil inorganic N because of greater interspecific competitive ability explained by a faster and deeper root growth and higher N demand of the cereal (Corre-Hellou and Crozat 2005), whereas the intercropped legume should increase its symbiotic N_2 fixation to satisfy its N requirements (Crozat et al. 1994; Voisin et al. 2002) as compared with sole cropping conditions.

In Europe, many studies on spring barley-pea intercrops have shown that relative yield and grain protein concentration of intercropped barley are higher than in sole crops (e.g. Hauggaard-Nielsen et al. 2003) and that the yield advantage depends greatly on N fertilization. In particular, Hauggaard-Nielsen and Jensen (2001) showed that spring barley-pea intercrop advantage for yield was maximum without N fertilization and significantly reduced when N was applied, mostly due to pea yield decrease with N supply. Similar results were found for spring wheat-pea intercrops (Ghaley et al. 2005). However, no information on winter wheat-grain legume intercrops is available, despite the fact that winter crops are more suited to southern European conditions in order to avoid water stress.

The aim of our study was to evaluate the effects of N availability as modified by fertilization (quantity and splitting of doses) on a durum wheat-winter pea intercrop compared with sole crops by analyzing: i) N resource use, ii) crop production, iii) potential advantages for total yield, dry weight and grain protein concentration and iv) functional relationships between N acquisition and intercropping performances for yield and cereal grain protein concentration in order to better understand species complementarities for N use.

Materials and methods

Site and Soil

The experiment was carried out on two experimental fields of the Institut National de la Recherche Agronomique station in Auzeville (SW France, 43°31'N, 1°30'E) in 2005-2006 (Exp. I) and 2006-2007 (Exp. II). The 25-year mean annual rainfall in Auzeville is 650 mm and the mean annual air temperature is 13.7 °C with a mean maximum daily air temperature of 21.9 °C in August and a mean minimum of 6.0 °C in January. The rainfall during the growing seasons was 361 mm and 468 mm for Exp. I and II, respectively, while the 25-year mean was 489 mm for the same period (November-July). Exp. I was characterized by a cold winter and a dry, warm spring, whereas the winter was warm and dry and spring particularly wet during Exp. II. In Exp. I, soil water content was lower during the growing season and water stress higher in spring.

Exp. I was carried out on a plot with loamy soil (23% clay, 29% silt and 46% sand) with an available water capacity of 223 mm (0-150 cm). Soil pH in water was 8.0, indicating a calcareous soil as illustrated by the $CaCO_3$ content (20 g kg^{-1}) mainly in the 90-120 cm layer (65 g kg^{-1}). The topsoil (0-30 cm) contained 9.4 g kg^{-1} total C, 0.93 to 1.09 g kg^{-1} total N, a satisfactory phosphorus and potassium content and a cation exchange capacity (CEC) of 16.0 cmol+ kg^{-1}. Exp. II was conducted on another plot with clay loam soil (26% clay, 34% silt and 28% sand) with an available water capacity of 207 mm (0-150 cm). Soil pH in water was 8.3 with a large amount of $CaCO_3$ (87 g kg^{-1}), mainly in the 60-120 cm layer (165 g kg^{-1}). The topsoil (0-30 cm) contained 9.9 g kg^{-1} total C, 1.07 g kg^{-1} total N, adequate contents of phosphorus and potassium and a CEC of 21.3 cmol+ kg^{-1}. For both experiments, phosphorus, potassium and CEC values were assumed to be non-limiting. The four previous crops on the experimental sites were durum wheat (*Triticum turgidum*), sunflower (*Helianthus annuus*), durum wheat and sorghum (*Sorghum bicolor*) for Exp. I and sunflower, durum wheat, sorghum and sunflower for Exp. II. In Exp. I, 7 t ha^{-1} sorghum residues with a C:N of 63 were incorporated on September 26, 2005 by tillage (20-25 cm depth). In Exp. II, 4 to 7 t ha^{-1} of sunflower residues - with a C:N varying between 31 to 55 according to the previous sunflower experiment - were incorporated on September 25, 2006 by tillage (20-25 cm depth) (see details in Table 1).

Experimental design

Durum wheat (W) (*Triticum turgidum* L, cv. Nefer, authority Eurodur) and winter pea (P) (*Pisum sativum* L., cv. Lucy, authority GAE recherche) were grown as sole crops (SC) and as a mixed crop (IC) in a row-replacement design. Three main treatments were compared: i) durum wheat (cv. Nefer) sole crops sown at the recommended density (336 grains m^{-2}), ii) winter pea (cv. Lucy) sole crops sown at the recommended density (72 grains m^{-2}) and iii) durum wheat-winter pea intercrops, each species sown at half of the sole crops densities in alternate rows. In Exp. I, final plant densities were 51 for sole cropped pea, 27 for intercropped pea, 226 for sole cropped wheat and 112 plants m^{-2} for intercropped wheat. In Exp. II, plant densities were 56 for sole cropped pea, 27 for intercropped pea, 202 for sole cropped wheat and 101 plants m^{-2} for intercropped wheat.

Wheat stages were identified according to the Zadoks scale (Zadoks et al. 1974).

In both experiments, different fertilizer N sub-treatments were evaluated on intercrops and wheat sole crops while pea sole crops were grown only without any N application. In Exp. I we compared: i) no fertilizer-N (N0), ii) low N fertilization (N100) split into two applications of 50 kg N ha^{-1} at '1 cm ear' (E1cm, Zadoks 30) and 'flag leaf visible' (FLV, Zadoks 37) and iii) moderate N fertilization (N180) split into 3 applications of 30 kg N ha^{-1} at wheat tillering (Zadoks 23), 100 kg N ha^{-1} at Zadoks 30 and 50 kg N ha^{-1} at Zadoks 37. In Exp. II, four treatments were evaluated: i) no fertilizer-N (N0), ii) one application of 60 kg N ha^{-1} (N60) at Zadoks 37 aimed at increasing grain protein, iii) one application of 80 kg N ha^{-1} (N80) at Zadoks 30 to increase yield and iv) a moderate N fertilization (N140) split into two applications of 80 kg N ha^{-1} at Zadoks 30 and 60 kg N ha^{-1} at Zadoks 37. In Exp. II, the previous crop was rainfed sunflower grown with four levels of fertilizer N: 50, 150, 0 and 100 kg N ha^{-1} for N0, N60, N80 and N140, respectively, which led to contrasting dynamics of N availability. As a consequence, the N60 treatment was more than the simple effect of a late N supply due to the previous treatment with sunflower, so we chose to name it N60+ in order to underline this point. The two experiments (I and II), combined with various N treatments, aimed to cover a wide range of N availabilities, which can be considered as low-N-input systems for durum wheat, a very N-demanding crop (up to 300 kg N ha^{-1} for a 8 t ha^{-1} grain target).

The experimental layout for both experiments was a randomized split-plot design with N application as main plots and crops as subplots, with five replicates (4 for wheat sole crops in N0 and intercrops in N180) in Exp. I and three replicates (5 for pea sole crops) in Exp. II. N treatments and replicates were separated by a barley (*Hordeum vulgare*) strip (6 and 12 m wide in Exp. I and II, respectively) in order to avoid border effects due to N fertilization. Each subplot (5 m x 1.84 m) consisted of 11 rows spaced 14.5 cm apart. Seeds were sown using a 6-row pneumatic precision experimental prototype drill with 29 cm row separation. Sowing was done in two passes by moving to the right (14.5 cm) for the second pass and by blocking one row of the drill. The intercrop treatment consisted of 6 rows of wheat and 5 rows of pea spaced 14.5 cm apart, with alternate wheat and pea rows.

Fungicide-treated seeds were sown on November 8, 2005 (Exp. I) and on November 9, 2006 (Exp. II). In Exp. II, 20 mm of irrigation water was applied after sowing because of the low water content in the topsoil. Weeds were controlled with a mixture of trifluraline (900 g ha^{-1}) and linuron (450 g ha^{-1}) before emergence. Diseases and green aphids were controlled as much as possible with appropriate pesticides.

Measurements and analysis

The number of seedlings in four rows of 1 m length was counted 1 month after emergence.

