# Chickpea and faba bean nitrogen fixation in a Mediterranean rainfed Vertisol: effect of the tillage system

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## Abstract

Efficient management of legumes in order to maximize benefits depends on a correct field assessment of $N_2$ fixation. A field experiment was conducted during a 6-year period (2001-2002 to 2006-2007) in Córdoba (Southern Spain) on a rainfed Vertisol within the wheat-chickpea and wheat-faba bean rotation framework of a long-term experiment started in 1986. The aim was to determine the effect of tillage systems [no tillage (NT) and conventional tillage (CT)] on chickpea and faba bean $N_2$ fixation. Fixation was calculated using the $^{15}N$ isotopic dilution (ID) and $^{15}N$ natural abundance (NA) methods with the reference being the wheat crop. The strong inter-annual rain variation caused great differences in the behaviour of both leguminous plants with regard to grain yield, nodule biomass and $N_2$ fixation. The NT system showed more nodule biomass than the CT system in both legumes. The ID method was more accurate than the NA method in determining $N_2$ fixation. The average amount of fixed N in faba bean (80 kg $ha^{-1}$ $year^{-1}$) was much greater than that in chickpea (31 kg $ha^{-1}$ $year^{-1}$). The Vertisol under the NT system offered more favourable conditions for the stimulation of the $N_2$ fixation, with fixed N values that were significantly higher than under CT. The N added to the system through $N_2$ fixation was low in faba bean and virtually nonexistent in chickpea, only in terms of above-ground biomass.

Keywords: Grain yield, Nodules, N uptake, $^{15}N$ enrichment, $^{15}N$ natural abundance.

## 1. Introduction

Understanding the role of legumes in supplying N to soil depends on the correct field assessment of $N_2$ fixation. This information not only provides insight into legume N economy but also enhances our understanding of the general N cycle (Peoples and Herridge, 1990). However, as indicated Peoples et al. (2009a), there is likely to be considerable variability in the net return of fixed N to soil in different geographic regions, which reflects the impact of local farming practices, or soil and climatic effects, on the ability of different legume species to grow and fix $N_2$ (Evans et al., 2001; Peoples et al., 2001).

Much of the N fixed by legumes is usually removed at harvest in high-protein seed, so that the net residual contributions of fixed N to agricultural soils after harvest may be relatively small. Nonetheless, the inclusion of legumes in a cropping sequence generally improves the productivity of the following crops (Peoples et al., 2009b). According to Unkovich et al. (2008), $N_2$ fixation of legumes can be influenced by the previous cropping sequence, periods of fallow, cropping intensity, and reduced tillage.

Soils under no tillage (NT), offer extremely favourable conditions for symbiosis, such as reduced soil temperature and greater water availability, resulting from increased aggregate stability and a higher number of macropores (Souza et al., 2003). Kessel and Hartley (2000) suggest that under zero tillage, a reduction in available N and an increase in soil moisture have the potential to increase the biological fixation of $N_2$. Rupela and Saxena (1987) maintain that straw mulch can indirectly affect nodulation and $N_2$ fixation by affecting the soil's physical, chemical and biological environment, although Horn et al. (1996) indicated that $N_2$ fixation is not significantly affected by the tillage system.

Isotopic techniques such as $^{15}N$ natural abundance (NA) and $^{15}N$ isotopic dilution (ID) have been used to study many different annual and perennial $N_2$-fixing species growing in a diverse range of farming systems across the globe. Unkovich et al. (2008) claimed that the ID technique is a valuable tool for estimating $N_2$ fixation of field-grown crops. While its use has been somewhat overtaken by the NA technique, it remains an important tool because this methodology is less sensitive than $^{15}N$ NA to sample handling and management problems, and sample analysis is also easier. According to Peoples et al.

(2009b), the ID method is more suitable for legumes grown in soils with low N content, which is expected to be high $N_2$ fixation.

Unkovich et al. (2008) argued that the δ $^{15}N$ NA technique is a powerful method for assessing $N_2$ fixation in the field. However, this technique has a number of important limitations that should be recognised before it is used. With $^{15}N$ NA, the accuracy of $N_2$ fixation measurements tends to be most uncertain when %Ndfa is high because of difficulties in accurately defining the extent of isotopic fractionation that occurs when the target species is totally dependent on $N_2$ fixation. Conversely, errors with the $^{15}N$ ID method are greatest when the %Ndfa is low (Evans et al., 1987; Peoples et al., 1996).

Faba bean $N_2$ fixation in dryland agriculture has been reported to range between 50 and 200 kg N $ha^{-1}$, by a number of authors, with a mean value of between 90 and 120 kg N $ha^{-1}$ in terms of only above-ground biomass (Schwenke et al., 1998; Carranca et al., 1999; Kessel and Hartley, 2000; Kumar and Goh, 2000; Unkovich and Pate, 2000; Herridge et al., 2008; Peoples et al., 2009a). Of the grain legumes usually grown in southern Europe, faba bean have shown the highest N yields and the best rotation effect as a wheat-preceding crop in dryland Vertisols (López-Bellido et al., 2000).

Chickpea can fix up to 140 kg N $ha^{-1}$, although reported values usually range from 20 to 60 kg N $ha^{-1}$ (Rupela and Saxena, 1987; Herridge et al., 1995; Marcellos et al., 1998; Pilbeam et al., 1998; Carranca et al., 1999; Kumar and Abbo, 2001; Herridge et al., 2008; Peoples et al., 2009a). Of the legume crops grown in wheat-based rotations, chickpea are often considered the poorest in terms of $N_2$ fixation (Walley et al., 2007). Pilbeam et al. (1998) claimed that a negative soil N budget is still likely in chickpea because the amount of N removed in the grain is usually greater than the amount of atmospheric N fixed. Consequently, chickpea do not appear to supply a net input of N within the cereal-legume system. López-Bellido and López-Bellido (2001) reported a rotation effect smaller than that of fallows and faba bean. The reasons for low $N_2$ fixation by chickpea are not well known, although farmers are familiar with their effects in comparison to those of other grain legumes.

The use of 2-yr cereal-legume crop rotations, specifically with chickpea or faba bean under rainfed Mediterranean conditions, has proven to be an efficient system in N fertilizer economy. However, further studies are required for a better understanding of the real fixation of $N_2$ by both legumes under dryland conditions and different tillage systems. The objectives of the study within the scope of a long-term experiment started in 1986 in southern Spain were to: (i) determine which of the two $^{15}N$ isotopic methods (isotopic dilution and natural abundance) is the most accurate and (ii) determine the influence of conventional tillage and no tillage on $N_2$ fixation by chickpea and faba bean crops grown in rotation with wheat on a Mediterranean dryland Vertisol.

