

Wheat response to nitrogen splitting applied to a Vertisols in different tillage systems and cropping rotations under typical Mediterranean climatic conditions

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Abstract

The application of an adequate rate and splitting of nitrogen (N) is essential for the efficient use of N fertiliser and to maintain the economic sustainability of cropping systems. A 3-year field experiment was conducted on a Vertisol under Mediterranean conditions to determine the effect of tillage systems, crop rotation, and variations in N timing on the grain yield and N recovery of ¹⁵N-labelled fertiliser (NR) in hard red spring wheat (*Triticum aestivum* L.). The experiment was designed as a randomised complete block with a split-split plot arrangement and 3 replications. The main plots were tillage system (no-tillage [NT] and conventional tillage [CT]), and the subplots were the preceding crop with 2-year rotations (wheat-sunflower WS, wheat-chickpea WC, and wheat-faba bean WFB). Sub-subplots were the N timing (all at the same rate of 100 kg N ha⁻¹), and the fertiliser was applied 50% at sowing and 50% at stem elongation and 50% at tillering and 50% at stem elongation. The area of each basic plot was 50 m² (5 m x 10 m). The residual NO₃ –N content (0–90 cm) was significantly higher in CT than in NT, the opposite occurring with grain yield. The NR of NT was greater than that of CT. According to the previous crop, the NR was WS > WFB = WC. The soil nitrate content was twice as much when the preceding crop was a legume compared with sunflower and the wheat yields were as follows: WFB > WC > WS. Although the N timing did not have an effect on overall grain yield,

the interactions with year, tillage system and previous crop were significant. The average recovery of ^{15}N fertiliser by wheat was 44.6%, with 33.7% obtained in the grain (41% in stem elongation, 32% in tillering and 27% in sowing). The habitual use of high rates of N fertiliser and the frequency of dry years in the agrosystem studied generated a progressive accumulation of residual in the soil profile. This can represent an important source of mineral N for the cereal and can reduce the need to apply N fertiliser to the crop.

Keywords: no tillage; soil nitrate; ^{15}N recovery; grain yield; sunflower; chickpea; faba bean

Introduction

Cereal yields are low and vary in response to inadequate and erratic seasonal rainfall under Mediterranean conditions. Available soil water is the principal constraint to the yield potential of wheat and its response to N (Garabet et al., 1998). According to Campbell et al. (1993), there is a strong interaction between the use of soil water and the response of crops to fertilisers in semi-arid regions. The agronomic efficiency of N fertiliser in a Mediterranean climate may be lower than that in temperate zones.

The response of wheat to N fertiliser is also influenced by factors including N fertiliser management, soil type, crop sequence, and the supply of residual and mineralised N. The effect of N fertiliser on cereal during wet years was more marked in wheat rotations without legumes. Conflicting reports exist regarding the N balance and use efficiency of crops grown under no till systems compared with conventional tillage systems. Some researchers have reported that conservation tillage systems have increased fertiliser N rates to prevent yield limitations from short-term N immobilisation (Fageria and Baligar, 2005). However, Torbert et al. (2001) reported that there was no indication of N limitations in no-tillage systems compared to other tillage systems. Several studies reviewed by Hansen et al. (2011) report decreases of N mineralisation in non inverted soils compared with conventionally tilled soils. Direct-drilled crops may therefore require greater N inputs compared with crops in ploughed systems, although Thomsen and Christensen (2007) concluded that conversion from mouldboard ploughing to shallow tillage had little influence on fertiliser N balance. Malhi et al. (2009), Giacomini et al. (2010) and Hansen et al. (2011) have observed that tillage system had little effect on fertiliser N dynamics of the soil in spring cereals.

Over the past several decades, numerous researches have sought to improve nitrogen-use efficiency (NUE) by developing fertiliser management strategies based on a better synchronisation between the supply of N and its requirement by a given crop (Ladha et al., 2005). Many studies have shown that split applications of N fertiliser result in higher rates of plant recovery and higher grain yields than under single applications. However, the proportions of the split should be determined locally, with due consideration of the initial soil fertility (Campbell et al., 1993; Mahler et al., 1994; Stockdale et al., 1997; Recous and Machet, 1999). López-Bellido et al. (2000) maintain that under dry Mediterranean conditions, a small application of N could be used at seeding, and additional N fertiliser could be applied as a top dressing at the end of tillering or at the beginning of stem elongation, depending on the winter rainfall, the previous culture in the rotation, and the amount of residual N in the soil at the end of winter.

The isotopic labelling method provides the most accurate measure of the relative contributions of soil N and fertiliser N to plant uptake. Numerous studies on the split application of N suggest that top-dressing applications in spring, whether during stem elongation and/or tillering (depending on the weather), improve the recovery of N fertiliser and the NUE when compared to application at sowing only (Sowers et al., 1994; Tran and Tremblay, 2000; Blankenau et al., 2002; Jia et al., 2011). Under a Mediterranean climate and in semi-arid regions, the amount of ^{15}N recovered is less than 50% (Garabet et al., 1998 and Grageda-Cabrera et al., 2011). In a study on dry Vertisols in southern Spain, López-Bellido et al. (2005) obtained recovered ^{15}N values that, on average, did not exceed 40% and found that more ^{15}N -labelled fertiliser was recovered following a split N application than after an application of N during the fall.

Many approaches have been suggested for increasing nitrogen-use efficiency (NUE), such as using the optimal time, rate, and method of application for matching N supply with crop demand. Because some of the main factors, such as climate controlling crop needs for N, are largely outside farmer control, it remains difficult to predict the amount of N to apply for optimum growth. Appropriately managing N fertiliser for wheat production in dry areas is critical to obtaining the maximum economic yield and for improving water efficiency. As a consequence, we investigate agronomic factors and strategies of fertiliser N application that we hypothesised may influence the N fertiliser dynamic. The present study sought to determine in hard red spring wheat cultivated on a Mediterranean Vertisol under dry conditions: (i)

fractionation of the N fertiliser rate more appropriate for better fertiliser efficiency and (ii) the effects of tillage system, crop rotation, and variation in N timing on the grain yield and N recovery of ^{15}N -labelled fertiliser.

Material and methods

2.1. Location and experimental design

Field experiments were conducted in Cordoba in southern Spain (37°46. N, 4°31. W, 280 m a.s.l.) on a Vertisol (Typic Haploxererts) typical to the Mediterranean region, where rainfed cropping is the standard practice. The study took place over a 3-year period (2003–2004, 2005–2006 and 2006–2007) within the framework of a long-term experiment named “Malagón”, which started in 1986. The year 2004–2005 was not considered in the study because of bad growing season: only 263 mm of rain, most of which occurred in the fall-winter period, were precipitated in the area causing crop failure.

The properties of the Vertisol used in our field experiments were collected by López-Bellido et al. (1997). The experiment was designed as a randomised complete block with a split-split plot arrangement and 3 replications. The main plots were the tillage system (no-tillage NT and conventional tillage CT), and the subplots were the preceding crop with 2-year rotations (wheat-sunflower WS, wheat-chickpea WC, and wheat-faba bean WFB). The sub-subplots were the N timing by the application of 100 kg N ha⁻¹ at 50% between sowing and stem elongation (50–0–50) and between tillering and stem elongation (0–50–50). The area of each basic plot was 50 m² (5 m x 10 m) with a total size of 1800 m².

