Nitrogen management in double-annual cropping system (barley-maize) under irrigated Mediterranean environments

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Abstract

Improving nitrogen (N) use efficiency (NUE) is an agricultural necessity, as it can contribute to increasing crop productivity, while decreasing environmental degradation. Double-annual cropping systems can reduce N losses while increasing productivity, and profitability per land unit. The objective of this study was to evaluate annual N fertilization strategies in a double-annual cropping system (barley-maize) under irrigated Mediterranean environments. A three-year N fertilization experiment (2013-2016) was implemented with combinations of different N rates applied to barley (0 and 100 kg N ha−1) and maize (0, 100, 200 and 300 kg N ha−1). Grain and biomass yields and N content, plant N uptake, residual N, N efficiency, soil organic carbon (SOC) and economic return (ER) of the double-annual barley-maize cropping system were determined. The optimum rate of N to achieve maximum yields in the global barley-maize system was 230-240 kg
N ha$^{-1}$, split between barley and maize. Potential annual yields of the double-annual barley-maize cropping system under irrigated Mediterranean environments could be as high as 20 Mg ha$^{-1}$ yr$^{-1}$ for grain (6.71 and 13.42 Mg ha$^{-1}$ for barley and maize, respectively) and 35 Mg ha$^{-1}$ yr$^{-1}$ for biomass. The long growing period of the double-cropping systems contributed to promote high recovery of the post-harvest residual N. Barley yield increased with high residual N of the maize, whereas maize yield was not affected by the residual N of the barley. Non-N fertilized barley achieved up to 5.79 Mg ha$^{-1}$ when 300 kg N ha$^{-1}$ were applied to the previous maize. After three years of the study, SOC did not change in any of the N treatments, even in the treatments with the highest N deficiency (0 kg N ha$^{-1}$ yr$^{-1}$ applied). Further research is needed to fine-tune the N fertilization strategy over long-term periods for the double-annual barley-maize cropping system.

**Highlights:**

- Annual grain yields of 20 Mg ha$^{-1}$ can be achieved in barley-maize annual rotation.
- Double-annual barley-maize system used efficiently the N fertilizer.
- High soil residual N after maize harvest increased yields of the non-N fertilized barley.

**Keywords:** Biomass, corn, double cropping, economic return, fertilizer efficiency, grain, winter cereal.

**Abbreviations:** ANR, apparent nitrogen recovery; C, carbon; EONR, economic optimum nitrogen rate; ER, economic return; GS, growing season; N, nitrogen; N$_{\text{available}}$, available nitrogen; N$_{b}$, barley nitrogen treatment; N$_{m}$, maize nitrogen treatment; N$_{\text{rate}}$, nitrogen rate;
NRE, nitrogen recovery efficiency; NUE, nitrogen use efficiency; OM, organic matter; OM; SOC, soil organic carbon, SOC.

1. Introduction

World agriculture is currently facing unprecedented challenges. There is a need to increase food production to meet global food demand (Bodirsky et al., 2014), while reducing production costs and pollution. Nowadays, the main method to increase crop yields while maintaining or restoring soil nutrients is probably the application of mineral fertilizers, mainly N (Hirel et al., 2011).

The reliable supply of N and other macronutrients, as well as plant breeding improvements, has allowed a large increase in crop production per land unit over the past century. Nitrogen fertilization has promoted economic development, allowing the increase of populations, and sparing forests that would probably otherwise have been converted to agricultural land to meet food demand (Foley et al., 2011). In the most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant and may be lost by leaching, denitrification or volatilization (Martínez et al., 2017; Raun and Johnson, 1999). This means that more than half of the N used for crop fertilization is maybe lost into the environment (Lassaletta et al., 2014). The apparent nitrogen recovery (ANR) has been estimated in 65% and 57% for maize and wheat, respectively (Ladha et al., 2005). Hence, improving nitrogen use efficiency (NUE) in cropping systems across the globe is an absolute necessity, as it is one of the most effective means of increasing crop productivity while decreasing environmental degradation (Cassman et al., 2002; Davidson et al., 2015).