## 2. Material and methods

### *2.1. Site and experimental design*

Field experiments were conducted in Córdoba, southern Spain (37º 46’ N and 4º 31’ W, 280 m a.s.l.), on a Vertisol (Typic Haploxererts) typical of the Mediterranean region, where rainfed cropping is the standard practice. The study took place over a 6-year period (2001-2002 to 2006-2007), within the framework of a long-term experiment named “Malagón” that was started in 1986 and designed as a randomized, complete block, with a split-split plot arrangement and four blocks. The main plots were tillage system [no-tillage (NT) and conventional tillage (CT)]; subplots were crop rotation, with two 2-yr rotations (wheat-chickpea and wheat-faba bean); sub-subplots were used for the calculation of $N_2$ fixation [$^{15}N$ dilution (ID) and $^{15}N$ natural abundance (NA)]. Each rotation was duplicated in a reverse crop sequence to obtain data for all crops on a yearly basis. The area of each sub-subplot was 50 $m^2$ (10 by 5 m).

### *2.2. Crop management*

No-tillage plots were seeded with a no-till seed drill. Weeds were controlled with glyphosate [N-(phosphonomethyl) glycine] + MCPA [(4-chloro-2-methylphenoxy) acetic acid] at a rate of 0.5 + 0.5 L a.i./ha prior to planting. The conventional tillage treatment included moldboard plowing and disk harrowing and/or vibrating tine cultivation to prepare a proper seedbed. During the chickpea and faba bean growing season, weeds were controlled by means of cyanazine [2-(4-chloro-6-ethylamin0-1,3,5-

triazin-2-yl-amino)-2-methyl propionitrile] at 2 L a.i./ha. Ascochyta blight (*Ascochyta rabiei*) in chickpea was controlled with chlorothalonil [2, 4, 5, 6 – tetra–chloroisophthalonitrile] at 0.75 a.i $ha^{-1}$. Glyphosate was applied at rate of 0.065 L a.i. $ha^{-1}$ as a post-emergence spray on faba bean plots when broomrape (*Orobanche crenata* Forsk) was about 0.5-1 cm high (García-Torres et al., 1987).

As is common practice in the area, kabuli chickpea (cv Zoco) were planted in 0.38-cm-wide rows in February, at seeding rate of 80 kg $ha^{-1}$; faba bean (cv Alameda) were planted in 50 cm-wide rows in early December, at 170 kg $ha^{-1}$, and hard red spring wheat (cv Gazul) were planted in 18 cm-wide rows in late November at 150 kg $ha^{-1}$. Every year, wheat plots were also supplied with P fertilizer at a rate of 65 kg P $ha^{-1}$; the fertilizer was incorporated following the standard practice in conventional tillage soil and banded with drilling in no-tillage plots. Phosphorus soil analysis has shown that P (Olsen) is adequate in both tillage methods (7.8 and 10.4 mg/kg in NT and CT, respectively). The high rate applied of P fertilizer assures that P is not a limiting factor. The soil-available K was adequate (530 mg/kg).

Isotopic dilution method microplots (1 x 2 m) were established within each main wheat, chickpea and faba bean plot, and 100 and 30 kg $ha^{-1}$ of $^{15}N$-labeled fertilizer, were applied to wheat and both legumes, respectively, immediately after sowing. The fertilizer solution was formulated with $^{15}N$-enriched urea (2.5 atom % excess $^{15}N$). Treatments were applied to the soil surface of the microplot area in 4 L of distilled water per microplot, using a hand sprayer.

Chickpea and faba bean seeds were harvested at maturity, in July and June respectively, using a Nurserymaster Elite Plot Combine (30 $m^2$ per plot).

*2.3. Plant and soil analysis*

Two soil core samples were collected prior to sowing and after harvesting from each wheat plot, at a depth of 0-90 cm, using a manual Eijkelkamp auger. Soon after sampling, soils were frozen at -30ºC until analysis. Soils were analyzed for both nitrate and ammonium content using the Griess-Illosvay colorimetric method as modified by Barnes and Folkard (1951) and Kempers' colorimetric method (1974), respectively, using a Bran & Luebbe II Auto-Analyzer.

Nodulation was assessed at the late flowering stage of the chickpea and faba bean. Ten randomly selected plants from each plot were uprooted by excavating with a spade a circle with a radius of approximately 15 cm around the plant to a depth of about 30 cm. Soil adhered to the root was removed via washing with tap water (Somasegaran and Hoben, 1994). Nodules attached to each plant root were removed, and the total number of nodules as well as the dry weight was recorded (after drying to constant weight at 80 ºC)

At maturity, a 0.5 $m^2$ portion at the center of each chickpea and faba bean plot was sampled. From this sample, the biomass, harvest index and N uptake were measured (dropped leaves were included in the analyses). Dry matter was determined by drying the sampled plants to constant weight at 80 ºC. N uptake was determined by the analysis of N content of straw and grain, using the Dumas combustion method (Leco FP-428 analyzer).

*2.4. Nitrogen fixation*

The proportion of N in the chickpea and faba bean derived from $N_2$ fixation was calculated by the $^{15}$N-dilution and $^{15}$N natural abundance methods, using wheat as a reference crop (Rennie and Dubetz, 1986; Peoples and Herridge, 1990; Pilbeam et al., 1998; Unkovich and Pate, 2000).

For the $^{15}$N-dilution method, percentage $N_2$ fixation (Ndfa) was calculated as described by Larue and Patterson (1981):

$$\%\text{Ndfa} = 100\ (1 - \frac{\text{atom\% excess } ^{15}\text{N legume}}{\text{atom\% excess } ^{15}\text{N wheat}})$$

where atom% $^{15}$N excess = (% atom $^{15}$N sample - % atom $^{15}$N $N_2$ air) and atom% $^{15}$N of air $N_2$ = 0.3663.

For the $^{15}$N natural abundance method, percentage $N_2$ fixation was calculated using the equation proposed by Ledgard and Peoples (1988):

$$\% \text{ Ndfa} = 100 \times \left( \frac{\delta\,^{15}\text{N wheat} - \delta\,^{15}\text{N legume}}{\delta\,^{15}\text{N wheat} - \text{B}} \right)$$

where $\delta\,^{15}N$ wheat and $\delta\,^{15}N$ legume are parts per 1000 $^{15}N$ enrichment of N in wheat and chickpea and faba bean crops. The value B is a measure of isotopic fractionation during $N_2$ fixation and was determined by the analysis of the $\delta\,^{15}N$ of total plant N accumulated by a nodulated legume grown in N-free media, as applied to chickpea (B= -1.8 ‰) and faba bean (B = -1.7 ‰) and according to López-Bellido et al. (2010).