2.2. Crop management

The NT plots were seeded with a no-till drill (Great Plains). Weeds were controlled with glyphosate + MCPA at a rate of 0.5 + 0.5 l a.i. ha⁻¹ prior to sowing. Conventional tillage treatment included mouldboard ploughing as well as disk harrowing and/or vibrating tine cultivation to prepare a proper seedbed. Information on cultivar, planting and herbicides applied during the growing season is provided in Table 1. Nitrate values before the start of the experiment were: 131 kg N ha⁻¹ and 123 kg N ha⁻¹ in faba bean rotation, 134 kg N ha⁻¹ and 117 kg N ha⁻¹ in chickpea rotation and 45 kg N ha⁻¹ and 46 kg N ha⁻¹ in sunflower rotation for CT

and NT, respectively. Nitrogen fertiliser only was applied to wheat plots, as ammonium nitrate (34.5% N). When the 50% was applied before sowing, the N was incorporated by disk harrowing in conventional tillage and surface broadcast in no-tillage plots. The remaining N was applied as top dressing at tillering corresponding to stage 21 of Zadoks' scale (Zadoks et al., 1974) and stem elongation (stage 31 of Zadoks' scale) depending on N timing. Every year, wheat plots were also supplied with P fertiliser at a rate of 65 kg ha⁻¹; this was incorporated in conventional tillage following the standard practice and banded with drilling in no-tillage plots. Soil-available K was adequate (530 mg kg⁻¹). Wheat was harvested early in June each year, using a 1.5 m wide Nursemaster elite plot combine. For each plot, a total area of 30 m² was collected from two adjacent strips of 15 m² (1.5 m x 10 m) each.

2.3. Plant and soil analysis

Soil samples were collected at a depth of 0–90 cm prior to wheat sowing. Soils were analysed for nitrate content using the Griess-Illosvay colourimetric method as modified by Barnes and Folkard (1951), with a Bran and Luebbe II Auto Analyser. At harvest, a biomass portion of 0.5 m² at the centre of each wheat sub-subplot was sampled to determine the N content of straw and grain using the Dumas combustion method (Leco FP-428 analyser).

2.4. Labelled nitrogen experiment

72 microplots (1 m × 2 m each) were established within the main experiment area to monitor the uptake of ¹⁵N-labelled fertiliser. Microplots were arranged in a randomised complete block with 3 replications of 4 treatments. All microplots received 100 kg N ha⁻¹, with the following application timings: (1) 50% sowing (¹⁵N-labelled) – 50% top dressing (TD) at stem elongation; (2) 50% sowing – 50% TD at stem elongation (¹⁵N-labelled); (3) 50% TD at tillering (¹⁵N-labelled) – 50% TD at stem elongation, and (4) 50% TD at tillering – 50% TD at stem elongation (¹⁵N-labelled). The data from treatments (1), (2), (3), and (4) were combined to determine the total contribution of sowing-applied and top-dressed N fertiliser to plant N from this application regime. Fertiliser solutions were formulated with ammonium nitrate (34.5% N) and ¹⁵N-enriched ammonium nitrate (2.5 atom% excess ¹⁵N) for sowing and TD applications. Sowing applications were implemented immediately after this, and top dressings were implemented at the stages of tillering (stage 21 of Zadoks' scale) and stem elongation (stage 31 of Zadoks'

scale), depending on N timing (Zadoks et al., 1974). The treatments were applied to the soil surface of the microplot area in 4 L of distilled water per microplot using a hand sprayer.

Upon maturity, a 0.5 m² sample of plants was harvested from each microplot and threshed, dried, and ground. All samples were analysed for ¹⁵N content and total N concentration using a Carlo Erba 1108 Elemental Analyser coupled to a VG Isochrom isotope-ratio mass spectrometer in continuous flow and using the Dumas combustion method (EA 3000 Eurovector SpA, Milan, Italy).

Labelled-fertiliser N recovery (NR) in the plant on an area basis and percentage basis was calculated as follows, after Hauck and Bremner (1976):

$$N_R = N_t \times \frac{c-b}{a-b} \quad \text{and} \quad \%N_R = \frac{N_R}{f} \times 100$$

where, N_t is the total plant N at maturity in kg ha⁻¹, a the atom% N in the fertiliser, b the atom% ¹⁵N in the unfertilised plant, c the atom% ¹⁵N in the fertilised plant, and f the fertiliser rate in kg N ha⁻¹ applied to the crop.

The percent of total plant N derived from ¹⁵N fertiliser (NF), was calculated as:

$$N_F = \frac{N_R}{N_t} \times 100$$

2.5. Statistical analyses

We analyzed data using Mixed-Model ANOVA with three fixed factors, tillage system, crop rotation, and N timing of application treatment. Year was considered as a random factor in this work due to unpredictable weather conditions under rainfed Mediterranean conditions (Gómez and Gómez, 1984). The NR, NF and the rest of the parameters studied were subjected to analysis of variance (ANOVA) using a split-split plot design combined over the years, tillage system, preceding crop and N timing, following error term according to McIntosh (1983). When they had significant effects, the treatment methods were compared using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Analyses of variance were performed using Statistix 8.1 (Analytical Software, 2005) to determine the treatment effects.

Results

3.1. Weather conditions

Significant year-to-year variations in rainfall were recorded over the study period. Figure 1 shows the monthly distribution of rainfall and changes in mean temperature (maximum and minimum) during the 3 years studied. The total rainfall was 741, 412, and 403 mm for the 2004, 2006, and 2007 years, respectively. The mean annual rainfall of the study area over the last 30 years is 584 mm. The period from 2004 was therefore a wet year, and the period from 2006 and from 2007 were considered to be dry, with the corresponding values of mean annual rainfall 172 and 181 mm below the average annual rainfall, respectively. Rainfall distribution also differed between years (Fig. 1).

Temperature differed only slightly between the 3 study years (Fig. 1). The mean minimum winter and mean maximum spring of the study area over the last 30 years are 4.8 °C and 22.9 °C, respectively. Mean minimum winter temperatures ranged between 2 and 5°C over the 3 years, with a range of 5 to 6°C in 2004, 3 to 4°C in 2006 and 1 to 4°C in 2007. During the grain filling period (April-May), the mean temperature was 17°C in 2004, 20 °C in 2006, and 16 °C in 2007. The mean maximum temperature during this period was 25°C in 2004, 27°C in 2006, and 22°C in 2007 (Fig. 1).

3.2. Tillage effect

The level of nitrates at sowing was overall significantly greater in conventional tillage (CT) (153 kg ha⁻¹) than in no tillage (NT) (132 kg ha⁻¹) (Table 2 and Fig. 2). In the year x tillage system x preceding crop interaction, the highest nitrate contents were found under NT and CT treatments respectively when faba bean and chickpea were used as a preceding crop only in the year 2007 (Fig. 2). Adversely, no effect of tillage system was found on the nitrate content of soil at sowing when sunflower was used as the preceding crop (Fig. 2).

The tillage system had a significant overall effect on yield (Table 2), with NT (3.2 t ha⁻¹) having a 2.7% higher yield than CT (3.1 t ha⁻¹) (Fig. 3) According to year, wheat yield was higher in NT in 2004 and 2006 and in CT in 2007 (Table 3 and Fig. 3A).

The influence of tillage system on N uptake varied depending on the year and the previous crop in rotation (Table 2). When faba bean was used in the rotation, wheat under the NT treatment had higher N uptake level than that under the CT treatment in 2004, with the inverse occurring in 2007 (Fig. 4). In the chickpea and sunflower rotations, there were no significant differences in the N uptake of wheat in any year and in either tillage system (Fig. 4).

The NT produced a higher total recovery of N-labelled fertiliser (NR) than CT (47.3% and 41.9%, respectively) (data not shown). There were significant differences only in 2004 (Table 3). The N derived from labelled fertiliser (NF) was higher in NT (49.4%) than in CT (42.7%) (data not shown).