Crop samples taken from 0.5 m² (7 rows, 1.015 m total width, 0.5 m long) were harvested by cutting plants just above the soil surface at: i) the beginning of pea flowering (BPF) (1104 °C d^{-1} after wheat emergence (AWE) in Exp. I and 1281 °C d^{-1} AWE in Exp. II), coinciding with ‘flag leaf visible’ stage of wheat (Zadoks 37) and ii) at wheat flowering (WF; Zadoks 69) coinciding with the end of pea flowering (1401 °C d^{-1} AWE in Exp. I and 1746 °C d^{-1} AWE in Exp. II). At maturity, plots were mechanically harvested to determine total grain yield. pea sole crops were harvested at pea physiological maturity (1938 °C d^{-1} AWE in Exp. I and 2143 °C d^{-1} AWE in Exp. II) while wheat sole crops and intercrops were harvested at wheat physiological maturity (Zadoks 92; 2429 °C d^{-1} AWE in Exp. I and 2824 °C d^{-1} AWE in Exp. II). Outside rows (2 rows on each side of the plot) were not harvested in order to avoid border effects.

Samples were divided into pea and wheat and into grain and straw and dried at 80 °C for 48 h. At crop maturity, DW, yield, N and ^{15}N excess of straw and grain were determined on 150 wheat straws (ears) and 20 pea plants, allowing the calculation of harvest index, N harvest index and grain protein concentration. ^{15}N excess and total-N accumulated in shoots were also measured at the BPF and at WF. Total N and C were analyzed in sub-samples of finely ground plant material using the Dumas combustion method with a Leco-2000 analyser (LECO Corporation, St. Joseph, USA). ^{15}N concentration was determined using an elemental analyzer (Euro-EA, Eurovector, Milan, Italy) coupled to a mass spectrometer (Delta advantage, Thermo-Electron, Bremen, Germany).

Soil samples (0-120 cm depth) were collected with a hydraulic coring device with a 15-mm diameter auger (MCL3, Geonor, Oslo, Norway) a few days after sowing on November 14, 2005 (Exp. I) and on November 15, 2006 (Exp. II) and shortly after harvest on July 8, 2006 (Exp. I) and July 19, 2007 (Exp. II). Soil cores were divided into four layers: 0 to 30, 30 to 60, 60 to 90, and 90 to 120 cm. For each sample, five soil cores were taken at a distance of 1 m from each other to take into account soil variability. The five corresponding cores were then pooled before determining water content and mineral-N analysis. Soil mineral N content was determined after KCl (*1 M*) extraction by colorimetric reactions (Griess and Berthelot reactions for nitrate and ammonium, respectively) in a continuous flow autoanalyzer (Skalar 5100, Skalar Analytic, Erkelenz, Germany).

Calculations

The data used to calculate N balances are shown in Table 1. Mineralization of N residues, humus N mineralization and N leaching over the growing period were estimated using the STICS soil-crop model (Brisson et al. 2008) and parameter values recently proposed by Justes et al. (2009) for mineralization of N residues. Mineral N available ($N_{available}$) was estimated for the two experiments as follows:

$$N_{available} = InitialNmin + Nmineralization - Nleaching + N \times FUE$$

with FUE (apparent Fertilizer-N Use Efficiency) calculated as follows:

$$FUE = \frac{\left(Nac_{W-SC(N)} - Nac_{W-SC(N0)}\right) - \Delta InitialNmin_{N-N0} - \Delta Nmineralization_{N-N0} - \Delta Nleaching_{N-N0}}{N}$$

where $Nac_{W-SC(N)}$ is the N accumulated by the wheat sole crop with N fertilization and $Nac_{W-SC(N0)}$ without N fertilization; Δ is the difference between fertilizer-N and N0 treatments for: i) initial mineral N in soil ($\Delta InitialNmin_{N-N0}$), ii) net N mineralization from humus plus residues - which could lead to N immobilization - ($\Delta Nmineralization_{N-N0}$) and iii) nitrate leaching below 120 cm depth ($\Delta Nleaching_{N-N0}$).

The percentage of plant N derived from N_2 fixation (%Ndfa) was determined using the ^{15}N natural abundance method for un-fertilized treatments (Amarger et al. 1979; Unkovich et al. 2008). In N-fertilized intercrops treatments a similar approach was used with some adaptation, i.e. taking into account as a reference crop the durum wheat in the intercrops fertilized at the same rate, making the rather dubious assumption that pea can take up the same mineral N in soil as durum wheat by exploring the same soil volume. The %Ndfa in sole cropped and intercropped pea was calculated using the natural variation in ^{15}N abundance expressed in terms of δ units, which are the parts per thousand (‰) deviation relative to the nominated international standard of atmospheric N_2 (0.3663% of ^{15}N), for pea ($\delta^{15}N_{pea}$) and for a reference crop ($\delta^{15}N_{ref}$). The correction factor β reflecting the $\delta^{15}N$ of legume shoots that are fully dependent upon N_2 fixation was assumed equal to be -1‰ for pea according to Voisin et al. (2002). In this way it is possible to determine the degree of isotopic discrimination between the stable isotopes ^{14}N and ^{15}N to calculate the %Ndfa according to the equation provided by Shearer and Kohl (1986):

$$\%Ndfa = 100 \times \left(\frac{\delta 15N_{ref} - \delta 15N_{pea}}{\delta 15N_{ref} - \beta} \right)$$

The calculation assumes that the $\delta^{15}N_{ref}$ provides a suitable measurement of the $\delta^{15}N$ of soil mineral N available for pea (Peoples et al. 2001; Unkovich et al. 2008). At wheat flowering and pea physiological maturity, the %Ndfa was calculated using as reference the average value between intercropped wheat harvested at wheat flowering and that harvested one month later at wheat physiological maturity. For the unfertilized treatments we also used a non-fixing mutant of pea (P2 cv. Frisson) as reference crop. We considered each N treatment separately in order to take into account the effect of N fertilizer on the $\delta^{15}N$ of soil mineral N. To eliminate variations due to soil heterogeneity over short distances we took as $\delta^{15}N_{ref}$ the average of all the replicates of the intercropped wheat harvested at wheat flowering and of all the replicates of the intercropped wheat harvested at wheat physiological maturity and only one value for pea Frisson which did not grow very well (and with a developmental shift in comparison with cv. Lucy).

Finally, N accumulated from air (QNdfa) was calculated as the product of accumulated shoot N and %Ndfa.

The land equivalent ratio (LER) is defined as the relative land area required when growing sole crops to produce the dry weight or yield achieved in intercrop (Willey 1979a). Dry weight LER for a wheat-pea intercrop is the sum of the partial LER values for wheat (LER_{DW-W}) and pea (LER_{DW-P}), in accordance with De Wit and Van Den Bergh (1965):

$$LER_{DW-W} = \frac{DW_{W-IC}}{DW_{W-SC}}$$

$$LER_{DW-P} = \frac{DW_{P-IC}}{DW_{P-SC}}$$

$$LER_{DW} = LER_{DW-W} + LER_{DW-P}$$

where DW_{W-IC} and DW_{P-IC} are the intercrops (IC) dry weight per unit area for wheat and pea, respectively; DW_{W-SC} and DW_{P-SC} the dry weight per unit area achieved in sole crops (SC) for wheat and pea, respectively. LER_{DW} was calculated separately for each IC replicate using the replicate values of DW for the numerators and the mean sole crops values across all replicates for the denominators to eliminate the variation in the ratio attributed to sole crop DW variability. Moreover, for LER_{DW-W} we considered the same N treatment for the intercrops and the sole crops while LER_{DW-P} was calculated with the unfertilized pea sole crop as reference because we hypothesized that N is not a limiting resource for legumes and did not affect pea DW. A value of LER_{DW} higher than 1 indicates an advantage to intercrop in terms of improved use of environmental resources (light, carbon, water and N) for plant DW growth. Conversely, when LER_{DW} is lower than 1, it indicates that resources are used more efficiently by sole crops than by intercrops. Moreover, partial LER_{DW} values for wheat and pea can be compared with 0.5 because in intercrop each species is sown at half of the sole crops densities. As a consequence, a partial LER_{DW} above 0.5 indicates that a mixed crop produces more than a sole crop (on a row or plant basis), and vice versa when partial LER_{DW} is below 0.5. By analogy, we calculated the LER by considering the grain yield (Y) and, in order to evaluate the complementary N use between the crops, the accumulated N. We then chose to name them LER_Y and LER_N, respectively.