The amount of N fixed was calculated as follows:

$$\text{N fixed (kg ha}^{-1}\text{)} = (\%\text{Ndfa}/100) \times \text{N yield (kg ha}^{-1}\text{) of chickpea or faba bean crop}$$

*2.5. Statistical analysis*

Annual data for each parameter over the whole 6-year period were subjected to analysis of variance (ANOVA), using a year-combined randomized complete block design following McIntosh (1983). Treatment means were compared using Fisher´s protected least significant difference (LSD) test at a $P < 0.05$. LSDs for different main effect and interaction comparisons were calculated using the appropriate standard error terms following Gómez and Gómez (1984). The Statistix v. 8.1 (Analytical Software, 2005) package was used for this purpose.

*2.6. Weather conditions and soil nitrates*

The average annual rainfall in the area is 584 mm. Total rainfall in the study years ranged from 263 mm (2004-2005) to 702 mm (2003-2004) (Fig. 1). Three years (2001-2002, 2002-2003 and 2003-2004), had higher than average annual rainfalls, whereas the other three years (2004-2005, 2005-2006 and 2006-2007) had lower than average annual rainfalls (Fig. 1). The period 2004-2005 was extremely dry, and this severely affected the growth and yield of chickpea and faba bean. The annual rain distribution throughout the growing period also varied over the six years of the study. Overall, the autumn period had the highest percentage of annual rain. This period showed variations from 33% (2005-2006) to 53% of the total rainfall (2003-2004). During winter, this percentage fluctuated between 22% (2006-2007) and 40% of the total rainfall (2005-2006). During spring, as is typical for Mediterranean weather, a lower percentage of rain fell; the amount fluctuated between 7% (2002-2003) and 20% of the total (2004-2005). In 2006-2007, however, this percentage was as high as 33%.

Winter temperatures oscillated from 3 to 6 ºC for the mean minimum temperature and 16 to 19 ºC for the mean maximum temperature (Fig. 1). In the flowering pod fill period that encompasses the months of March and April for both legumes, the average minimum temperature fluctuated from 10 to 12 ºC, and the maximum varied from 22 to 27 ºC (Fig. 1).

The nitrate content before sowing in the soil profile (0-90 cm) for the chickpea plants during the 6 years of the study was 92 kg ha$^{-1}$, and does not significantly differ between the NT (81 kg ha$^{-1}$) and CT (104 kg ha$^{-1}$) systems. For the faba bean, the average nitrate content was 129 kg ha$^{-1}$, which was significantly higher in the NT system (141 kg ha$^{-1}$) than in the CT system (116 kg ha$^{-1}$).

## 3. Results

### *3.1. Comparison of $^{15}N$ isotopic dilution and $^{15}N$ natural abundance methods*

A significantly higher percentage of Ndfa was obtained with $^{15}N$ isotopic dilution (ID) respect to $^{15}N$ natural abundance (NA) for both legumes (Table 1). The remarkable differences recorded, around 20%, result in some uncertainty as to the most reliable method for the estimation of $N_2$ fixation.

In chickpea, %Ndfa ranged from 58 to 83% over the 6 years of study with the ID method, with a mean of 70% and standard deviation (SD) of ± 16.7 (Table 1). The %Ndfa values obtained with the NA method ranged from 36 to 67%, with a mean of 50 ± 18% (Table 1). The %Ndfa values for chickpea were generally higher in the first 3 years of study (Fig. 1.), which had the highest rainfall, when the ID method was used. The opposite occured with the NA method (Table 1).

In faba bean, %Ndfa ranged from 81 to 96% with the ID method, with a mean of 89 ± 9.9% (Table 1). With the NA method, %Ndfa ranged from 50 to 90%, with a mean of 68 ± 23.8%. The year-on-year %Ndfa values for faba bean were more homogeneous when the ID method was used; however, year-on-year, these values showed greater variability with the NA method, unrelated to the wet or dry status of the years.

The $\delta^{15}N$ values for both legumes and the reference plant did not differ in any case according to tillage system. In chickpea, $\delta^{15}N$ varied according to the years of study between 0.39 and 4.25‰, with a mean of 1.68 ± 1.33‰. In faba bean, $\delta^{15}N$ varied between -0.05 and 5.19 ‰, with a mean of 0.92 ± 1.7‰.

The reference plant had $\delta^{15}N$ values between 1.92 and 33.3‰ and a mean of 8.5 ± 5.6‰. The rainfall had a strong influence on the wide annual variation: the dry years (Fig. 1), especially during the active growth period of the wheat, showed considerably higher $\delta^{15}N$ values (Fig. 2). However, $\delta^{15}N$ values for chickpea were lower during those years, while $\delta^{15}N$ values for faba bean were not affected by the rainfall; however, 2005, the driest year with practically zero yield, had a higher average value (Fig. 2).

The soil $\delta^{15}N$ value did not differ between both tillage systems and had a mean of 6.98 ± 4.54‰. This data, apart from its strong variability, differs from the above-mentioned mean $\delta^{15}N$ value of the reference plant (8.5 ±5.6%).

Overall, the above-mentioned results show: (i) strong heterogeneity in the $\delta^{15}N$ values of the reference plant and, to a lesser degree, in the legumes, which can affect isotopic composition and $N_2$ fixation; (ii) high variability of $\delta^{15}N$ in the soil N available by the plant, limiting the accuracy of the NA method, which ultimately depends on the magnitude and uniformity of $\delta^{15}N$ in the plant-available soil N pool; (iii) variation between the $\delta^{15}N$ values of the reference plant and $\delta^{15}N$ of the soil N, which is a basic requirement for the use of the NA method; and (iv) consequently, the use of the $^{15}N$ NA method is unreliable under field conditions, therefore, the ID method was adopted for the determination of $N_2$ fixation by the legumes.

**3.2. Chickpea**

*3.2.1. Nodules*

The number and dry weight of the nodules per plant (nodules/plant) differed according to the tillage system. The interaction between year and tillage system was also significant (Table 2). Overall, the NT system had a higher number and dry weight of nodules/plant than the CT system, which also occurred in the years 2005 and 2006 for the number of nodules and in 2005, 2006 and 2007 for the dry weight of the nodules (Table 3). In 2004, chickpea plants showed a higher number and weight of the nodules in relation to other years (Table 3). The year of 2004 also showed the highest rainfall (Fig 1). However, the number and dry weight of the nodules did not clearly show a relation with seed yield in any of the years studied.

*3.2.2. Crop yield*

As a whole, seed yield for the chickpea was significantly influenced by the year but not by the tillage system although the interaction between year and tillage was highly significant (Table 2). In the years of 2002 and 2003, the NT system had a higher seed yield than the CT system; the result was inverse in 2006 (Table 3). The amount and distribution of annual rainfall influenced the chickpea's performance because this crop is sensitive to climatic variations during the short growing season and particularly during the flowering period, which takes place during the months of April and May. Grain yield was generally higher in the years that had greater amounts of rain in the months of April and May, which was more evident in 2002 and 2007 (Fig 1 and Table 3). In the years in which the effect of the tillage system on the seed yield was significant, the study did not observe a clear relation with rainfall.