In highly productive irrigated lands, excessive N rates are frequently applied. Data from surveys in the Ebro Valley (a semi-arid irrigated area in NE Spain), where irrigated
maize is one of the most important and high N-demanding crops (Maresma et al., 2016), indicate that farmers normally apply rates of 318-453 kg N ha$^{-1}$ yr$^{-1}$ (Cavero et al., 2003; Isidoro et al., 2006; Sisquella et al., 2004). Normal maize grain yields in the area range from 12 to 15 Mg ha$^{-1}$, with total plant N uptake of 250-300 kg ha$^{-1}$ (Berenguer et al., 2008; Cela et al., 2011; Martínez et al., 2017; Yagüe and Quílez, 2010). Therefore, when excess N fertilizer is applied, there is a high risk of N leaching during the maize intercrop period (October to April) (Martínez et al., 2017; Moreno et al., 1996) depending on the rainfall distribution during the time there is not crop in the field (Salmerón et al., 2011).

To avoid post-harvest leaching of residual N and to increase production and profitability per land unit, double-annual cropping systems could be implanted. Winter cover crops after summer crops can provide environmental benefits that make them suitable for using the residual N and enhance NUE (Miguez, 2005; Quemada et al., 2013). In double-annual cropping systems, soil is covered during a longer period of the year than with mono-cropping systems. This entails several benefits, including prevention of soil erosion by wind and water (Hirel et al., 2011), increase of total dry matter production (Lloveras, 1987a, 1987b; Yagüe and Quílez, 2013), increase of land gross margin per land unit (Gil, 2013), and reduction of NO$_3^-$-N losses (Gabriel and Quemada, 2011; Krueger et al., 2012), among other aspects. However, the increased uptake of N and other nutrients with double-cropping systems, coupled with higher productivity, presents a significant challenge for maintenance of soil fertility, requiring higher rates of fertilization, and potentially leading to reductions in soil organic C if crop residues are not retained in fields (Heggenstaller et al., 2008).

Double-annual forage cropping strategies (summer crop-winter crop) have been increasingly applied in southern Europe during recent years (Ovejero et al., 2016). A summer crop (sorghum or maize) is grown from June to October, and in November a
winter cereal such as barley or triticale is subsequently sown as in other forage production areas (Lloveras, 1987a, 1987b; Monaco et al., 2008; Trindade et al., 2001). Double-annual forage crop production is usually associated with dynamic livestock farming, where animals are fed with forages and their faeces, usually mixed with straw, are applied to crops as fertilizer (Lloveras, 1987a; Perramon et al., 2016, Raphalen, 1980). Several authors have reported studies of double-annual cropping systems with N organic fertilization in Mediterranean environments (Grignani et al., 2007; Ovejero et al., 2016; Perramon et al., 2016; Yagüe and Quílez, 2010). However, there is limited research on double-annual cropping system unlinked to livestock farming. Thus, there is a need to evaluate the fertilization strategy, productivity, sustainability and economic profitability of a double-annual cropping system under irrigated Mediterranean environments unlinked to livestock farming.

The objectives of the present research were i) to determine the effect of annual N fertilization on grain and biomass yields, N uptake, soil NO$_3^-$-N content, N efficiencies, soil organic carbon (SOC) and economic return (ER), and ii) to assess and optimize the N management, in a double-annual cropping system (barley-maize) under irrigated Mediterranean environments.

2. Materials and methods

2.1. Study area

A three-year experiment (2013-2016) was conducted in Algerri (Lleida, NE Spain) under irrigated conditions (41º 46.5’ N, 0º 38.7’ E). The experiment was implemented in a commercial field and comprised an area of approximately 190 m × 18 m, with an individual plot size of 60 m$^2$. The sprinkler irrigation system (18 m × 18 m
grid) was built in 2004. Since that time, the farmer practiced annual rotation of winter cereals and maize, where the stover of the cereal crops was incorporated after each harvest.