Statistics

Analysis of variance was carried out using the AOV procedure of the 2.7.1 version of R software (R development Core Team 2007) for each year, considering N treatments as the main factor, crops as a sub-factor and interaction between N treatments and crops. All data were tested for normal distribution using the Shapiro–Wilk test and pairwise comparisons were performed using a two-tailed t-test (P=0.05 or P=0.10) to compare N treatments within crops and crops within N treatments. According to Sheskin (2004), the significance of differences between treatments can be estimated using simple planned comparisons when comparisons have been planned beforehand, regardless of whether or not the omnibus F value is significant. Correlation coefficients calculated from linear regressions were statistically analysed using the table proposed by Fisher and Yates (1938). Finally, confidence intervals for the means of LER values and partial LER values were calculated from replicates assuming normal distribution according to Sheskin (2004) in order to compare the means of LER with 1 and partial LER values with 0.5.

Results

N availability according to treatments

Apparent N available depended greatly on the preceding crops and the differences in their N treatments, experimental N fertilization, N fertilizer efficiency, soil N mineralization (soil + crop residues), initial N mineral content and weather conditions. In Exp. I, soil N mineral content at sowing was 37 kg N ha^{-1} on average for all N treatments, while in Exp. II it was ca. 30 kg N ha^{-1} for N0 and N80 and ca. 50 kg N ha^{-1} for N60+ and N140 (Table 1). Considering the whole growing period, apparent N fertilizer-use efficiency (FUE) was ca. 63% for N100 and N180 in Exp. I and 11%, 58% and 56% for N60+, N80 and N140 in Exp. II, respectively.

The mineralization simulated using STICS soil-crop model indicated that ca. 50% of residues and humus net N mineralization would have occurred between sowing and BPF and the other 50% between BPF and harvest due to increasing soil temperature. Throughout the growing period, residues and humus net N mineralization calculated in Exp. I were lower in N0 than in N-fertilized treatments, due to a lower soil organic N content. In Exp. II, net N mineralization calculated was lowest for N0 and N80, highest for N60+ and intermediate for N140.

Finally, apparent N available over the whole growing period was lowest for N0 for both experiments (ca. 92 kg N ha^{-1}), highest for N180 (223 kg N ha^{-1}) and intermediate for N60+ and N80 (ca. 147 kg N ha^{-1}) and for N100 and N140 (ca. 170 kg N ha^{-1}). N treatments differed also in the N availability dynamics; indeed, apparent N available calculated from sowing to BPF represented 46% of apparent N available over the growing period for N100, 58% for N180, 65% for N0 and N60+ and 90% for N80 and N140.

Finally, residual soil mineral N content measured at harvest on 120 cm depth was different between treatments (Table 1). Without N fertilizer, pea sole crop soil mineral N at harvest was significantly higher than that of the intercrop itself higher than that of the wheat sole crop. No difference was found between intercrop and wheat sole crop for N60+ and N80 while mineral N content at harvest was higher by 10 kg N ha^{-1} on average in intercrop than in wheat sole crop for N100 and N180 (Exp. I) and for N140 (Exp. II).

N complementarities in intercrop

N acquisition and N accumulation in shoots

As expected, sole cropped wheat N uptake and then N accumulation in shoots was positively correlated with N fertilization in both experiments (Fig. 1). Similar results were obtained for the intercropped wheat in Exp. I, while in Exp. II the maximum N uptake was obtained with N60+ and the minimum with N0. Without N fertilizer, sole cropped pea always accumulated significantly ($p<0.10$) more N than the sole cropped wheat and than the whole intercrop. In N-fertilized plots, the whole intercrop accumulated more N than the sole cropped pea in Exp. I, but less or a similar amount in Exp. II, due to the decrease in the intercropped pea's apparent accumulated N. The intercrop as a whole always acquired more N than the sole cropped wheat and the difference was reduced and became non-significant with the increase in N availability (N140 and N180). The intercropped wheat accumulated more than 50% as much N as the sole cropped wheat (70% and 78% on average for Exp. I and II, respectively). The higher the N availability, the larger was the difference between intercropped and sole cropped wheat. Finally, intercropped pea N acquisition was reduced with N fertilization compared to N0 except in Exp. I where the maximum was in N100. Moreover, in Exp. II no difference was found between N treatments for pea N accumulated. On average, for all N treatments, crops and years, N harvest index was 0.58 for wheat and 0.76 for pea. In Exp. I, wheat N harvest index was 0.75 for both sole crop and intercrop while in Exp. II it was 0.66 for sole cropped wheat and only 0.58 for intercropped wheat. N harvest index of the intercropped pea was ca. 0.78 whatever the N treatment and experiment while sole cropped pea N harvest index was 0.73 and 0.64 for Exp. I and II, respectively.

N_2 fixation of pea

We clearly observed that in our experiments, soil heterogeneity and N-fertilization affected $\delta^{15}N_{ref}$ more than the choice of crop reference or stage of sampling (Table 2). Indeed, we found that the non-fixing pea Frisson $\delta^{15}N$ was similar to that of the intercropped wheat in N0. No difference was found between intercropped wheat $\delta^{15}N$ at flowering and at maturity (Table 2). Moreover, intercropped wheat $\delta^{15}N$ was reduced with N fertilization compared with N0, except for N60+ in Exp. II, while no significant difference was found in N-fertilized treatments. The values of sole cropped pea $\delta^{15}N$ were slightly lower in Exp. II than in Exp. I and no difference was found between the two sampling dates for both experiments.

The calculated percentage of total above-ground N acquisition derived from N_2 fixation (%Ndfa) of the intercropped pea calculated was higher than that of the sole cropped pea for all N treatments (on average 85% and 64%, respectively in Exp. I and 75% and 52%, respectively in Exp. II). In Exp. I, the %Ndfa of the intercropped pea was almost the same in N-fertilized plots and in N0 while in Exp. II, there was a large difference between the N treatments. A key point is that in Exp. II, N fertilization applied at the 'visible flag leaf' wheat stage (N60+), corresponding to the beginning of pea grain filling, seems not to have affected the legume %Ndfa compared with the unfertilized treatment (85 and 84%, respectively). Conversely, N fertilization (80 kg N ha^{-1}) applied earlier at the beginning of wheat stem elongation (N80 and N140 in Exp. II) seems to have reduced the %Ndfa compared with N0 (60% for N80 and 70% for N140)[0].

Finally, the quantity of above-ground N accumulated derived from air (QNdfa) was maximum for the sole cropped pea and greater in Exp. I than in Exp. II (Table 1). In Exp. I, the QNdfa of the intercropped pea was greater in N100 than in N0 and N180.

On the other hand, in Exp. II, the QNdfa of the intercropped pea was the highest for N0, intermediate for N60+ and the lowest for N80 and N140.

Land equivalent ratio for N accumulated in shoots (LER_N)

LER values calculated from shoot N accumulation (LER_N) were always greater than 1, i.e. 1.15 on average for all N treatments and experiments, indicating an advantage of intercrops compared with sole crops for N accumulation (Fig. 2a). However, LER_N were lower when a large amount of N fertilizer was applied (1.08 for N140 in Exp. I, 1.06 for N80 and 0.88 for N140 in Exp. II) compared with N0 (1.32 and 1.16 in Exp. I and II, respectively). Wheat partial LER_N values were always greater than 0.5, i.e. 0.73 and 0.78 on average for Exp. I and II, respectively. On the other hand, pea partial LER_N values were close to or less than 0.5 (0.48 and 0.31 on average for Exp. I and II, respectively). Wheat partial LER_N values were the highest for N0 in Exp. I and for N60+ in Exp. II and lowest in Exp. I for N100 and N180 and for N0 and N140 in Exp. II. Finally, pea partial LER_N values were slightly affected by N fertilization in Exp. I compared with N0 while values were significantly reduced with N fertilization in Exp. II (0.26) compared to N0 (0.46).