Similar to seed yield, the N uptake by shoot and the harvest index (HI) were significantly influenced by the year but not by the tillage system. The interaction effect between year and tillage was also highly significant (Tables 2 and 3). The chickpea under the NT system had a higher extraction of N when compared to the CT system in 2003 and 2004, but it was the opposite in 2005 and 2006. The HI was only higher for the NT in relation to the CT system in 2003 and was lower in 2005 and 2006.

*3.2.3. Nitrogen fixation*

The percentage of Ndfa varied significantly according to the year and the tillage system (Table 2). The average percentage of Ndfa for the six years of the study was 70% according to the $^{15}N$ isotopic dilution method. The %Ndfa was higher for the NT system (76%) when compared to the CT system (64%). In the years 2002, 2003 and 2007 which had higher seed yields, the percentage of Ndfa was higher for the NT system in relation to the CT system (Fig.3).

The amount of fixed N is expressed in kg $ha^{-1}$ and varied significantly according to the year and with the tillage method (Table 2). Likewise, the interaction year x tillage was significant (Table 2). The amount of fixed N for chickpea was 31 kg N $ha^{-1}$ averaged over the 6 years of study. The amount of fixed N by the NT system resulted in a higher value with respect to the CT system (35 and 27 kg N $ha^{-1}$, respectively). In the years 2002, 2003 and 2004, the NT system had a higher amount of fixed N than the CT system. In 2006, however, the results were the opposite (Fig. 3). In the first case, both the higher Ndfa percentage and the higher seed yield in the NT system contributed to these results.

The contribution of shoot of the chickpea to soil N, as measured by the N balance (fixed N – seed N), was scarce (8 kg N $ha^{-1}$ as an average). Differences between the tillage systems were significant and favoured the NT system (data not shown).

## 3.3. Faba bean

*3.3.1. Nodules*

The number and dry weight of faba bean nodules/plant, similar to chickpea, differed according to the year and the tillage system. These differences were significantly higher for the interaction between year and tillage (Table 2). The NT system also showed a higher number and dry weight of nodules/plant than the CT method (Table 4). The average number of faba bean nodules/plant was more than 2.5 times higher than in chickpea although the faba bean dry weight was only about 70% higher than that of the chickpea (Tables 3 and 4). Similar to chickpea, the rainiest year (2004) had the highest number and dry weight for nodules, which was very different when compared to other years (Table 4). This was not clearly related to the seed yield of the faba bean because 2007 showed greater yields even with the lowest values for number and dry weight of the nodules/plant across the whole study (Table 4).

*3.3.2. Crop yield*

Faba bean seed yield was significantly influenced by the year and the tillage system. The interaction between year and tillage system was also significant (Table 2). Overall, the NT system had a notably higher seed yield than the CT system. This occurred in 4 of the 6 years of the study, three of which were the rainiest (Table 4). Unlike chickpea, the longer growing period for faba bean and the fact that they are planted at the end of autumn led the seed yield to be more dependent on the total amount of rainfall registered during the growing season (2001-2002, 2002-2003 and 2003-2004) (Fig. 1 and Table 4). However, in the year 2007 the faba bean had an elevated seed yield even though it was a relatively dry year. Although it was a dry year, the rain had good distribution throughout the growing season and was optimum during the period of formation and pod filling (Fig. 1, Table 4). In the year 2005, which was extremely dry, there was a drastic crop decrease that practically resulted in 0 values for the CT system (Table 4).

The HI and the N uptake by crop were both significantly influenced by the year and the tillage system, and the interaction between these factors was also significant (Table 2). In the NT system, faba bean had higher shoot N and obtained higher HI than in the CT system (Table 4).

*3.3.3. Nitrogen fixation*

The percentage of Ndfa was not significantly affected by year and the tillage system. The interaction was not significant either (Table 2). The average percentage of Ndfa for the 5 years of the study using the $^{15}N$ enrichment method was 89% (Figure 4). The percentage of Ndfa in faba bean showed a significant positive correlation with the number of nodules/plant (r = 0.80**).

The amount of fixed N (kg ha$^{-1}$) for faba bean differed according to the year and the tillage method (Table 2). The amount of fixed N for faba bean was 80 kg N ha$^{-1}$, averaged over the 5 years of study (Fig. 3). There was a significant direct correlation between the amount of fixed N and the seed yield (r = 0.98**) (Table 4 and Fig. 4). Values for fixed N were significantly higher in the NT system when compared to the CT system (91 and 69 kg N $ha^{-1}$, respectively) (Fig. 4).

The contributions of the shoot of faba bean to soil N measured by N balance was higher than in chickpea and had an average of 21 kg N $ha^{-1}$ (data not shown)

**4. Discussion**

The consistent differences found between the %Ndfa values using the $^{15}N$ ID and $^{15}N$ NA methods required the determination of the most reliable method according to the experimental data obtained and the criteria stated by Unkovich et al. (2008). Walley et al. (2001) and Holdensen et al. (2007) pointed out that the heterogeneity in the $\delta^{15}N$ of the reference plants and sometimes legumes may affect both the isotopic composition of soil mineral N and legume $N_2$ fixation. This heterogeneity may be associated with slight variations in topography, inducing localized differences in water content and soil mineral N, typical in dryland Vertisols. Our results show strong variability in the $\delta^{15}N$ values of both legumes and, above all, the reference plant, on a sample level (within each year) and interannually, with a clear influence of the dry and wet years.

Högberg (1997) also stated that high temperatures and low precipitation frequently create high values of $\delta^{15}N$ in the reference plants. Such conditions cause a high rate of $NH_3$ volatilization, especially in upper soil layers, which produces great enrichment of foliar $^{15}N$ in plants exposed to the aforementioned stress. The years 2004-2005 and 2005-2006, which had higher values for $\delta^{15}N$ (10.9 and 18.7 ‰, respectively), were the driest years, especially in the final period (winter-spring), which is the most active for wheat. The same occurred with the $\delta^{15}N$ for faba bean (3.7‰) but only in the year 2004-2005. Peoples et al. (2009b) also indicated a trend for higher $\delta^{15}N$ in cropping soils associated with the greater cycling, turnover and losses of N.

Lastly, Unkovich et al. (2008) pointed out that the accuracy of the $^{15}N$ NA technique ultimately depends upon the magnitude and uniformity of $\delta^{15}N$ in the plant-available soil N pool. The $^{15}N$ NA method assumes that the $\delta^{15}N$ of non $N_2$-fixing reference plants is identical to the $\delta^{15}N$ of soil N utilised by the legume. In our study the values of $\delta^{15}N$ of soil N showed great variability (6.98 ± 4.54‰) and differed from the mean value for the $\delta^{15}N$ of the reference plant (8.5 ± 5.6‰) almost by 18%. For these reasons the $^{15}N$ ID technique was adopted as the most reliable and solid method for the estimation of %Ndfa under the study conditions.