3.3. Preceding crop effect

The soil nitrate content was significantly affected by preceding crop before sowing wheat, as the significance of interactions year x preceding crop, tillage system x preceding crop, and year x tillage system x preceding crop show (Table 2). Rotations with legumes produced higher levels of soil nitrates (an average of 170 and 175 kg ha⁻¹ in the 0–90 cm profile of soil sown with faba bean and chickpea, respectively) compared to sunflower used as a preceding crop (averagely 81 kg ha⁻¹ of nitrate in the same profile) (Fig. 2).

The wheat yield was significantly affected by the biannual rotation (Table 2), with yield increases of 16.5% and 31.4% if the wheat was preceded by the faba bean (3.6 t ha⁻¹) rather than by the chickpea (3.1 t ha⁻¹) or the sunflower (2.7 t ha⁻¹), and 12.7% if preceding crop was the chickpea rather than the sunflower (Fig. 3). In 2004, the sowing of wheat preceded by both legumes resulted in a significant increase in yield than when sunflower was used as the previous crop (Table 3).

The N uptake of wheat was significantly different depending on the year and the preceding crop (Table 2). The highest N uptake occurred when wheat was sown in rotation with faba bean (120 kg N ha⁻¹) which differed significantly from chickpea (101 kg N ha⁻¹) and sunflower (86 kg N ha⁻¹) as previous crops, the latter two also different from each other (Table 3).

Overall, the NR by the wheat was significantly greater in the sunflower rotation (47%) compared to that recorded in the faba bean and chickpea rotations, which were approximately

equal to one another (44.2% and 42.6%, respectively) (Fig. 5). A significantly higher NF value (55.7%) was obtained from wheat-sunflower rotation compared to wheat-faba bean and wheat-chickpea ones, both significantly different from each other (38% and 44.4%, respectively) (Fig. 6).

3.4. N timing effect

Wheat yield was not affected by the N timing of the fertiliser, although the interactions year x N timing, tillage system x N timing, preceding crop x N time, year x tillage system x N timing and year x preceding crop x N timing were significant (Table 2). The influence of N timing on grain yield varied as a function of year and tillage system (Fig. 3A). In the year 2004, the best grain yield response was obtained by NT without significant differences between the two forms of N-fertiliser splitting (sowing-stem elongation and tillering-stem elongation), followed by the response to CT when N fertiliser was applied at sowing and stem elongation (Fig. 3A). In 2006, the highest yield was always obtained from NT but with the treatment which provided N applied at sowing and stem elongation (Fig. 3A). In 2007, the result was favourable to CT in both split N treatments, with the application at tillering-stem elongation having the highest wheat yield (Fig. 3A). As regards rotation, faba bean with the N splitted between sowing and stem elongation (5.2 t ha^{-1}) and chickpea with the N splitted between tillering and stem elongation (5.1 t ha^{-1}) were the combinations that provided the highest wheat yields (Fig. 3B). Unlike legumes, wheat yield was notably reduced by the rotation with sunflower (approximately 1 t of grain), obtaining the highest yields when N was applied to the sowing-stem elongation (4.1 t ha^{-1}) compared to the tillering-stem elongation (3.8 t ha^{-1}). In 2006, there was no clear influence of the previous crop or N timing on grain yield (Fig. 3B). In 2007, wheat yield was significantly affected by faba bean as the preceding crop compared to chickpea and sunflower (which produced comparable results) in both treatments of N fertiliser splitting (sowing-stem elongation and tillering-stem elongation). The latter also showed significant differences in grain yield (2.8 and 3.4 t ha^{-1} , respectively) when faba bean was used as the preceding crop (Fig. 3B).

The split application of N fertiliser in tillering-stem elongation produced a significantly higher NR than the N splitted between sowing and stem elongation (46% and 43.2%, respectively) (Fig. 5). The interaction year x preceding crop x N timing (Fig. 5) shows the strong influence of the year and, to a lesser extent, the rotation on NR as a function of N timing. The NR by the wheat in rotation with faba bean was significantly greater in the sowing-stem elongation split

of N fertiliser in 2004 and 2006 (Fig. 5). In the rotation with chickpea, the N applied at the tillering-stem elongation in 2004 produced the highest NR value, followed by the values measured in 2006, in which no difference in NR was found between both splitting treatments. In 2007, the lowest NR values were measured, with a significantly lower NR value when the N was splitted at the tillering-stem elongation than at the sowing-stem elongation (Fig. 5). Finally, in the sunflower rotation, the highest NR value was produced with the sowing-stem elongation application in 2004. In the 2006 and 2007 years have been obtained the lowest NR values, although no difference was found between the different application methods (Fig. 5). The NR of the grain had a very similar behaviour to the total percentage of N recovered by the entire plant (Fig. 5). As a general trend, the N fertiliser applied during stem elongation in both splitting treatments led to significantly higher NR values than the N applied at the sowing or tillering (indicated by an asterisk in Fig. 5).

On average, the application of fertilizer N splitted between the stage of sowing or tillering and stem elongation did not lead to significant differences in NF values (45% and 47.1%, respectively) (Table 2). The interaction between year x preceding crop x N timing (Fig. 6) shows the strong influence of the year and preceding crop on NF, with a lesser influence on N timing. Only during 2006 and in the chickpea and sunflower rotations the NF value was significantly higher when the N application was splitted between the tillering-stem elongation than the sowing-stem elongation application (Table 3 and Fig. 6). Generally, the N fertiliser applied in the stem elongation led to significant increases of NF (indicated by an asterisk in Fig. 6) in both treatments compared to the N applied in the sowing or tillering stage.

Discussion

As Craswell and Godwin (1984) and Campbell et al. (1993) observed, the high interannual variability of rain, typically as occurs in Mediterranean climate, induced large variations in grain yield, soil N content, and the efficiency of N use by wheat. In several experiments carried out on dry Vertisols of southern Spain, López-Bellido et al. (1996, 2000) and Garrido-Lestache et al. (2004) found a relationship between wheat yield and seasonal trend: during the wet years (i.e., growing season precipitations greater than 500 mm) was seen a positive increase in wheat yield up to the rate of 100 kg N ha⁻¹, while a little or no effect of N fertiliser during the dry years (i.e., growing season precipitations less than 450 mm) was observed. In fact, in the year 2006, the low grain yield of wheat (Fig. 3) was mainly due to the scarcity of rain (Fig. 1),(Fig. 3), but

in 2007 this resulted in a significant accumulation of residual mineral N (especially as nitrates) in the soil. The carry-over effect of residual N has been observed by Corbeels et al. (1998) and López-Bellido et al. (2000) as a characteristic of Mediterranean rainfed Vertisols. The significant accumulation of N in the soil profile is a product of its high clay content and the generally scarce rains in the Mediterranean region. This dynamic generates an elevated stock of mineral N that dampens, and sometimes reduces or even eliminates, the response of wheat to N fertiliser. Johnson and Mattern (1987) found that a high N residual in soil was due to the cumulative effect of N fertiliser applications over many years in order to provide less N fertiliser to wheat. Under dry soil conditions, much of the N fertiliser not absorbed by wheat may remain in the soil as nitrates available to subsequent crops, particularly when high rates of N are applied (Olson et al., 1976). Additionally, in Vertisols the N residual is progressively distributed throughout the soil profile occupied by roots, and soil cracks contribute significantly to this process. For this reason, wheat can utilise this N more efficiently than fertiliser applied during the growing season. In another experiment carried out in Vertisols under dry conditions in southern Spain, López-Bellido et al. (2005) observed the substantial contribution of soil N to total plant N, which varied between 50% (when the N fertiliser was applied to wheat entirely as a top dressing) and 83% (when applied at sowing).