Intercropping dry weights and yields and wheat grain quality

Dry weight (DW) and yield (Y)

Our results indicate that intercrops shoot biomass dry weight (DW) and yield depended on N availability (Fig. 3). On average, for all N treatments and crops, harvest index was 0.43 for wheat and 0.52 for pea. For both sole cropped and intercropped wheat, harvest index was 0.45 and 0.41 for Exp. I and II, respectively. Sole cropped pea harvest index was 0.49 and 0.47 in Exp. I and II, respectively, while intercropped pea harvest index was 0.52 and 0.54 in Exp. I and II, respectively and on average for all N treatments.

The sole cropped and intercropped wheat DW and yield were significantly ($p<0.10$) increased by fertilizer N in Exp. I (Fig. 3). In Exp. II, sole cropped wheat DW and yield were significantly increased ($p<0.10$) from N0 to N80, while intercropped wheat DW and yield were highest in N60+ and clearly lowest in N0. For both experiments, intercropped pea DW and yield were significantly reduced with N fertilization ($p<0.10$), mostly when large amounts were applied (N180 in Exp. I and N140 in Exp. II). Thus, in Exp. I, total intercrop DW and yield were increased when fertilizer N was applied. In Exp. II, total intercrop DW and yield were the highest in N60+ and, surprisingly, the lowest in N140. Finally, wheat and pea sole crops DW and yield were always significantly higher ($p<0.10$) than their corresponding intercrop DW and yield, but seemed lower than the total intercrop DW and yield for treatments with little or no N fertilizer (N0, N60+ and N100). Conversely, increasing the amount of fertilizer N

(N180 in Exp. I, N80 and N140 in Exp. II), the sole cropped wheat produced significantly more DW and yield than the whole intercrop (p<0.10).

Dry weight and yield land equivalent ratios (LER_{DW} and LER_{Y})

LER values calculated from shoot biomass dry weight (DW) produced at harvest (LER_{DW}) were approximately 1 or more in all treatments (p<0.05) except for N180 where it was significantly (p<0.05) less than 1 (Fig. 2b). This indicates that resources were used for DW production up to 17% more efficiently in intercrops than in sole crops in low-N conditions. On the whole, LER_{DW} values were reduced with increasing N fertilization, particularly for treatments N180 (Exp. I) and N140 (Exp. II). For all N treatments, wheat partial LER_{DW} values ($LER_{DW\text{-}W}$) were always above 0.5 (p<0.05) and not significantly different from 0.5 (p>0.10) for N0 and N140 in Exp. II. On the other hand, $LER_{DW\text{-}P}$ values were always equal to or significantly below 0.5 (p<0.05).

LER_{Y} were 1.19, 1.17 and 1.01 for N0, N100 and N180, respectively in Exp. I and 1.19, 1.11, 0.92 and 0.75 for N0, N60+, N80 and N140, respectively in Exp. II (Fig. 2c), indicating that resources were finally used more efficiently in intercrops for yield when little or no N fertilizer was applied. Partial $LER_{Y\text{-}P}$ were 0.49 and 0.64 in N0 in Exp. I and II, respectively and only 0.36 and 0.23 for N180 and N140, respectively while partial $LER_{Y\text{-}W}$ were always about 0.5 or more (p<0.05).

The advantage of intercrops over sole crops was greater for N accumulation than for yield or DW, as already mentioned. Indeed, considering all the N treatments and experiments, LER values were 1.15 on average for LER_{N}, but only 1.02 and 1.05 for LER_{DW} and LER_{Y}, respectively. On average, wheat partial LER values were higher for N than for DW or yield (0.76, 0.63 and 0.62, respectively), while pea partial LER values were higher for yield (0.43) than for N (0.38) or DW (0.39).

Intercropping advantage for wheat grain protein concentration

Wheat grain protein concentration was on average 13% (Exp. I) and 15% (Exp. II) higher (p<0.05) in intercrops than in sole crops (Fig. 4) except for N180 (Exp. I). On average for both experiments, the linear regression (Fig. 4) indicates that the lower the sole crop grain protein concentration in N0, the greater was the increase in intercrop wheat grain protein concentration. Both sole cropped and intercropped wheat grain protein concentration were higher in N-fertilized plots compared with N0. The late split of N (N60+) in Exp. II resulted in a large increase in wheat grain protein concentration compared with N0 (28% in sole crop and 24% in intercrop) and a similar result was found for N140 in Exp. II (49% in sole crop and 37% in intercrop). On the other hand, the single early split of N (N80) in Exp. II had a small effect on wheat grain protein concentration compared with N0 (10% and 16% for sole cropped and intercropped wheat, respectively). In Exp. I, the increase in wheat grain protein concentration compared with N0 was about 64% and 27% for sole cropped and intercropped wheat, respectively on average for N100 and N180.

Functional relationships

LER values of intercrops for yield (LER_Y) were strongly negatively correlated ($p<0.01$) with N accumulated by the whole intercrop at the beginning of pea flowering (Fig. 5a). This was mainly due to the significant reduction of partial LER_Y values of pea (LER_{Y-P}) with N accumulated by the intercrop ($p<0.01$), while partial LER_Y values of wheat (LER_{W-P}) remained stable whatever the N accumulated by the whole intercrop ($p>0.10$). Similar results were found when plotting LER_Y and partial LER_Y values with mineral N available until BPF (Fig. 5b). As an interesting result, the two regressions obtained in Figs 5a and 5b indicate that LER exceeded 1 when the N accumulated in intercrop or the early mineral-N available was less than 120 kg N ha^{-1}.

On the other hand, LER_Y was slightly positively correlated ($p<0.05$) with the percentage of plant N derived from N_2 fixation of the legume (Fig. 5c) while LER_{Y-W} and LER_{Y-P} were not correlated with the %Ndfa ($p>0.10$). When considering the amount of atmospheric N acquired by pea (Fig. 5d) a significant positive correlation was observed with LER_Y and LER_{Y-P} ($p<0.05$), but not for LER_{Y-W} ($p>0.10$).

Finally, for both experiments and all N treatments, there was a negative correlation between wheat yield and wheat grain protein concentration for a given N level (Fig. 6). In Exp. I, correlations were highly significant for N0 ($p<0.05$) (Fig. 6a), but not for the N-fertilized treatments ($p>0.10$). In Exp. II, correlations were significant for N0 ($p<0.01$), N80 ($p<0.01$) and N140 ($p<0.05$) (Fig. 6b) and seemed to become weaker as N availability increased.

Discussion

N complementarity in intercrop (IC)

As expected, sole cropped wheat N accumulation was positively correlated with N availability (amount of soil mineral N and fertilizer N) and the intercropped wheat accumulated more than 50% more N than the sole cropped wheat. This confirms that the cereal had access to a greater proportion of soil inorganic N when intercropped as compared with the sole cropping situation, supported by the increase in the percentage of plant N derived from N_2 fixation (%Ndfa) of pea which agrees with several other studies (e.g. Corre-Hellou 2005; Hauggaard-Nielsen et al. 2003; Corre-Hellou and Crozat 2005). Hence, due to the complementary use of N sources by intercrop components, N accumulated by the whole intercrop was only slightly affected by N fertilization.

The calculations of %Ndfa and the choice of reference crop must be analysed carefully (Shearer and Kohl 1986). In order to evaluate the quality of %Ndfa estimation, a sensitivity analysis of the calculation was carried out using i) a non-fixing pea, characterized by very low DW production and early physiological maturity, or ii) the intercropped wheat and iii) two stages of plant sampling. This analysis indicated that the $\delta^{15}N$ difference remained the same between intercropped and sole cropped pea and between stages. Thus the %Ndfa of the intercropped pea can be assumed to be always higher than that of the sole cropped pea even if absolute values of calculated %Ndfa are debateable. Indeed, we observed that the variability of $\delta^{15}N$ values within a crop stage was similar to that between stages for both wheat and pea in sole crops or intercrops due to i) soil heterogeneity over short distances, ii) crop dynamics and iii) variability in chemical analysis due to sampling. We can assume that the mean of the $\delta^{15}N$ values measured at the two stages (wheat flowering and wheat maturity for wheat and WF and pea maturity for pea) was a better estimate of the real value of crop $\delta^{15}N$ than when considering stages separately due to spatial heterogeneity and plant sampling bias, as recommended by some authors (e.g. Peoples et al. 2001).