There are very few sources of information about the influence of tillage systems on the number and dry weight of nodules for the grain legume crops. Our study has confirmed that a no tillage system generates a higher number and dry weight of nodules/plant than CT systems for chickpea and faba bean. As indicated by Kessel and Hartley (2000) and Souza et al. (2003), the NT system can create an enabling environment in the soil for rhizobial because it preserves more humidity and favourably alters the temperature. Similarly, the relation between the number and biomass of nodules and the grain yield of the legumes has not been well studied in areas where they have long been cultivated and where it is not a normal practice to artificially inoculate seeds with rhizobial. The lack of a significant relationship between both parameters in our study has also been indicated by Ryan et al. (2009) in the Mediterranean for several legumes (including chickpea and faba bean). This could be attributed to the complexity of intervening factors under field conditions, such as low efficiency or ineffectiveness of the native rhizobial strains, or other aspects related to environmental and management conditions such as droughts, systematic application of fertilisers to crops that include cereal, other soil nutrient deficits, the endemic attack of the Sitona weevil and the application of herbicides among others.

For both legumes, the percentage of Ndfa varied according to the year. This variation was greater in chickpea (70 ± 17‰) than in faba bean (89 ± 10‰), and more related to the yearly rainfall in the first than in the second. The active growth period of chickpea is more dependent on spring rainfall, which is more erratic and limiting under Mediterranean weather conditions. On the contrary, the longer growth period of faba bean, planted at the end of autumn, permits better crop establishment and symbiosis with rhizobia. The mean %Ndfa of chickpea was located within the ranges indicated by Turpin et al. (2002), Aslam et al. (2003) and Peoples et al. (2009a), and is higher than the mean value of 65% cited by Herridge et al. (2008) (Fig. 3). In faba bean, our mean %Ndfa was higher than that reported for Europe by Peoples et al. (2009a) (74%) and West Asia (69%) and was above the range of 19-85% indicated by Rennie and Dubetz (1986) and Kessel and Hartley (2000) (Fig. 4).

The disparity between the $N_2$ fixation responses of chickpea and faba bean to the tillage system must be attributed to the particular physical and chemical characteristics of Vertisols, which may lead to similar behaviours by both tillage systems, and the previously mentioned differences in growth periods of both legumes. In this sense, the chickpea crop shows greater sensitivity to water shortage in the spring; therefore, the greater water availability of NT versus CT favours $N_2$ fixation. Also, the $NO^-_3$-N content of the soil in the wheat-chickpea rotation was lower under NT with respect to CT (81 and 104 kg N $ha^{-1}$,respectively), as indicated in the Material and Methods section ("weather conditions and soil nitrates"). However, in the wheat-faba bean rotation, N availability, expressed by the $NO^-_3$-N content of the soil, was greater under NT versus CT (140 and 116 kg N $ha^{-1}$, respectively); contrary to Kessel and Hartley (2000) and Souza et al. (2003) for other environmental conditions and types of soil.

The amount of fixed N varied considerably due to the marked year-on-year difference in biomass yield. Unlike %Ndfa, the average amount of fixed N in our experiments (Figs. 3 and 4) was notably lower than the values cited by Herridge et al. (2008) and Peoples et al. (2009a): between 40 and 50 kg N $ha^{-1}$ $yr^{-1}$ for chickpea and 100-150 kg N $ha^{-1}$ $yr^{-1}$ for faba bean. These low amounts of fixed N can be attributed to the year-on-year variability of seed yield in both crops caused by abiotic and biotic stress, especially the water deficit. However, under the optimal growth conditions in the years 2002 (for chickpea) and 2003 (for faba bean), particularly in terms of the amount and distribution of rainfall (Fig. 1), the means for fixed N obtained under NT (75 and 146 kg N $ha^{-1}$ $yr^{-1}$, for chickpea and faba bean, respectively) (Figs. 3 and 4) can be considered to reliably represent potential atmospheric $N_2$ fixation by chickpea and faba bean crops on Mediterranean Vertisols in southern Spain.

Faba bean crops had higher levels of $N_2$ fixation than chickpea as noted by Walley et al. (2007) and Herridge et al. (2008). Only in terms of above-ground biomass, we can affirm that faba bean can positively contribute to the overall N economy in dry Mediterranean systems, particularly when a cropping system is evaluated long-term. In contrast, chickpea can be considered a legume that typically achieves only modest levels of $N_2$

fixation because it is either N neutral or contributes to N deficit, as also pointed out Horn et al. (1996) and Pilbeam et al. (1998).

However, it is well known that the N balance fixed by legumes is underestimated when only the shoot contribution is considered and the N provided by roots and rhizodeposition is not taken into account. It therefore seems more realistic to also consider the below-ground pool of legume N when evaluating the real contribution of fixed N by legumes to agricultural systems. This is particularly important in the case of grain legumes such as chickpea and faba bean where large amounts of N tend to be removed in the seed at harvest. A parallel study performed during a 3-year period (2003-2004, 2005-2006 and 2006-2007) in the same experiment (López-Bellido et al. 2010) showed average values for the below-ground biomass N content of 124 and 106 kg N $ha^{-1}$ $yr^{-1}$ (NT and CT, respectively) for chickpea, and 146 and 74 kg N $ha^{-1}$ $yr^{-1}$ (NT and CT, respectively) for faba bean. This data supports the positive contribution of N to the soil by both legumes and highlighting the more favourable effect of no tillage.

## 5. Conclusions

The $^{15}N$ natural abundance method is less reliable than $^{15}N$ isotopic dilution for determining $N_2$ fixation by chickpea and faba bean under the conditions of Mediterranean dryland Vertisols.  The specific characteristics of these soils and the climatic variations, with a predominant water deficit, generate strong heterogeneity and differences in the $\delta^{15}N$ values of the soil N of the reference plant.

Strong inter-annual variations in the quantity and distribution of rain during the years of the study caused severe differences in the behaviour of both legumes in terms of grain yield, nodule biomass and $N_2$ fixation.

No tillage had a clear positive influence on the nodular biomass and $N_2$ fixation by both legumes with repect to conventional tillage. These differences should be attributed mainly to the great availability of water in the no tillage soil, since the availability of soil N did not vary much between both tillage systems. Nevertheless, chickpea exhibited a more consistent response to no tillage than faba bean, due to its greater sensitivity to environmental variables, especially water shortage in spring.

The interannual variation of fixed N expressed in kg N $ha^{-1}$ $yr^{-1}$ was greater than that of %Ndfa, due to the more marked differences in the biomass yield of both crops; which is more sensitive to water stress than the ability of the rhizobium to fix $N_2$. This would explain why the values for %Ndfa from our study are similar or even superior to those referenced in the literature, while the kg N $ha^{-1}$ $yr^{-1}$ of fixed N are generally lower.