In 2004, year with the highest yield, the N uptake per kg of wheat grain was notably reduced (28 g kg^{-1}) compared to other years (36 and 39 g kg^{-1} , in 2006 and 2007, respectively). The more favourable growth conditions during the 1st year were induced by increased rainfall that led to increased N uptake efficiency in wheat. López-Bellido and López-Bellido (2001) and López-Bellido et al. (2005, 2008) reported N uptake values of wheat grain ranging $28\text{--}33 \text{ g kg}^{-1}$ under dry Mediterranean conditions in southern Spain, with lowering of values under irrigated condition (Jia et al., 2011).

The average, overall value of N derived from labelled fertiliser (NF) (46.1%) recorded in the experiment was higher than that obtained in the previous experiment mentioned above (28.6%) (López-Bellido et al., 2005). Under irrigation, Jia et al. (2001) obtained slightly higher NF values (34–55%) by using different applications at sowing (15–22%) and top dressing (16–40%).

Generally in the CT system the nitrate content was significantly higher at sowing than in the NT ones (approximately 15% greater), but this phenomenon did not manifest consistently

across years and among preceding crops, as indicated by the significant existing interactions (Fig. 2). However, in the same experiment but at previous periods López-Bellido and López-Bellido (2001) did not find differences in the soil content between tillage systems. Giacomini et al. (2010) also observed that, in Central Europe, the type of tillage system had little effect on N fertiliser dynamics in the soil.

The tillage system influenced the recovery of ^{15}N by wheat, with overall NT values significantly higher than CT values (47% and 42%, respectively), although this difference only occurred in 2004, the year of the greatest grain yield. In the other years covered by this study, the recovery of ^{15}N -labelled fertiliser, expressed as a percentage (NR) was similar in both tillage systems. In northern Europe, Thomsen and Christensen (2007) reported NR values between 59–64%, and the values were lower under shallow tillage than under mouldboard ploughing.

An interesting aspect regards the strong influence that the preceding crop had on soil nitrate content. In agreement with López-Bellido and López-Bellido (2001), both legumes tested in this study, and especially faba bean, generated soil nitrate levels that were notably higher than those produced by sunflower. Giacomini et al. (2010) found similar results, indicating that the previous crop in the rotation had a greater role on the N uptake of wheat than the tillage system. In fact, the soil N content was more than double with the legume as preceding crop compared with sunflower, resulting in an average of approximately 90 kg ha^{-1} in the 0–90 cm profile. This demonstrates the importance of the previous crop in a rotation when establishing the N needs of the grain as well as the efficient role of legumes in fixing N.

The NR was higher in the wheat-sunflower rotation, and residual was notably lower than that in other legume rotations at wheat sowing (Fig. 5). When sunflower was the preceding crop, the reduced availability of mineral N for wheat increased the efficiency of N fertiliser and illustrates the importance of residual to the balance and fertilisation of cereal in Mediterranean rainfed Vertisols.

Previous studies undertaken both in the long-term Malagón experiment and in other locations have demonstrated that the threshold for a significant response of wheat yield to rates of N fertiliser was never greater than 100 kg N ha^{-1} (López-Bellido et al., 1996; López-Bellido et al., 2000; Garrido-Lestache et al., 2004). Nevertheless, wheat yields differed significantly when the total N rate was splitted between sowing and tillering-stem elongation, as compared to a single

application upon sowing. Using ^{15}N -labelled fertiliser with a single rate of 150 kg N ha^{-1} , López-Bellido et al. (2005) found that recovery of ^{15}N by wheat was 3 times greater when applied as a top dressing (stem elongation) compared to application at sowing.

Wheat yields remained generally consistent also when the wheat was tested against different application times of a 100 kg ha^{-1} rate of N fertiliser, ascribing this result to elevated levels of residual $\text{NO}_3\text{-N}$ recorded at sowing in the 3 years of the study. The significant interactions between the year and tillage system (Fig. 3) show the strong influence of annual weather conditions, especially rain, in the variable response of yield to applied N fertiliser. Moreover, springtime drought stress, especially in dry years (2006 and 2007), diminished the yield response upon later application of the fraction of N fertiliser (stem elongation). In general, in dry years (especially during the spring), the application of N splitted between sowing and stem elongation is more favourable than the tillering-stem elongation splitting, demonstrating the greater efficiency of N fertiliser applied at sowing.

The NR across both split treatments of N fertiliser (44.6%), was lower than the 51.7% average recorded in previous experiments undertaken in the same area (López-Bellido et al., 2005). The fraction of NR was highest in stem elongation followed by that applied during tillering, with the least fraction obtained when applied at sowing. Of the total ^{15}N recovery by wheat measured in this study, 27% corresponded to sowing application, 32% to tillering, and 41% to stem elongation. Ladha et al. (2005) conducted an exhaustive review of the efficiency of N fertiliser in cereals and reported average values that vary by 20–30% under dry conditions and 30–40% under irrigation worldwide. In Europe, total NR values were 61%, and grain NR was 44%.

Conclusions

The NT system produced greater grain yields and higher ^{15}N recovery than CT. Legumes play an important role in the agrosystem sustainability because they produce higher yield and savings of N fertiliser. However, ^{15}N recovery efficiency was higher with sunflower as preceding crop (most common crop in the area in rotation with wheat) thanks to the characteristics of their root system that allows synchronization in the use of water and residual N.

The splitting of N fertiliser in sowing-stem elongation produced higher grain yields in dry years, while tillering-stem elongation led to greater yields in wet years. The recovery of ^{15}N fertiliser was always higher if the N was mainly applied during stem elongation.

Due to the characteristics of Vertisol, an increase of N is generated in the soil and tends to accumulate along the profile due to cracks. The high rates of N fertiliser applied to wheat by the farmers lead to overfertilisation in soils already rich in nitrates.

Consequently, under Mediterranean rainfed Vertisol conditions, the quantity of nitrates in the soil profile, the type of preceding crop, and the projected precipitation during the growing season should be understood prior to sowing wheat. Determining these conditions can establish the optimal rate and splitting of N fertiliser, with significant economic and environmental implications. In view of our results, future works undertaken aim to further reduce the rate of N fertiliser, especially when the winter is dry, and apply it mainly in top dressing.

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References

- Alcoz, M.M., Hons, F.M., Haby, V.A., 1993. Nitrogen fertilisation, timing effect on wheat production, nitrogen uptake efficiency, and residual soil nitrogen. *Agron. J.* 85, 1198–1203.
- Analytical Software, 2005. Statistix 8.1. Analytical Software, Tallahassee, FL, USA.

- Barnes, H., Folkard, A.R., 1951. The determination of nitrates. *Analyst* 76, 599–603.
- Blankenau, K., Olf, H.W., Kuhlmann, H., 2002. Strategies to improve the use efficiency of mineral fertilizer nitrogen applied to winter wheat. *J. Agron. Crop. Sci.* 188, 146–154.
- Campbell, C.A., Zentner, R.P., Selles, F., McConkey, B.G., Dyck, F.B., 1993. Nitrogen management for spring wheat grown annually on zero-tillage: yield and nitrogen use efficiency. *Agron. J.* 85, 107–114.
- Corbeels, M., Hofman, G., Van Cleemput, O., 1998. Analysis of water use by wheat grown on a cracking clay soil in a semi-arid Mediterranean environment: weather and nitrogen effects. *Agric. Water Manage.* 38, 147–167.
- Craswell, E.T., Godwin, D.C., 1984. The efficiency of nitrogen fertilizers applied to cereals in different climates, in: Tinker, P.B., Läuchli, A. (Eds.), *Advances in Plant Nutrition*, vol. 1. Praeger, New York, pp. 1-55.
- Fageria, N.K., Baligar, V.C., 2005. Enhancing nitrogen use efficiency in crop plants. *Adv. Agron.* 88, 97–185.
- Garabet, S., Ryan, J., Wood, M., 1998. Nitrogen and water effects on wheat yield in a Mediterranean-type climate. II. Fertiliser-use efficiency with labelled nitrogen. *Field Crops Res.* 58, 213–221.