A second critical point concerns the calculations of the pea %Ndfa in N-fertilized treatments considering intercropped wheat for the same treatment as the reference plant. This assumption means that wheat and pea used the same proportion of fertilizer-N and soil mineral N. This hypothesis is certainly debatable because of: i) the localization and dynamics of the fertilizer-N in the soil, ii) the interaction between soil mineral N content and symbiotic fixation, iii) soil heterogeneity and iv) differences in crop dynamics. Moreover, $\delta^{15}N$ of the N fertilizer is very important; it was -0.4 ± 0.1‰ in Exp. II which agrees with the decrease observed in the $\delta^{15}N$ values of wheat in N-fertilized treatments (N applied early) compared with N0. The $\delta^{15}N$ of the N fertilizer was not measured in Exp. I, but it must have been negative judging by the decrease in wheat $\delta^{15}N$ value in N-fertilized plots; an analysis of the same type of fertilizer in the following year indicated a $\delta^{15}N$ value of -0.9 ± 0.1‰. This confirms that the ^{15}N natural abundance method is not very suitable when N fertilizer is applied, even though in our experiment the differences in calculated

%Ndfa were in good agreement with the total N content of plants. A multi-enrichment technique using labelled ^{15}N application must therefore be carried out in these situations for obtaining a more precise estimate of legume %Ndfa (Salon C, pers. comm.).

Durum wheat-winter pea intercrops seems to be more efficient than sole crops to improve N use, particularly in low-N systems (Hauggaard-Nielsen et al. 2006), although some other results only showed a small benefit from intercrops (Jensen 1996; Andersen et al. 2004). In particular, intercrops seems more stable over the years than sole crops for N accumulation. Indeed, whatever the N treatments and experiments, N accumulated by the whole intercrop was less variable than by sole crops. Moreover, intercrops appeared more efficient than sole crops for the use of N sources due to the complementary use of soil mineral N and the increase in the %Ndfa of the intercropped pea when the soil mineral N content was low (<30 kg N ha^{-1} for 0-30 cm depth, in agreement with sole cropped pea results obtained by Voisin et al. (2002)) during early intercrop growth (until the booting stage of wheat). Indeed, N fertilization (80 kg N ha^{-1}) applied at the beginning of wheat stem elongation clearly lead to a decrease in %Ndfa. However, when N fertilizer was applied later, at the 'visible flag leaf' wheat stage, corresponding to the beginning of pea grain filling, no reduction was observed in the %Ndfa. This is in keeping with: i) the strong decrease in N_2 fixation activity after the beginning of pea pod filling (Vocanson et al. 2005), ii) the slower N accumulation in later stages of growth (Vocanson et al. 2005) and iii) the increase in weevil damage on nodules observed in Exp. I, also noted by other authors (Corre-Hellou and Crozat 2004).

The complementary use of N sources by intercrop components was particularly efficient for the unfertilized treatment indicating that intercropping is well adapted to low-N-input systems. Moreover, the soil mineral N content at harvest was similar for the sole cropped wheat and the intercrops, confirming that intercropping is as efficient as wheat in using soil mineral N. Finally, intercropping could reduce i) nitrate leaching compared to sole cropped pea due to its lower soil mineral N content at harvest and ii) gaseous N losses, by reducing the use of fertilizer N.

Intercropping production

The LER can be considered as an indicator of crops resource use for plant growth all over the growing season. In our experiments, resources (light, CO_2, water, nutrients and N) were used up to 17% more efficiently in intercrops than in sole crops for DW production in low-N conditions. Our results show that wheat took advantage of intercropping by using available resources more efficiently than pea, regardless of N availability. Moreover, wheat benefited from N fertilization indirectly by the increased growth of the wheat improving light and water captures ability and then suppressing pea growth (Ghaley et al. 2005).

The yield of wheat depends heavily on N supply as already observed for many cereals (e.g. Gate 1995; Jeuffroy and Bouchard 1999; Le Bail and Meynard 2003), and consequently N fertilization increased total grain yield of intercrops due to its strong effect on wheat yield, which exceeded the reduction in pea yield. Hence the yield of the whole intercrop was always at least to the same as that of the sole crops, except when a large amount of N was applied. LER values calculated from yield (LER_Y) indicates that resources were used up to 20% more efficiently for yield production in intercrops compared with sole crops when little or no N fertilizer was applied. The negative effect of N fertilization was mainly due to the reduction of pea shoot biomass and yield corresponding to a reduction in N_2 fixation. This confirms that intercropping efficiency depends mostly on the complementary use of N between crops and the capacity of the legume to increase the rate of N_2 fixation (%Ndfa) for its N nutrition which is enhanced by the fact that the advantage of intercrops compared with sole crops was greater for N accumulation than for yield.

Functional relationships

The intercrop efficiency for grain production was estimated by LER_Y and partial LER_Y values. LER_Y and $LER_{Y\text{-}P}$ were negatively correlated with N accumulated by the intercrop at the beginning of pea flowering. This indicates that, in our experiments, the final efficiency for yield of the whole intercrop and of the intercropped pea were already determined at the beginning of pea flowering even when N was applied later on and whatever the weather conditions from the beginning of pea flowering to harvest. This suggests that is possible to predict the final efficiency of the whole intercrop and of the intercropped pea at this stage. However, in order to manage the intercrops, it would be interesting to determine the final efficiency earlier than at the beginning of pea flowering. We hypothesized that N accumulated by the whole intercrop at beginning of pea flowering depends on mineral N available at beginning of pea flowering. This was confirmed by the similar relation observed when plotting LER_Y and partial LER_Y against early available N. However, this calculation assumes that apparent N-fertilizer-use efficiency was similar for the sole cropped and the intercropped wheat which seems reasonable since N-fertilizer-use efficiency depended mostly on the weather conditions when N fertilizer was applied which can lead to N losses by volatilization. It is well known that N-fertilizer-use efficiency also depends on crop N demand in relation to physiological stage and varies according to the crop growth rate (Limaux et al. 1999). However, we can assume that N demand of the whole intercrop and of the intercropped wheat were fairly similar in early stages due to row intercropping where plant competition would be almost the same within the row in sole crops and intercrops until stem elongation. Hence, our results confirm that early available N strongly determines the performance of the intercropped pea and of the whole intercrop in comparison with sole cropping situation, but does not significantly modify the growth of intercropped wheat. These results are in keeping with the fact that intercropping efficiency, estimated for total grain production (LER_Y), was increased when the %Ndfa of pea increased and more specifically when the amount of N derived from air was increased. As a first estimate, in our conditions, early mineral N available or N accumulated in intercrops at beginning of pea flowering must be lower than 120 kg N ha^{-1} to observe an advantage for yield.

It is well known that wheat grain protein concentration depends not only on the amount of N fertilizer but also on N splitting (e.g. Gate 1995), partly due to smaller N losses (Limaux et al. 1999). This was confirmed by the late split of N (N60+ treatment) in

Exp. II which resulted in a large increase in wheat grain protein concentration for both sole crops and intercrops. It has been demonstrated by many authors over the last two decades that for sole wheat crops, yield and grain protein concentration are negatively linearly correlated (e.g. Gate 1995). This was confirmed by the negative correlation between wheat yield and wheat grain protein concentration for a given N level, in particular for low N supplies. This result was also observed for the intercropped wheat. Moreover, as N availability increased the correlation became weaker, indicating that N was not a very limiting resource when a large amount of N was applied. As a consequence, it is likely that the higher grain protein concentration in intercropped wheat than in sole cropped wheat can be mainly explained by the reduction in intercropped wheat yield, which was about 40% lower than that of wheat sole crop. However, it must be assumed that wheat grain protein concentration depends on the interaction with N availability. Indeed, only 15% of the N absorbed by the intercropped pea is unavailable for the intercropped wheat which in our conditions represented only ca. 10 kg N ha^{-1} on average for both experiments and all N treatments. It seems also that the N dynamics were altered in intercrops because of the changes in the timing of N_2 fixation of the legume. Moreover, intercrop allowed a better synchrony of wheat N demand and supply due to the changes in wheat growth as a consequence of inter- and intraspecific competition, leading to a reduced number of ears per square metre for the intercropped wheat. Finally, the wheat grain protein concentration was significantly higher in intercrops than in sole crops, because a larger amount of N was remobilized by each plant and ear due to: i) fewer wheat plants, ears and grains per unit area, but ii) with only slightly less available soil N per square metre than for sole crops, so that more N was available for each grain of wheat.