In terms of above-ground biomass, faba bean positively contributes to the N economy in Mediterannean dryland agriculture when the crop system is evaluated long-term. On the contrary, chickpea, which only achieves modest levels of $N_2$ fixation, contributes little or nothing to the supply of net N inputs to the cereal-legume system. However, the panorama would be significantly more favourable if we consider radicular N and rhizodeposition.

Further investigations are required to determine and improve the behaviour of the native rhizobial strains in both legumes, traditionally cultivated without artificial inoculation, with the objective of better explaining the relationship between the nodular biomass and seed yield, response of $N_2$ fixation to droughts and how environmental factors and crop management interact with the genetic, physiological and agronomic aspects of new strains introduced by inoculation.

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**References**

Aslam, M., Mahmood, I.A., Peoples, M.B., 2003. Contribution of chickpea nitrogen fixation to increased wheat production and soil organic fertility in rain-fed cropping. Biol. Fertil. Soils 38, 59–64.

Analytical Software, 2005. Statistix 8.1. Tallahassee, FL, USA.

Barnes, H., Folkard, A.R., 1951. The determination of nitrates. Analyst 76, 599–603.

Carranca, C., Varennes de, A., Rolston D., 1999. Biological nitrogen fixation by faba bean, pea and chickpea, under field conditions, estimated by the $^{15}N$ isotope dilution technique. Eur. J. Agron. 10, 49–56.

Evans, J., Taylor, A.C., 1987. Estimating dinitrogen ($N_2$) fixation and soil accretion of nitrogen by grain legume. J. Aust. Inst. Agric. Sci. 53, 78–82.

Evans, J., McNeill, A.M., Unkovich, M.J., Fettell, N.A., Heenan, D.P., 2001. Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. Aust. J. Exp. Agric. 41, 347–359.

Gómez, K.A., Gómez, A.A., 1984. Statistical procedures for agricultural research. Wiley, New York.

García-Torres, L., Romero, F., Mesa, J., 1987. Agronomic problems and chemical control of broomrape (*Orobanche* spp.) in Spain, studies review, in: Weber, H.C., Forstreuter, W. (Eds.), Proc. of the 4th Symp. on Parasitic Flowering.

Herridge, D.F., Marcellos, H., Felton, W.L., Turner, G.L., Peoples, M.B., 1995. Chickpea increases soil-N fertility in cereal systems through nitrate sparing and $N_2$ fixation. Soil Biol. Biochem. 27, 545–551.

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural system. Plant Soil 311, 1–18.

Holdensen, L., Hauggaard-Nielsen, H., Jensen, E.S., 2007. Short-range spatial variability of soil $\delta^{15}N$ natural abundance─effects on symbiotic $N_2$–fixation estimates in pea. Plant Soil 298, 265– 272.

Horn, C.P., Birch, C.J., Dalal, R.C., Doughton, J.A., 1996. Sowing time and tillage practice affect chickpea yield and nitrogen fixation 1. Dry matter accumulation and grain yield. Aust. J. Exp. Agric. 36, 695–700.

Högberg, P., 1997. $^{15}N$ natural abundance in plant-soil system. New Phytol. 137, 179–203.

Kempers, A.J., 1974. Determination of sub-microquantities of ammonium and nitrate in soils with phenol, sodium nitroprusside and hypochlorite. Geoderma 12, 201–206.

Kessel, C., Hartley, C., 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? Field Crop. Res. 65, 165–181.

Kumar, J., Abbo, S., 2001. Genetics of flowering time in chickpea and its bearing on productivity in semiarid environments. Adv. Agron. 72, 107–138.

Kumar, K., Goh, K.M., 2000. Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. Adv. Agron. 68, 197–319.

Larue, T., Patterson, T.G., 1981. How much nitrogen do legumes fix? Adv. Agron. 34, 15–38.

Ledgard, S.F., Peoples, M.B., 1988. Measurements of nitrogen fixation in the field, in: Wilson, J.R. (Ed.), Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford, UK, pp. 351–367.

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. Effects of crop rotation and nitrogen fertilization on soil nitrate and wheat yield under rainfed Mediterranean conditions. Agronomie 21, 509–516.

López-Bellido, F.J., López-Bellido, R.J., Redondo, R., López-Bellido, L., 2010. B value and isotopic fractionation in N2 fixation by chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.). Plant Soil 337, 425–434..

Marcellos, H. Felton, W.L., Herridge, D.F., 1998. Chickpea in wheat-based cropping systems of Northern New South Wales I. $N_2$ fixation and influence on soil nitrate and water. Aust. J. Agric. Res. 49, 391–400.

McIntosh, M.S., 1983. Analysis of combined experiments. Agron. J. 75, 153–155.

Peoples, M.B., Herridge, D.F., 1990. Nitrogen fixation by legumes in tropical and subtropical agriculture. Adv. Agron. 44, 155–223.

Peoples, M.B., Palmer, B., Lilley, D.M., Due, L.M., Herridge, D.F., 1996. Application of $^{15}N$ and xylem ureide methods for assessing $N_2$ fixation of three shrub legumes periodically pruned for forage. Plant Soil 182, 125–137.

Peoples, M.B., Bowman, A.M., Gault, R.R., Herridge, D.F., McCallum, M.H., McCormick, K.M., Norton, R.M., Rochester, I.J., Scammell, G.J., Schwenke, G.D., 2001. Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. Plant Soil 228, 29–41.

Peoples, M.B., Hauggaard-Nielsen, H., Jensen, E.S., 2009a. The potential environmental benefits and risks derived from legumes in rotations, in: Emerich, D.W., Krighman, H.B. (Eds.), Agronomy Monograph 52, ASA, Madison Wi, pp. 349–385.

Peoples, M.B., Unkovich, M.J., Herridge, D.F., 2009b. Measuring symbiotic nitrogen fixation by legumes, in: Emerich, D.W., Krighman, H.B. (Eds.), Agronomy Monograph 52, ASA, Madison Wi, pp. 125–170.

Pilbeam, C.J., Wood, M., Harris, H.C., Tuladhar, J., 1998. Productivity and nitrogen use of three different wheat-based rotations in North West Syria. Aust. J. Agric. Res. 49, 451–458.

Rennie, R.J., Dubetz, S., 1986. Nitrogen-15-determined nitrogen fixation in field-grown chickpea, lentil, fababean and field pea. Agron. J. 78, 654–660.

Rupela, O.P., Saxena, M.C., 1987. Nodulation and nitrogen fixation in chickpea, in Saxena, M.C., Singh, K.B. (Eds). The chickpea. CAB International. Wallingford, Oxon, United Kingdom, pp. 191–206.

Ryan, J., Ibrikci, H., Sovrimer, R., McNeill, A., 2009. Nitrogen in rainfed and irrigated cropping systems in the Mediterranean region. Adv. Agron. 104, 53–136.