- Garrido-Lestache, E., López-Bellido, R.J., López-Bellido, L., 2004. Effect of N rate, timing and splitting and N type on bread-making quality in hard red spring wheat under rainfed Mediterranean conditions. *Field Crops Res.* 85, 213–236.
- Giacomini, S.J., Machet, J.M., Boizard, H., Recous, S., 2010. Dynamics and recovery of fertilizer ^{15}N in soil and winter wheat crop under minimum *versus* conventional tillage. *Soil Tillage Res.* 108, 51–58.
- Gómez, K.A., Gómez, A.A., 1984. *Statistical Procedures for Agricultural Research*. Wiley, New York.
- Grageda-Cabrera, O.A., Vera-Núñez, J.A., Aguilar-Acuña, J.L., Macías-Rodríguez, L., Aguado-Santacruz, G.A., Peña-Cabriales, J.J., 2011. Fertilizer dynamics in different tillage and crop rotation systems in a Vertisol in Central Mexico. *Nutr. Cycl. Agroecosyst.* 89, 125–134.
- Hansen, E.M., Munkholm, L.J., Olesen, J.E., 2011. N-utilization in non-inversion tillage system. *Soil Tillage Res.* 113, 55–60.
- Hauck, R.D., Bremner, J.M., 1976. Use of tracers for soil and fertilizer nitrogen research. *Adv. Agron.* 28, 219–266.
- Jia, S., Wang, X., Yang, Y., Dai, K., Meng, C., Zhao, Q., Zhang, X., Zhang, D., Feng, Z., Sun, Y., Wu, X., Cai, D., Grant, C., 2011. Fate of labelled urea- ^{15}N as basal and topdressing

applications in an irrigated wheat-maize rotation system in North China Plain: I winter wheat. *Nutr. Cycl. Agroecosyst.* 90, 331–346.

Johnson, V.A., Mattern, P.J., 1987. Wheat, rye and triticale, in: Olson, R.A., Frey, K.J. (Eds.), *Nutritional quality of cereal grains: genetic and agronomy improvements n° 28*. American Society of Agronomy, Inc., Madison, WI, USA, pp. 133-182.

Kirda, C., Derici, M.R., Schepers, J.S., 2001. Yield response and N-fertiliser recovery of rainfed wheat growing in the Mediterranean region. *Field Crops Res.* 71, 113–122.

Ladha, J.K., Pathak, H., Krupnik, T.J., Six, J., van Kessel, C., 2005. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. *Adv. Agron.* 87, 85–156.

Limaux, F., Recous, S., Meynard, J.M., Guckert, A., 1999. Relationship between rate of crop growth at date of fertiliser N application and fate of fertiliser N applied to winter wheat. *Plant Soil* 214, 49–59.

López-Bellido, L., Fuentes, M., Castillo, J.E., López-Garrido, F.J., Fernández, E.J., 1996. Long-term tillage, crop rotation, and nitrogen fertilizer effects on wheat yield under Mediterranean conditions. *Agron. J.* 88, 783–791.

López-Bellido, L., López-Bellido, F.J., Fuentes, M., Castillo, J.E., Fernández, E.J., 1997. Influence of tillage, crop rotation and nitrogen fertilization on soil organic matter and nitrogen under rain-fed Mediterranean conditions. *Soil Tillage Res.* 43, 277–293.

- López-Bellido, L., López-Bellido, R.J., Castillo, J.E., López-Bellido, F.J., 2000. Effects of tillage, crop rotation, and nitrogen fertilization on wheat under rainfed Mediterranean conditions. *Agron. J.* 92, 1054–1063.
- López-Bellido, R.J., López-Bellido, L., 2001. Efficiency of nitrogen in wheat under Mediterranean conditions: effect of tillage, crop rotation and N fertilization. *Field Crops Res.* 71, 31–46.
- López-Bellido, L., López-Bellido, R.J., Redondo, R., 2005. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. *Field Crops Res.* 94, 86–97.
- López-Bellido, R.J., López-Bellido, L., Benítez-Vega, J., López-Bellido, F.J., 2007. Tillage system, preceding crop, and nitrogen fertilizer in wheat crop: I Soil water content. *Agron. J.* 99, 59–65.
- López-Bellido, R.J., Castillo, J.E., López-Bellido, L., 2008. Comparative response of bread and durum wheat cultivars to nitrogen fertilizer in a rainfed Mediterranean environment: soil nitrate and N uptake and efficiency. *Nutr. Cycl. Agroecosyst.* 80, 121–130.
- MacDonald, A.J., Poulton, P.R., Stockdale, E.A., Powlson, D.S., Jenkinson, D.S., 2002. The fate of residual N-15-labelled fertilizer in arable soils: it's availability to subsequent crops and retention in soil. *Plant Soil* 246, 123–137.
- McIntosh, M.S., 1983. Analysis of combined experiments. *Agron. J.* 75, 153–155.

- Mahler, R.L., Koehler, F.E., Lutcher, L.K., 1994. Nitrogen source, timing of application, and placement: effects on winter wheat production. *Agron. J.* 86, 637–642.
- Malhi, S.S., Soon, Y.K., Brandt, S., 2009. Effect of growing season rainfall and tillage on the uptake and recovery of (15)N-labelled urea fertilizer by spring wheat in a semi-arid environment. *Can. J. Soil Sci.* 89, 403–411.
- Mossedaq, F., Smith, D.H., 1994. Timing nitrogen application to enhance spring wheat yield in a Mediterranean climate. *Agron. J.* 86, 221–226.
- Olson, R.A., Frank, K.D., Deibbert, E.J., Dreier, A.F., Sander, D.H., Johnson, V.A., 1976. Impact of the residual mineral N in soil on grain protein yields of winter wheat and corn. *Agron. J.* 68, 769–772.
- Powlson, D.S., Hart, P.B.S., Poulton, P.R., Johnston, A.E., Jenkinson, D.S., 1992. Influence of soil type, crop management and weather on the recovery of N-15-labelled fertilizer applied to winter-wheat in spring. *J. Agr. Sci.* 118, 83–100.
- Recous, S., Machet, J.M., 1999. Short-term immobilization and crop uptake of fertilizer nitrogen applied to winter wheat: effect of date of application in spring. *Plant Soil* 206, 137–149.
- Sowers, K.E., Pan, W.L., Miller, B.C., Smith, J.L., 1994. Nitrogen use efficiency of split nitrogen applications in soft white winter wheat. *Agron. J.* 86, 942–948.

- Stockdale, E.A., Gaunt, J.L., Vos, J., 1997. Soil-plant nitrogen dynamics: what concepts are required. *Eur. J. Agron.* 7, 145–159.
- Thomsen, I.K., Christensen, B.T., 2007. Fertilizer ¹⁵N recovery in cereal crops and soil under shallow tillage. *Soil Tillage Res.* 97, 117–121.
- Torbert, H.A., Potter, K.N., Morrison, J.E.Jr., 2001. Tillage system, fertilizer nitrogen rate, and timing effect on corn yields in the Texas Blackland Prairie. *Agron. J.* 93, 1119–1124.
- Tran, T.S., Tremblay, G., 2000. Recovery of N-15-labelled fertilizer by spring bread wheat at different N rates and application times. *Can. J. Soil Sci.* 80, 533–539.
- Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14, 415–421.