Concluding remarks

Our results confirm that intercropping is more suited to low-N-input systems than to conventional highly fertilized systems. When N fertilizer is applied, the intercropped legume growth and yield were significantly reduced, while wheat was only slightly affected. On the other hand, when there was a shortage of N during early growth, e.g. when little or no fertilizer was applied late to preceding crops, leaving low residual mineral N, there was a marked complementarity between species, in particular for N acquisition. Intercropping efficiency for N use was greatest with low N availability, due to greater N uptake by wheat. This clearly allowed better wheat grain filling due to: i) the high pea N_2 fixation rate in intercrop, making available for the intercropped wheat almost as much soil mineral N per square meter as in the sole crop, ii) fewer wheat plants, ears and grains per unit area in intercrops compared with sole crops and hence iii) a higher efficiency of the cereal to recover N. Our results show that N fertilization of intercrops must be carried out after the end of pea flowering to prevent an adverse effect on N_2 fixation. Moreover when the N fertilization occurs after the end of wheat stem elongation (at the booting stage), the N taken up will be largely remobilized to the grain, causing a significant increase in grain protein concentration.

Our results must also be related to the species complementarity due to differences in their phenology and physiology. It can be postulated that if there are significant complementarities between the crops for the use of natural resources, particularly N, the optimum N fertilization level for the intercrops is probably lower than that of the average of the individual sole crop. This implies that intercropping may be advantageous when little or no N fertilizer is applied due to a high degree of complementary N use between the two species. Such results have been reported for several cereal-legume intercrops grown in arid, semi-arid, tropical and temperate climates (Fujita et al. 1992; Ofori and Stern 1987; Jensen 1996).

Finally, our results confirm that intercropping is a good way to improve the efficiency of N use in agroecosystems, particularly those with a low N availability, because of i) the increase in wheat grain quality, ii) the increase of free atmospheric N input through N_2 fixation and iii) the potential reduction of N leaching after legumes. We believe that it is important to investigate the interspecies dynamics that shape the final outcome of intercropping and more precisely inter- and intraspecific competition throughout the whole growing period. This may reveal dynamics in competition, which is critical to determine when the advantage of intercrop begins. Later on, this will be helpful to optimize these innovative agroecosystems, in particular for the choice of durum wheat and pea cultivar traits suited to intercropping, the ideal proportions of species and N fertilization management.

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Tables

Table 1. Detailed data used for N-balance calculation of the different N treatments (Nx where ‘x’ represents N applied in kg N ha^{-1}) for various periods: from sowing (S) to the beginning of pea flowering (BPF), or BPF to harvest (H) or S to H. Data are: i) characteristics of incorporated residues, ii) topsoil organic N content, iii) 0-120 soil N mineral content at sowing, iv) apparent N-fertilizer-use efficiency, v) apparent N fertilizer available and corresponding N fertilizer applied, vi) simulated N mineralization (humus and residues) using the STICS soil-crop model, vii) simulated N leaching using STICS model, viii) calculated apparent available N and ix) soil N mineral content at 0-120 cm depth at harvest for the intercrops (IC) and the sole crops of wheat (W SC) and pea (P SC).

		2005-2006 (Experiment I)			2006-2007 (Experiment II)			
		N0	N100	N180	N0	N60+	N80	N140
Residus incorporated	Specie	*Sorghum bicolor*			*Helianthus annuus*			
	Date	September 26, 2005			September 25, 2006			
	Mode	20-25 cm tillage			20-25 cm tillage			
	Amount (t ha^{-1})	7	7	7	5	7	4	6
	C:N	63	63	63	49	31	55	40
Soil organic N (g kg^{-1}) on 0-30 cm		0.93	1.09	1.09	1.07	1.07	1.07	1.07
Initial mineral N (kg N ha^{-1}) on 0-120 cm		37	37	37	30	52	28	46
Apparent N fertilizer use efficiency (% of N applied)	S to BPF		18 %	47 %			90 %	104 %
	S to H		62 %	64 %		18 %	72 %	40 %
Calculated efficient N fertilizer (kg N ha^{-1}) and *(N fertilizer applied)*	S to BPF		9 *(50)*	61 *(130)*			72 *(80)*	83 *(80)*
	S to H		62 *(100)*	115 *(180)*		11 *(60)*	58 *(80)*	56 *(140)*
Simulated N mineralization (humus + residues) (kg N ha^{-1})	S to BPF	36	44	44	33	43	32	36
	BPF to H	30	38	40	34	42	35	37
Simulated N leaching (kg N ha^{-1})	S to BPF	13	13	13	3	4	3	4
	BPF to H	0	0	0	0	0	0	0
Calculated apparent N available (kg N ha^{-1})	S to BPF	60	77	129	60	91	129	161
	S to H	90	168	223	94	144	150	171
Measured final mineral N (kg N ha^{-1}) on 0-120 cm	IC	29	46	61	24	25	19	35
	W SC	17	36	50	13	25	15	24
	P SC	43			49			

Table 2. Data of $\delta^{15}N$ values for the different N treatments (Nx where 'x' represents N applied in kg N ha^{-1}): i) ^{15}N excess ($\delta^{15}N$) for a non-fixing pea (Frisson) sole crop (SC), intercropped (IC) wheat, IC pea and SC pea at wheat flowering (WF), wheat harvest (WH) and pea harvest (PH), ii) fraction of plant N derived from air (%Ndfa) of SC and IC pea calculated as the mean of WF and PH using $\delta^{15}N$ average value of wheat at WF and WH and iii) amount of N derived from air (QNdfa) of SC and IC pea at pea harvest. Values are the mean (n=3 to 5) ± standard error.

Data	Crop		Stage	2005-2006 (Experiment I)			2006-2007 (Experiment II)			
				N0	N100	N180	N0	N60+	N80	N140
δ15N	Frisson	SC	WF	5.1***			3.1**			
	Wheat	IC	WF	5.0 ± 0.4	2.0 ± 0.5	2.2 ± 0.6	2.8 ± 0.4	3.0 ± 0.7	1.1 ± 0.7	1.1 ± 0.7
			WH	4.8 ± 0.6	2.5 ± 0.9	1.6 ± 0.4	2.3 ± 0.3	2.4 ± 0.2	1.2 ± 0.2	0.9 ± 0.4
			*Mean**	*4.9 ± 0.6*	*2.3 ± 0.8*	*1.8 ± 0.5*	*2.5 ± 0.4*	*2.7 ± 0.6*	*1.2 ± 0.4*	*1.0 ± 0.6*
	Pea	IC	WF	0.0 ± 0.2	-0.4 ± 0.3	-0.3 ± 0.2	-0.4 ± 0.3	-0.4 ± 0.6	-0.3 ± 0.0	-0.3 ± 0.2
			PH	0.1 ± 0.1	-0.7 ± 0.4	-0.8 ± 0.0	-0.4 ± 0.2	-0.5 ± 0.1	0.1 ± 0.4	-0.5 ± 0.3
			*Mean***	*0.1 ± 0.1*	*-0.6 ± 0.4*	*-0.6 ± 0.2*	*-0.4 ± 0.3*	*-0.5 ± 0.4*	*-0.1 ± 0.3*	*-0.4 ± 0.3*
		SC	WF	1.4 ± 0.4			0.6 ± 0.1			
			PH	1.0 ± 0.4			0.7 ± 0.2			
			*Mean***	*1.1 ± 0.4*			*0.7 ± 0.1*			
%Ndfa	Pea	IC	*Mean***	82 ± 2	87 ± 13	88 ± 5	84 ± 5	85 ± 7	60 ± 9	70 ± 9
		SC	*Mean***	64 ± 6			52 ± 4			
QNdfa	Pea	IC	*Mean***	66 ± 9	85 ± 7	65 ± 2	67 ± 9	42 ± 20	28 ± 7	28 ± 9
		SC	*Mean***	115 ± 29			90 ± 30			

* mean of WF and WH ; ** mean of WF and PH ; *** only one value

Figures

Figure 1. N accumulated (kg N ha^{-1}) in sole crops (SC) and intercrops (IC) of pea (P) and wheat (W) in straw and grain for the different N treatments (Nx where ‘x’ represents N applied in kg N ha^{-1}). Values are means (n=3 to 5) ± standard error for crops N accumulated in straw and grain.