Schwenke, G.D., Peoples, M.B., Turner, G.L., Herridge, D.F., 1998. Does nitrogen fixation of commercial dryland chickpea and faba bean crops in North-west New South Wales maintain o enhance soil nitrogen? Aust. J. Exp. Agric. 38, 61–70.

Souza, A., Colozzi-Filho, A., Giller, K.E., 2003. The soil microbial community and soil tillage, in: El Titi, A. (Ed.), Soil Tillage in Agroecosystems, CRC Press, London, pp. 51–81.

Somasegaran, P., Hoben, J.J., 1994. Handbook for rhizobia methods in legume-*Rhizobium* technology. Springer, Heidelberg, New York, pp. 450.

Turpin, J.E., Herridge, D.F., Robertson, M.J., 2002. Nitrogen fixation and soil nitrate interactions in field-grown chickpea (*Cicer arietinum*) and fababean (*Vicia faba*). Aust. J. Agric. Res. 53, 599–608.

Unkovich, M.J., Pate J.S., 2000. An appraisal of recent field measurements of symbiotic $N_2$ fixation by annual legumes. Field Crop. Res. 65, 211–228.

Unkovich, M.J. Herridge, D., Peoples, M., Cadisch, G., Boddey, B., Giller, K., Alves, B., Chalk, P., 2008. Measuring plant associated nitrogen fixation in agricultural systems. ACIAR Monograph Nº 136. pp. 258.

Walley, F., Fu, G., van Groenigen, J.-W., van Kessel, C., 2001. Short-range variability of nitrogen fixation by field-grown chickpea. Soil Sci. Soc. Am. J. 65, 1717–1722.

Walley, F.L., Clayton, G.W., Miller, P.R., Carr, P.M., Lafond, G.P., 2007. Nitrogen economy of pulse crop production in the Northern Great Plains. Agron. J. 99, 1710–1718.

Table 1. Influence of method for estimating $N_2$ fixation on N derived from atmosphere (%Ndfa) of chickpea and faba bean.

| $^{15}$N Method[a] | Year | | | | | | Mean[c] |
|---|---|---|---|---|---|---|---|
| | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | |
| *Chickpea:* | | | | | | | |
| ID | 73a[b] | 67a | 83a | 59a | 79a | 58a | 70A |
| NA | 39b | 36b | 38b | 65a | 67a | 55a | 50B |
| *Faba bean:* | | | | | | | |
| ID | 81a | 96a | 94a | - | 87a | 86a | 89A |
| NA | 50b | 78a | 59b | - | 90a | 62b | 68B |

[a] ID:$^{15}$N isotopic dilution; NA: $^{15}$N natural abundance
[b] Comparision for the same level of year and 15N method according to LSD (P<0.05)
[c] Capital letters indicate significant differences for mean according to LSD (P<0.05)

Table 2. Significant effects of year and tillage system on nodules plant$^{-1}$, shoot N, seed yield, harvest index (HI), N derived from the atmosphere (%Ndfa) and fixed N of chickpea and faba bean crops under rainfed Mediterranean conditions during 6 years.

| Source | Nodules plant$^{-1}$ | | Shoot N | Seed yield (kg ha$^{-1}$) | HI | %Ndfa | Fixed N (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | Nº | Dry weight (g) | | | | | |
| *Chickpea:* | | | | | | | |
| Year (Y) | *** | *** | *** | * | * | *** | *** |
| Tillage (T) | *** | ** | NS | NS | NS | * | ** |
| Y x T | * | ** | *** | *** | *** | NS | *** |
| *Faba bean:* | | | | | | | |
| Year (Y) | *** | *** | *** | *** | *** | NS | *** |
| Tillage (T) | ** | ** | *** | *** | *** | NS | *** |
| Y x T | *** | *** | * | * | *** | NS | NS |

Table 3. Effects of tillage system and year on number of nodules plant$^{-1}$, nodules weight plant$^{-1}$, seed yield, shoot N and harvest index of chickpea crop under rainfed Mediterranean conditions.

| Index | Tillage system[a] | Year[b] | | | | | | Mean | LSD[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | | |
| Nodules plant$^{-1}$ | NT | - | - | 50a | 31a | 28a | 20a | 32A | 6 |
| | CT | - | - | 49a | 16b | 18b | 15a | 24B | |
| Nodules weight plant $^{-1}$ (g) | NT | - | - | 0.53a | 0.10a | 0.13a | 0.17a | 0.23A | 0.05 |
| | CT | - | - | 0.57a | 0.02b | 0.07b | 0.09b | 0.19B | |
| Seed yield (kg ha$^{-1}$) | NT | 1248a | 872a | 723a | 256a | 311b | 673a | 680A | 451 |
| | CT | 878b | 498b | 560a | 511a | 971a | 830a | 708A | |
| Shoot N (kg ha$^{-1}$) | NT | 85a | 45a | 47a | 25b | 32b | 29a | 44A | 16 |
| | CT | 75a | 21b | 32b | 39a | 50a | 38a | 43A | |
| Harvest index | NT | 29a | 47a | 44a | 25b | 26b | 51a | 37A | 6 |
| | CT | 27a | 15b | 41a | 38a | 46a | 48a | 36A | |

[a] NT: no tillage; CT: conventional tillage
[b] For each year and average followed by the same letter are not significantly different at $P<0.05$ according to LSD
[c] LSD for different levels of year

Table 4. Effects of tillage system and year on number of nodules plant$^{-1}$, nodules weight plant$^{-1}$, seed yield, shoot N and harvest index of faba bean crop under rainfed Mediterranean conditions.

| Index | Tillage system[a] | Year[b] | | | | | | Mean | LSD[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | | |
| Nodules plant$^{-1}$ | NT | - | - | 156a | 56a | 93a | 17a | 80A | 24 |
| | CT | - | - | 164a | 49a | 33b | 11a | 64B | |
| Nodules weight plant$^{-1}$ (g) | NT | - | - | 0.33b | 0.08a | 0.20a | 0.04a | 0.16A | 0.04 |
| | CT | - | - | 0.39a | 0.04b | 0.05b | 0.02a | 0.12B | |
| Seed yield (kg ha$^{-1}$) | NT | 1135a | 2687a | 1935a | 230a | 1040a | 1935a | 1493A | 456 |
| | CT | 588b | 2321b | 1020b | 10a | 179b | 1565a | 947B | |
| Shoot N (kg ha$^{-1}$) | NT | 58a | 156a | 109a | 15a | 68a | 116a | 87A | 30 |
| | CT | 53a | 149a | 60b | 9a | 38b | 84b | 66B | |
| Harvest index | NT | 61a | 52a | 45a | 42a | 39a | 47a | 48A | 14 |
| | CT | 26b | 48a | 37a | 5a | 14a | 49a | 29B | |

[a] NT: no tillage; CT: conventional tillage
[b] For each year and average followed by the same letter are not significantly different at $P<0.05$ according to LSD
[c] LSD for different levels of year