The study area is characterized by a semi-arid climate with low annual precipitation (373 mm) and high annual average temperature (14.3 °C). During the first, second and third growing season (GS), the annual precipitation and average temperature were respectively 404 mm and 14.4 °C, 430 mm and 14.6 °C, and 427 mm and 14.5 °C. Each GS, around 150 and 650 mm of irrigation water (lacking nitrate) were respectively provided to barley and maize to avoid any hydric stress. Soils were classified as Petrocalcic Calcixerepts (Soil Survey Staff, 2014) and had a petrocalcic horizon (Bkm) at 0.82 m depth. Soil quality indicators and physicochemical parameters were analysed using standard methods (MAPA, 1994): soil texture, pH, electrical conductivity (EC), available P (Olsen P) and extractable K (NH₄Ac) (Table 1).

### 2.2. Experimental design

Eight different N combinations in the double-annual cropping system (barley-maize) were considered in a split-plot design with four replications. The N treatment in barley (winter crop) was the main plot (0 and 100 kg N ha⁻¹) while the N treatments in maize (summer crop) were the subplots (0, 100, 200 and 300 kg N ha⁻¹).

The barley and maize N fertilization treatments were randomized at the beginning of the experiment in 2013. Thereafter, the N treatments were applied in the same plots for the other GS. The N fertilizer used in both crops was ammonium nitrate (34.5%). In barley, sidedress was applied at one time in early February (DC 25-27 of the scale of Zadoks; Zadoks et al., 1974), whereas in maize the N fertilizer was split into two equal sidedresses (50% at V5 and 50% at V10 stage, 5 and 10 leaves with visible leaf collar).
Phosphorus and potassium were manually applied every year during winter over the barley crop at rates of 150 kg P\(_2\)O\(_5\) ha\(^{-1}\) and 250 kg K\(_2\)O ha\(^{-1}\), to avoid deficiencies of those elements.

2.3. Cropping system

Barley and maize were managed according to good and normal practices in the area, and maintained over the three years of the experiment.

- Barley: Conventional tillage was done before planting, after the maize harvest. It included disc ploughing and cultivation to a depth of 30 cm to incorporate previous maize stover and to prepare the soil for the sowing of the barley. The variety *Gustav* was sown at a rate of 230 kg seed ha\(^{-1}\), with 12 cm between rows. One herbicide treatment was applied post-emergence to control weeds (Fluroxipir 20%, at 1 L ha\(^{-1}\)). The grain and biomass were harvested between the first and second week of June.

- Maize: The barley stover was removed from the field. Maize was planted with no tillage to reduce the time gap between barley harvest and maize planting. The hybrid *PR32W86* (FAO cycle 600) was sown at a rate of 90,000 seeds ha\(^{-1}\), with 71 cm between rows. Two herbicide treatments were applied: one at pre-emergence to control the majority of weeds (S-Metolachlor 40% and Terbuthylazine 18.75%, at 3 L ha\(^{-1}\)) and the other at post-emergence to control *Abutilon theophrasti M.* and *Sorghum halepense L.* (Dimethylamine salt of dicamba 48.2% at 1 L ha\(^{-1}\) and Nicosulfuron 6% at 0.75 L ha\(^{-1}\)).

Biomass yield was measured at physiological maturity in the first week of October, and the grain was harvested between the last week of October and first week of November.

2.4. Sampling and analytical procedures
All the analysis were done for each individual plot. Barley and maize grain yields were measured by harvesting the central area (15 m²) with an experimental harvester. Moisture of the grains was determined in grain samples (250 g) using a GAC II grain analysis computer (Dickey-john, Auburn, IL, USA), and then grain yields were adjusted to 14% moisture. Barley and maize biomass yield was respectively determined in a 1.5 m² and 7 m² area. The dry matter content of the aboveground biomass was measured drying a sample of 250 g at 60ºC for 48h. Biomass (whole plant) and grain N concentration of barley and maize were determined in milled samples by near infrared (NIR) spectroscopy, using a previously calibrated 500 Infrared Analyser (Bran+Luebbe, Norderstedt, Germany). Total N uptake of the barley and maize was calculated multiplying whole plant N content by dry matter at harvest.