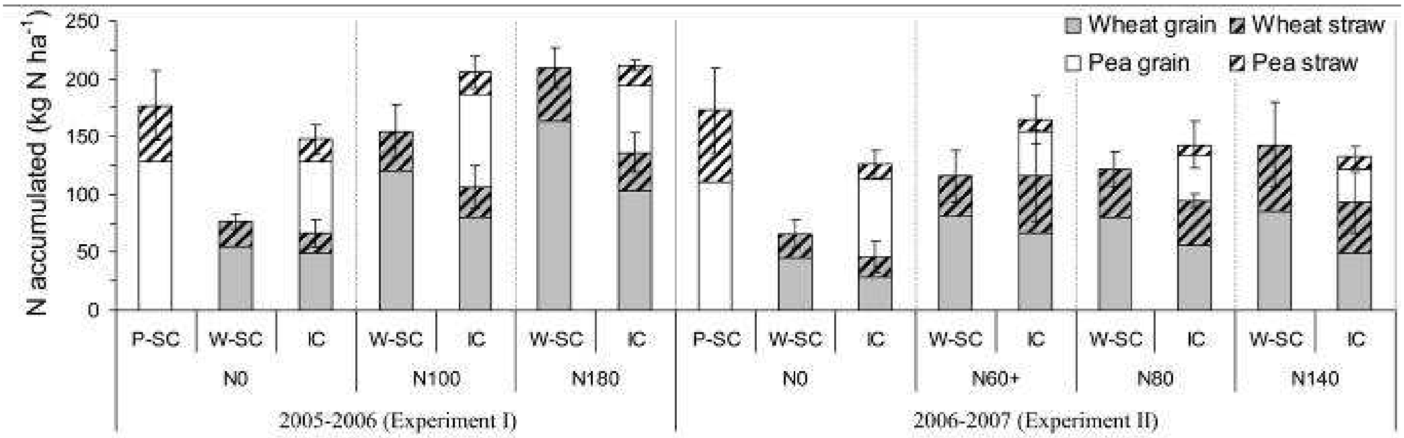

Figure 2. Partial land equivalent ratio (LER) for wheat and pea calculated from (a) N accumulated (LER_N), (b) dry weight (LER_{DW}), (c) grain yield (LER_Y) for the two experiments and N treatments (Nx where 'x' represents N applied in kg N ha^{-1}). Values are the mean (n=3 to 5) ± standard error. Single plus (+) and single asterisks (*) above the bars indicate that LER is significantly different from 1, at P<0.10 and P<0.05, respectively. Single plus (+) and single asterisks (*) inside the bars indicate that partial LER (either for wheat or pea) is significantly different from 0.5, at P<0.10 and P<0.05, respectively; 'ns' indicates non-significant (P>0.10).

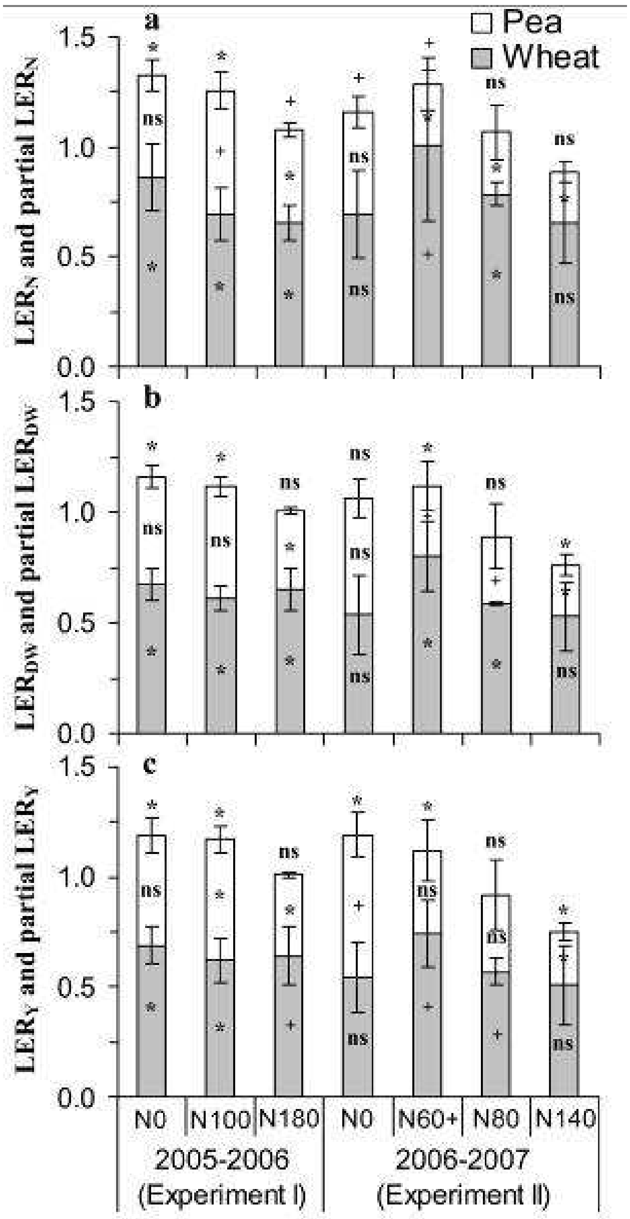

Figure 3. Dry weight (t ha^{-1}) of sole crops (SC) and intercrops (IC) of pea (P) and wheat (W) for straw and grain for the different N treatments (Nx where 'x' represents N applied in kg N ha^{-1}). Values are means (n=3 to 5) ± standard error for grain and for the whole dry weight.

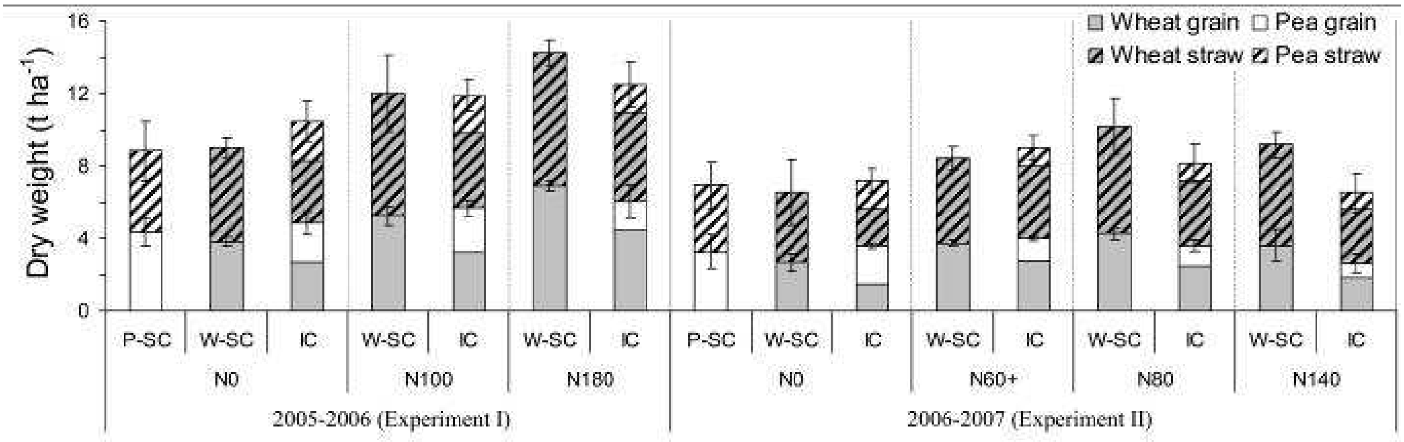

Figure 4. Relationship between grain protein concentration (% of dry weight) of the intercropped (IC) wheat and sole cropped (SC) wheat for the different N treatments (Nx where ‘x’ represents N applied in kg N ha^{-1}) of Exp. I and II. A linear regression was fitted including all N treatments and experiments. Double asterisk (**) indicate that linear regression is significant at P=0.01. Values are means (n=3 to 5) ± standard error. The first bisector y=x and the regression y=1.2x are indicated in order to illustrate the increased range of grain protein concentration in IC compared with SC.