Table 1. Information for each crop about cultivar, planting, herbicides, and others.

Crop	Cultivar	Planting and harvest dates	Planting density		Specific herbicides (quantity)	Others
			Wide row	Seeding rate		
			cm	kg ha ⁻¹		
Wheat	Gazul	early Dec.- early June	18	150	Diclofop Methyl [†] + Tribenuron [‡] (0.9 g a.i. ha ⁻¹ + 15 g a.i. ha ⁻¹)	—
Sunflower	Pioneer PR63A76	early Mar.- mid Aug.	50	5		—
Chickpea	Zoco	early Feb.- mid-July	35	80	Diluron [¶] (1.35 L a.i. ha ⁻¹)	Ascochyta blight [<i>Ascochyta rabiei</i> (Pass.) Labr.] was controlled with chlorothalonil [#] (0.75 L a.i. ha ⁻¹)
Faba bean	Alameda	late Nov.- early June	50	170	Diluron [¶] (1.35 L a.i. ha ⁻¹)	Broomrape (<i>Orobanche crenata</i> Forsk) was controlled with glyphosate ^{††} (0.065 L a.i. ha ⁻¹)

[†]Diclofop Methyl: [2-(4-(2,4-dichlorophenoxy)phenoxy)propanoic methyl].

[‡]Tribenuron: {methyl-2-[(N-4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylamino]carbonyl amino sulphonyl}benzoate}. [§]Trifluraline: [N,N-dipropyl-2,6-dinitro-4-trifluoromethylaniline].

[¶]Diluron: [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea].

[#]Chlorothalonil: [2,4,5,6-tetrachloroisophthalonitrile].

^{††}It was applied as a postemergence spray on faba bean plots when broomrape was about 0.5 to 1 cm high.

Table 2. Mean squares and significant effects of year, tillage system, preceding crop and N timing on soil nitrates, grain yield, total N uptake, N recovery and N derived from labeled fertiliser (N_F) in wheat crop over 3 year period.

Source	Soil NO ₃ (kg ha ⁻¹)	Grain yield (t ha ⁻¹)	Total N uptake (kg ha ⁻¹)	N recovery (N _R)		N _F (%)
				Total (%)	Grain (%)	
Year (Y)	190451***	60.77***	19 103***	915***	424***	2121***
Tillage (T)	11928**	0.49**	143	786***	181	1230**
Y × T	1150*	3.35***	2447**	823***	395*	427**
Error a	819	0.03	188	8	41	30
Preceding crop (P)	101813***	6.60***	10344***	177**	221**	2890***
Y × P	9426*	2.36***	2919***	112*	105*	193***
T × P	21265**	0.04	167	132*	116	116*
Y × T × P	13267*	1.04***	1923***	223***	92	19
Error b	3354	0.03	142	31	34	27
N timing (N)	-	0.04	147	208**	2 10 ⁻⁶	123
Y × N	-	0.71***	3172***	382***	256**	151*
T × N	-	0.39***	33	103	16	10
P × N	-	0.13*	1013**	351***	39	35
Y × T × N	-	0.24**	251	6	13	47
Y × P × N	-	0.41***	1672***	490***	218***	116*
T × P × N	-	0.06	765**	137**	2	7
Y × T × P × N	-	0.02	198	29	45	17
Error c		0.03	132	25	32	38

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level

Table 3. Soil nitrate, grain yield, total N uptake, N recovery and N derived from fertiliser influenced by tillage (NT, no tillage; CT, conventional tillage), preceding crop (Fb, faba bean; Ch, chickpea; Sf, sunflower) and N timing (50-0-50, N applied at sowing and stem elongation; 0-50-50, N applied at tillering and stem elongation) within each year^a.

		Soil NO ₃ (kg ha ⁻¹)	Grain yield (t ha ⁻¹)	Total N uptake (kg ha ⁻¹)	N recovery (N _R)		N _F (%)
					Total (%)	Grain (%)	
<u>2004</u>							
Tillage system	NT	95a	4883a	136a	55a	41a	43a
	CT	104a	4312b	122b	39b	31b	33b
Preceding crop	Sf	46b	3942b	103b	51a	40a	50a
	Fb	127a	4937a	144a	44b	33b	30b
	Ch	125a	4913a	140a	47ab	36ab	33b
N timing	50-0-50	-	4676a	127a	46a	37a	38a
	0-50-50	-	4519b	131a	48a	36a	37a
<u>2006</u>							
Tillage system	NT	85b	2735a	91a	47a	34a	52a
	CT	117a	2339b	92a	49a	36a	53a
Preceding crop	Sf	66b	2528ab	89a	51a	38a	58a
	Fb	125a	2660a	97a	47ab	34a	48b
	Ch	113ab	2423b	90a	46b	34a	52b
N timing	50-0-50	-	2656a	101a	49a	37a	49b
	0-50-50	-	2418b	83b	46a	33b	56a
<u>2007</u>							
Tillage system	NT	215a	1915b	77b	40a	30a	54a
	CT	238a	2477a	96a	38a	30a	42b
Preceding crop	Sf	131b	1663c	67b	39ab	32a	60a
	Fb	262a	3090a	119a	42a	33a	36c
	Ch	285a	1834b	74b	35b	25b	48b
N timing	50-0-50	-	2054b	77b	34b	27b	48a
	0-50-50	-	2338a	96a	44a	33a	48a

^a For each year and effect, means followed by the same letter are not significantly different at $p < 0.05$ according to LSD.

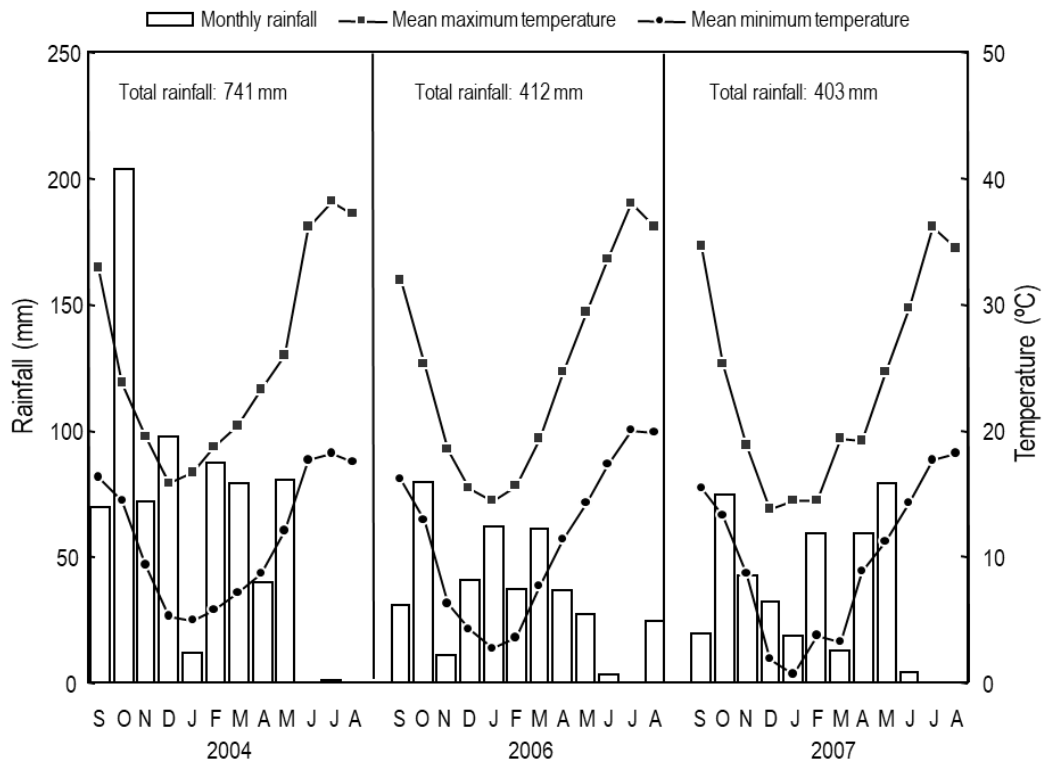


Figure 1. Monthly and annual rainfall, mean maximum and minimum temperatures over the 3-year study at Córdoba (Spain).