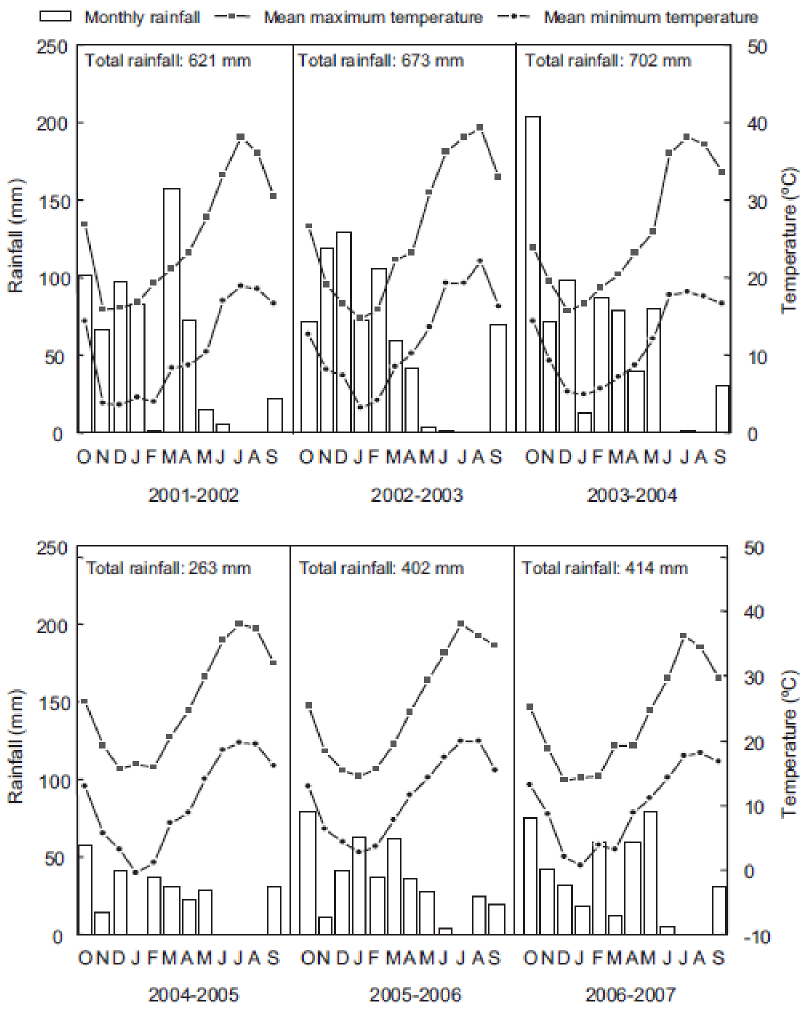


**Fig. 1.** Monthly and annual rainfall and mean maximum and minimum temperature for 6 years at Malagón Experiment, Córdoba, Spain.

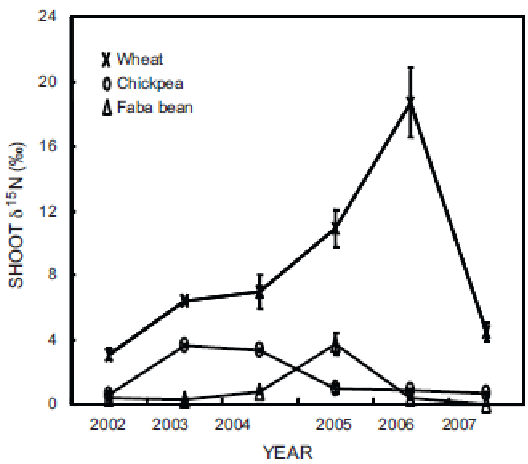


**Fig. 2.** Variation of $\delta^{15}N$ in chickpea, faba bean and reference plant (wheat) according to year. Vertical bars represent standard error.

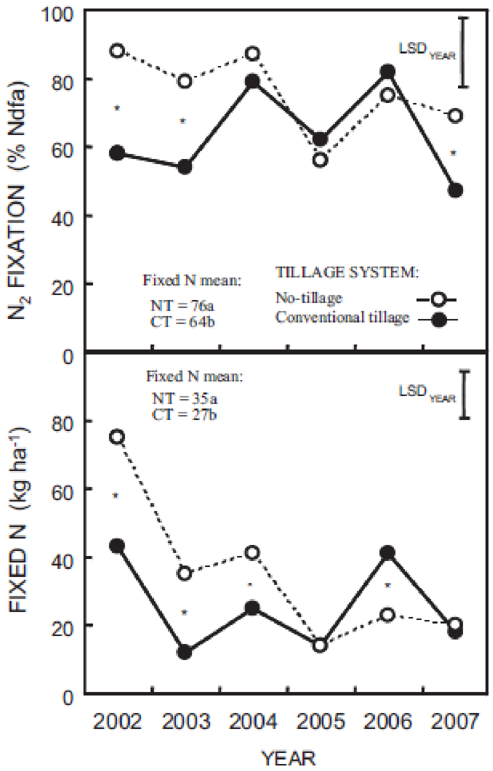


**Fig. 3.** Chickpea $N_2$ fixation as influenced by year and tillage system. The asterisk (*) represents significant difference between tillage systems. Vertical bar represent LSD for different levels of year.

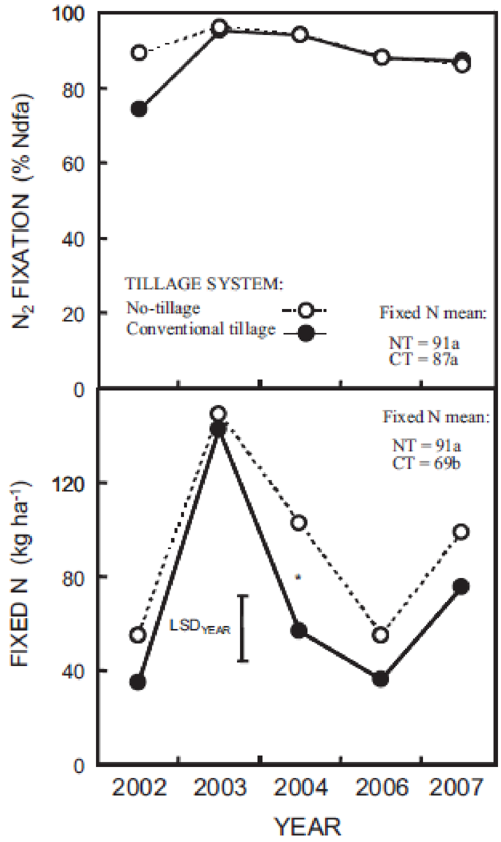


**Fig. 4.** Faba bean $N_2$ fixation as influenced by year and tillage system. The asterisk (*) represents significant difference between tillage systems. Vertical bar represent LSD for different levels of year.