Soil NO₃⁻-N was measured after the barley and maize harvest at a depth of 0-82 cm from three consecutive layers (0-30, 30-60 and 60-82 cm). The maximum sampling depth was 0.82 m due to the presence of a petrocalcic horizon at this depth. The soil NO₃⁻-N was determined using an individual soil sample comprised by five cores distributed among the plot. Soil nitrates were extracted using deionized water and measured using test strips with a Nitrachek® device calibrated according to the standard procedure (Bischoff et al., 1996). NH₄⁺-N was not measured because several previous research studies in the area had considered negligible the amount of N when compared to the N present in nitrate form (Villar-Mir et al., 2002; Berenguer et al., 2009). Each year after the maize harvest, individual soil samples of the 0-30 cm soil layer were used to determine the soil organic carbon (SOC) content by the Walkley-Black dichromate oxidation method (Allison, 1965). The N mineralized from organic matter (OM) was estimated every year for barley and maize in the control treatment (0 kg N ha⁻¹ yr⁻¹) assuming nitrate leaching, ammonia losses negligible. It was calculated summing final N
in the soil and uptaked N by the crop, and subtracting the initial N in the soil (Sexton et al., 1996).

Three N-efficiency parameters were calculated in both crops: the NUE (Quemada and Gabriel, 2016; Zhang et al., 2015; EUNEP, 2015), the N recovery efficiency (NRE) (Ladha et al., 2005) and the apparent N recovery fraction (ANR) (Fageria and Baligar, 2005; López-Bellido et al., 2005). The NUE was determined as the ratio between the total N removed by the aboveground crops divided by the sum of all N inputs (kg kg\(^{-1}\)). The NRE was calculated as the ratio between aboveground plant N uptake and fertilizer N input (kg kg\(^{-1}\)). ANR (kg kg\(^{-1}\)) was the ratio between aboveground plant N uptake at Nx – aboveground plant N uptake at N0 and the amount of mineral N applied at Nx.

The economic return (ER) was calculated as the difference between the income produced by the selling of the grain yield and the cost of the N fertilizer applied. The rest of the costs entailed in the double-annual cropping system barley-maize were fix and they were not included in the ER analysis. The ER does not correspond with the net income perceived by farmers, it is an estimation of the variable expenses and incomes. To calculate the farmer’s net income, the fixed costs of the farm will need to be discounted to the ER. The N:grain price ratio is defined as the price per kilogram of N divided by the price per kilogram of grain (price ratio = price of fertilizer N, € kg\(^{-1}\) N/price of grain, € kg\(^{-1}\) grain) (Sripada et al., 2008). In the present study, the N:grain price ratios used were 5.6:1 and 5.3:1 for barley and maize, respectively. The N price considered was 0.90 € kg\(^{-1}\) of N (N fertilizer plus application cost) and the grain prices were 0.16 and 0.17€ kg\(^{-1}\) for barley and maize, respectively. The prices were determined as the average prices of the three years of the experiment (2013-2016).

2.5. Statistical analysis
Data analysis was performed using the JMP Pro 12 software (SAS Institute, Cary, USA). The experiment was arranged as a randomized complete block design with four replications, and analysed as a split-plot in time (Steel and Torrie, 1980). A mixed-effects analysis of variance (ANOVA) was carried out to assess the responses to mineral N fertilization, with GS evaluated as repeated measurements. The N treatment was defined as between-subject (fixed) factor, the GS was the within-subjects (fixed) factor and the replicate (block) effect and the replicate by N treatment interaction were considered as random effects. The interaction between N treatment and GS was also included in the model as fixed effect. Means were compared by Tukey’s HSD test (p<0.05), where levels not connected by the same letter are significantly different.

Linear-plateau regression analyses were carried out between grain and biomass yields and the total N applied to determine the rate of N ($N_{\text{rate}}$) that achieve maximum yields and optimum economic return (EONR) in the double-annual (barley-maize) cropping system.