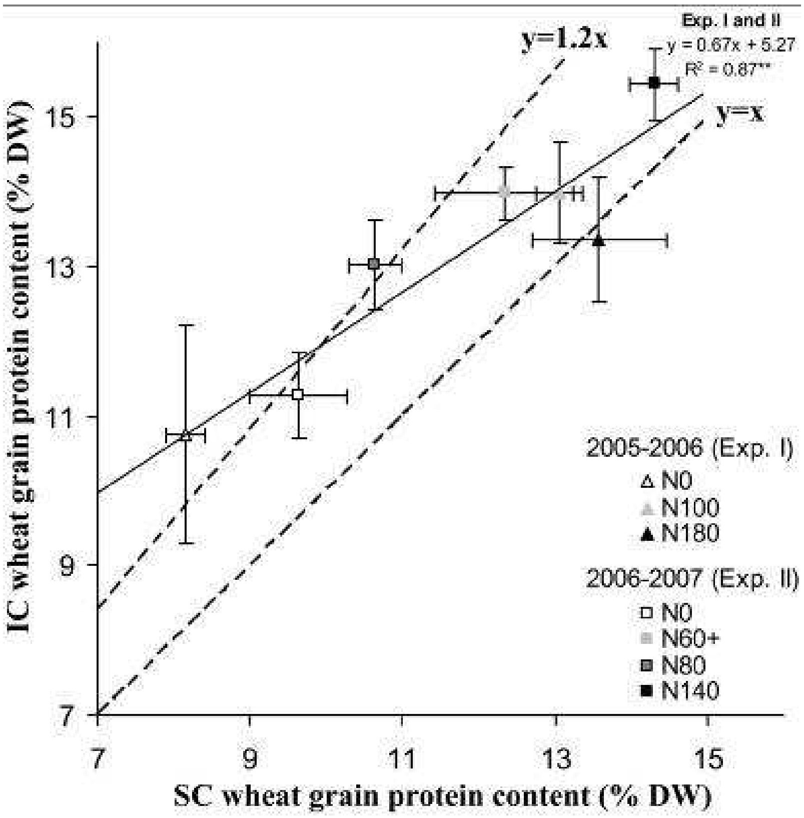

Figure 5. Land equivalent ratio calculated from yield (LER_Y) of the total intercrop (Total IC) and partial LER_Y values of intercropped wheat (Wheat IC) and intercropped pea (Pea IC) as a function of (a) N accumulated by the whole intercrop at the beginning of pea flowering (BPF); (b) mineral N available until BPF (mineral N at sowing + N fertilization applied before BPF + N mineralized from humus and residues until BPF – N leaching until BPF); (c) the percentage of pea N derived from air at physiological maturity and (d) the amount of pea N accumulated from air at physiological maturity (QNdfa). Linear regressions were carried out for LER_Y, $LER_{Y\text{-}W}$ and $LER_{Y\text{-}P}$. Values are the mean (n=3 to 5) ± standard error. Single plus (+), single asterisk (*) and double asterisk (**) indicate that linear regression is significant at P=0.10, P=0.05 and P=0.01, respectively.

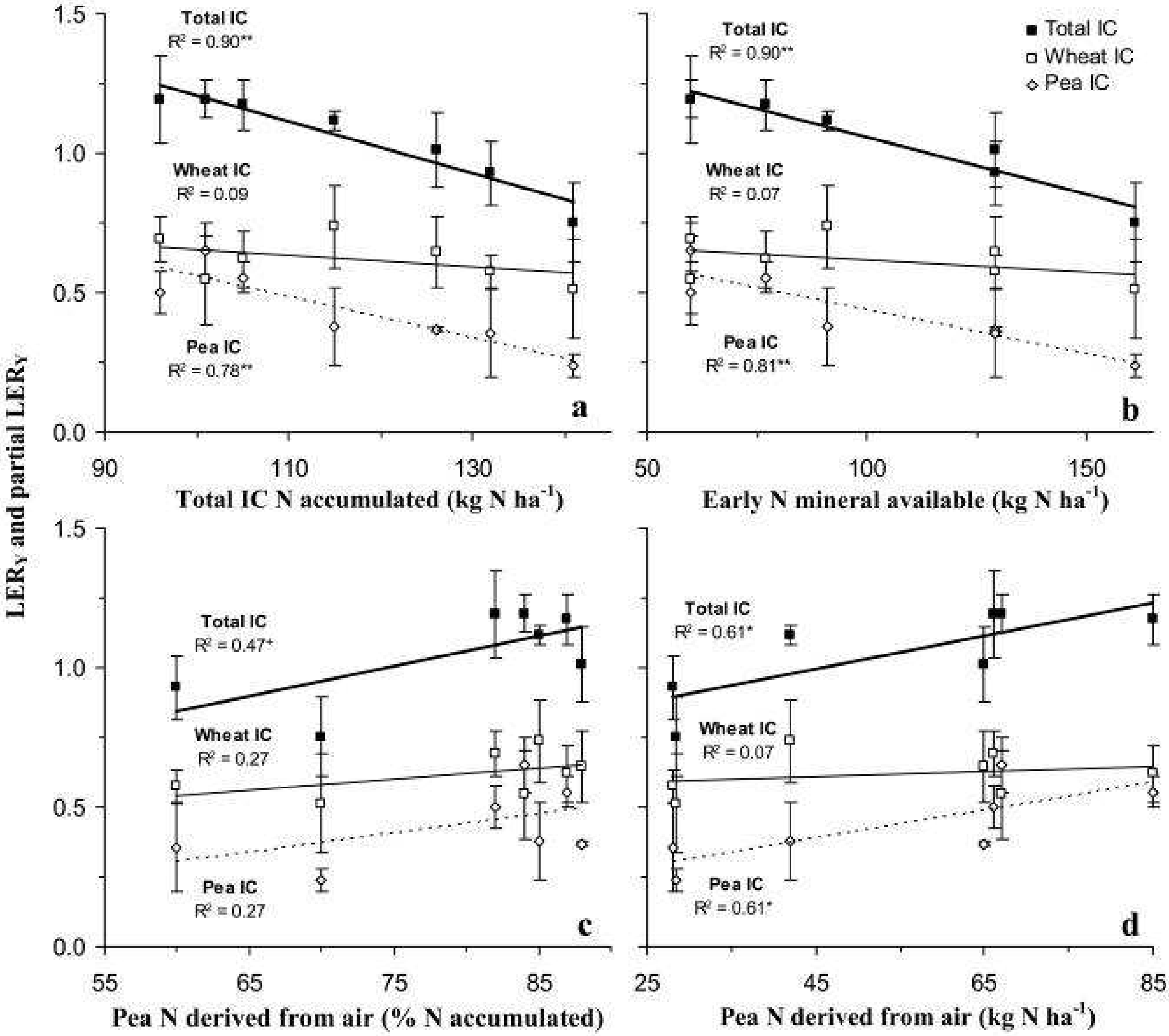

Figure 6. Grain protein concentration of wheat (% of dry weight) as a function of the dry grain yield (t ha^{-1}) for sole cropped (SC) wheat (solid symbols) and intercropped (IC) wheat (open symbols) for the different N treatments (Nx where 'x' represents N applied in kg N ha^{-1}) for Exp. I (a) and Exp. II (b). Linear regressions were carried out for each N treatment, including both sole and intercropped treatments. Single plus (+), single asterisk (*) and double asterisk (**) indicate that linear regression is significant at P=0.10, P=0.05 and P=0.01, respectively.