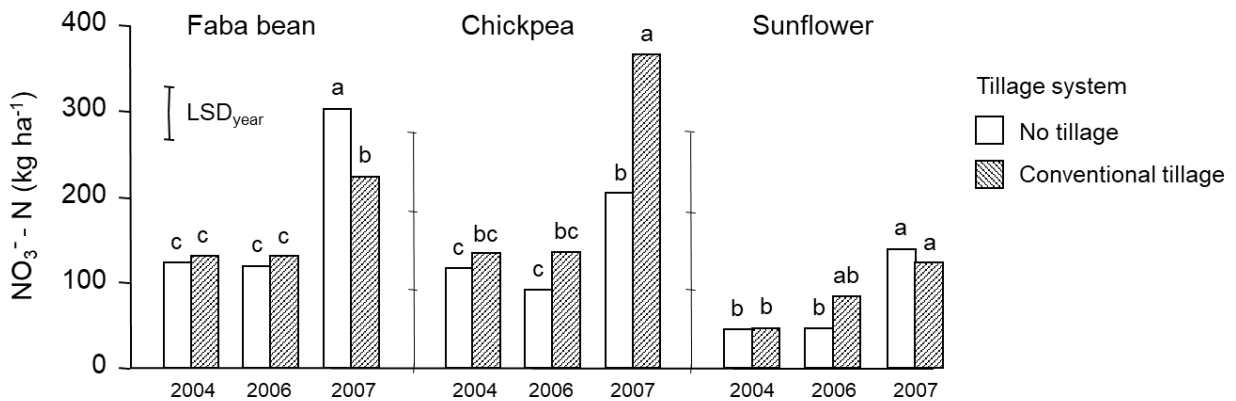


Figure 2. Soil nitrate content before wheat sowing as affected by year, tillage system and preceding crop. For each preceding crop means followed by the same letter are not significantly different at $P < 0.05$ according to LSD. Vertical bar represents LSD for the same year.

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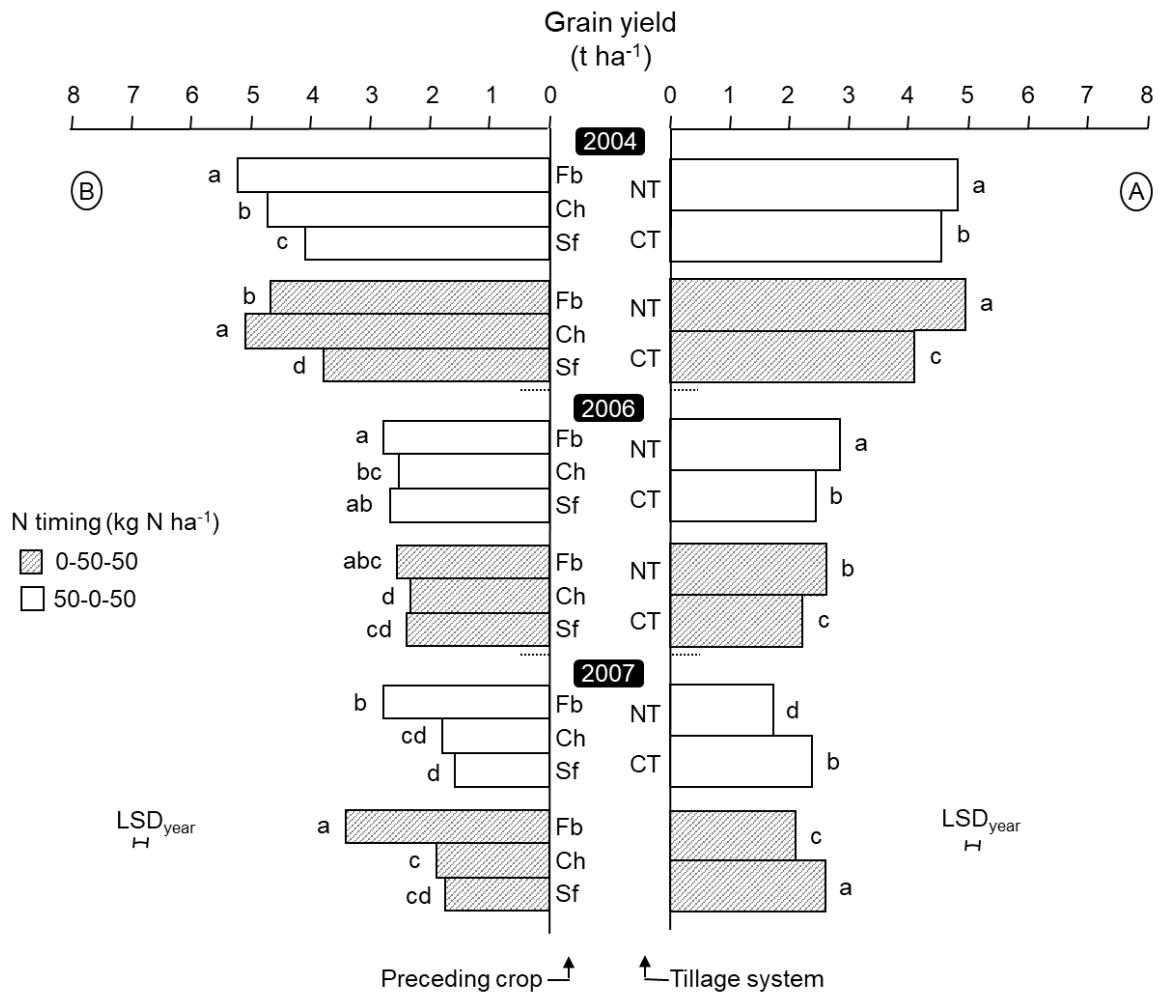


Figure 3. Wheat yield as affected by year, N timing (50-0-50, N applied at sowing and stem elongation; 0-50-50, N applied at tillering and stem elongation) and: A) tillage system (NT, no-tillage; CT, conventional tillage), B) preceding crop (Fb, faba bean; Ch, chickpea; Sf, sunflower). For each year means followed by the same letter are not significantly different at $P < 0.05$ according to LSD. Horizontal bars represent LSD for different years.

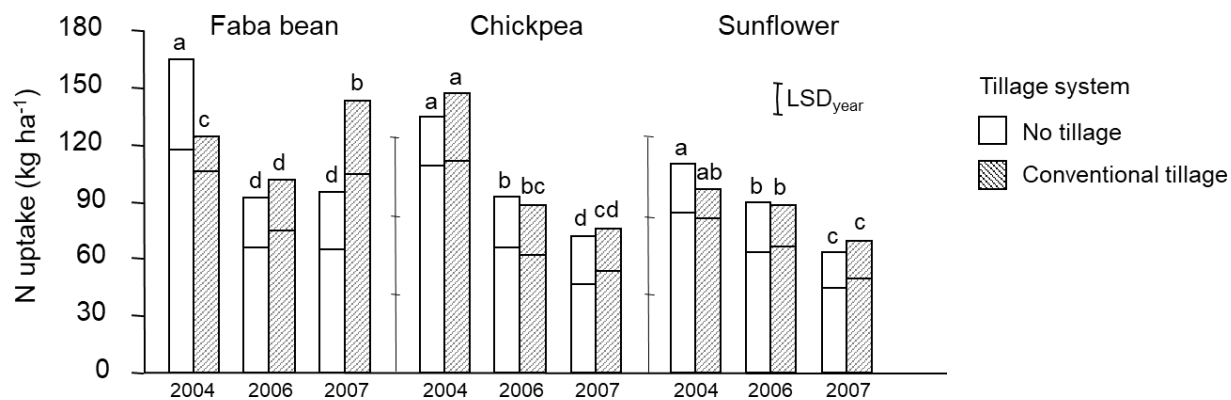


Figure 4. Total N uptake by wheat as affected by year, tillage system and preceding crop. The lower part of the bars represent grain N uptake and the top represent straw N uptake. For each preceding crop means followed by the same letter are not significantly different at $P < 0.05$ according to LSD. Vertical bar represents LSD for the same year.

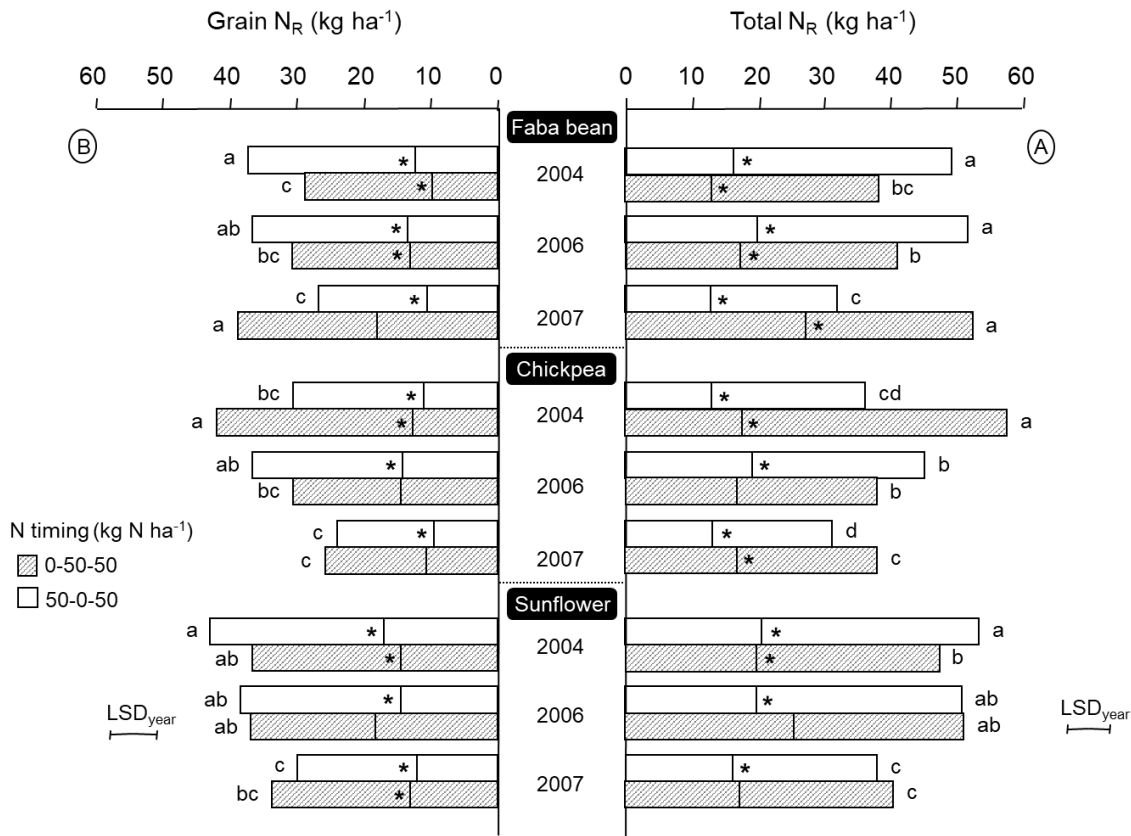


Figure 5. Total (A) and grain (B) nitrogen recovery (N_R) as affected by year, preceding crop and N timing (50-0-50, N applied at sowing and stem elongation; 0-50-50, N applied at tillering and stem elongation). The inside of the bars represents the N_R of the first application of N fertiliser and the outside represent the N_R of the second application for both splitting treatment; the asterisk represents significant differences between both applications. For each preceding crop means followed by the same letter are not significantly different at $P < 0.05$ according to LSD. Horizontal bars represent LSD for the same year.

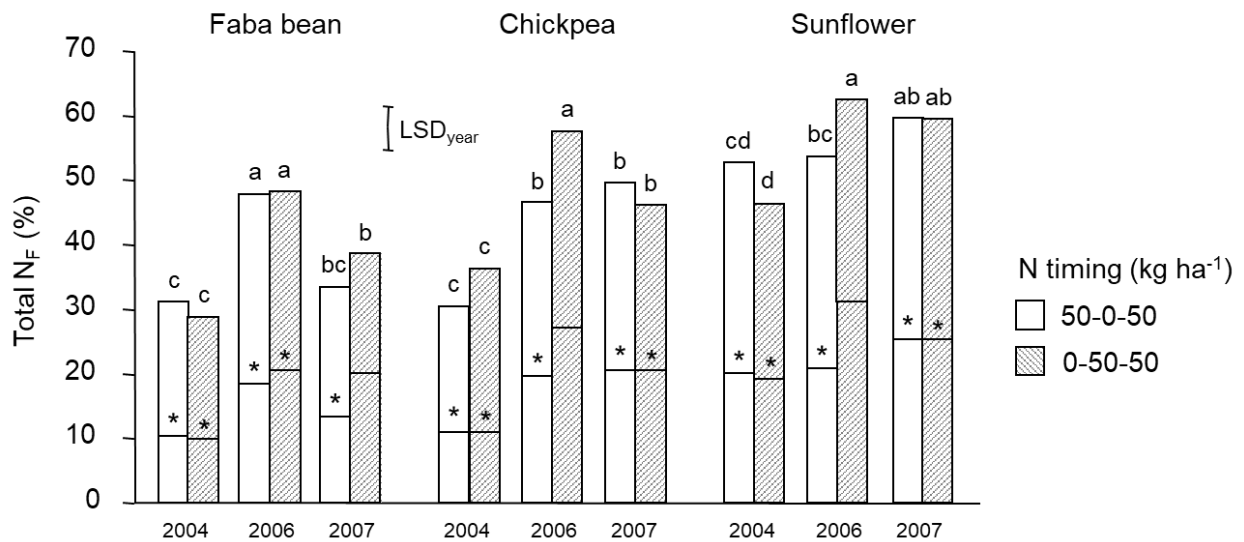


Figure 6. Total plant nitrogen derived from ¹⁵N fertiliser (N_F) as affected by year, N timing (50-0-50, N applied at sowing and stem elongation; 0-50-50, N applied at tillering and stem elongation) and crop preceding. The lower part of the bars represents the N_R of the first application of N fertiliser and the top represent the N_R of the second application for both splitting treatment; the asterisk represents significant differences between both applications. For each preceding crop means followed by the same letter are not significantly different at P<0.05 according to LSD. Vertical bar represents LSD for the same year.