3. Results

3.1. Grain and biomass yields

Grain and biomass yields varied over the years with the same N treatments for both studied crops (barley and maize). The highest average annual grain and biomass yields were achieved with annual $N_{\text{rate}}$ of 200 kg N ha$^{-1}$ or above (Table 2). The maximum average grain and biomass yields were 20.13 and 34.77 Mg ha$^{-1}$, respectively. The $N_{\text{rate}}$ applied to barley (0 or 100 kg N ha$^{-1}$) affected barley yields but did not affect maize yields. Independently of the maize treatment, the fertilized barley achieved yields above 5.7 Mg ha$^{-1}$ of grain and 8.9 Mg ha$^{-1}$ of biomass. However, the non-fertilized barley only achieved those yields when the previous maize received 300 kg N ha$^{-1}$. On average,
barley yielded 3.8 Mg of grain ha\(^{-1}\) and 6.4 Mg of biomass ha\(^{-1}\) with the non-fertilized (0 kg N ha\(^{-1}\)) treatment, whereas the fertilized treatment (100 kg N ha\(^{-1}\)) yielded 6.1 Mg of grain ha\(^{-1}\) and 9.5 Mg of biomass ha\(^{-1}\) (Table 2).

The N\(_{\text{rate}}\) applied to maize affected the grain and biomass yields of barley, maize and the annual sum of cereal. Maximum maize yields (about 12.5 and 22 Mg ha\(^{-1}\) of grain and biomass, respectively) were achieved with maize N\(_{\text{rate}}\) of 100 kg N ha\(^{-1}\) or above, independently of the N\(_{\text{rate}}\) applied to the barley.

The N\(_{\text{rate}}\) applied in barley and maize affected total annual grain and biomass yields per GS. The control N treatment (0 kg N ha\(^{-1}\) yr\(^{-1}\)) obtained the lowest annual yields. The N\(_{\text{rate}}\) that totalled 100 kg N ha\(^{-1}\) yr\(^{-1}\) between both crops yielded more than the control N treatment but less than higher N rates. The annual N\(_{\text{rate}}\) of 200, 300 and 400 kg N ha\(^{-1}\) applied to the system obtained the maximum annual yields. Annual average yields ranged from 17.7 to 20.1 Mg of grain ha\(^{-1}\) and from 30.6 to 34.8 Mg of biomass ha\(^{-1}\), for the 200 and 400 kg N ha\(^{-1}\) yr\(^{-1}\), respectively. The optimum total N\(_{\text{rate}}\) to achieve maximum grain and biomass yields was 232.5 and 240.5 kg N ha\(^{-1}\) yr\(^{-1}\), respectively (Figure 1).

The GS was significant in both grain and biomass yields for barley and maize (data not shown). Average standard deviation of 1.42, 1.40, 1.73, and 4.12 Mg ha\(^{-1}\) were observed for barley grain, maize grain, barley biomass and maize biomass yields, respectively. The control N treatment (0 kg N ha\(^{-1}\) yr\(^{-1}\)) showed the largest variability within the GS (up to 35% of variation for the barley biomass yield). On average, barley yields were more variable over the GS (29% and 23% for grain and biomass, respectively) than maize yields (13% and 19% for grain and biomass, respectively).
3.2. Biomass and grain N content and total N uptake

Biomass and grain N content (both barley and maize) varied from year to year and were affected by maize N$_{\text{rate}}$, but not by barley N$_{\text{rate}}$ (Table 2). Barley N content varied from 15.4 to 17.6 g kg$^{-1}$ (grain) and from 11.2 to 13.9 g kg$^{-1}$ (biomass), whereas the N content variation in maize was from 11.2 to 12.7 g kg$^{-1}$ in grain and from 9.0 to 10.7 g kg$^{-1}$ in biomass. The control N treatment (0 kg N ha$^{-1}$) showed the lowest maize N content in grain and biomass, but it did not show the lowest N content for barley. The N content among treatments was greater in grain than in biomass, and in barley than in maize.

The average N uptake from both crops during a GS ranged from 201.8 to 398.7 kg N ha$^{-1}$ depending on the N$_{\text{rate}}$ applied (Figure 2). Maize N uptake was affected by the GS and by the maize N$_{\text{rate}}$, but there was not interaction between them. Barley N fertilization affected barley N uptake but not maize N uptake. No differences were found in the total annual N uptake between N$_{\text{rate}}$ of 200 kg ha$^{-1}$ and 300 or 400 kg ha$^{-1}$, although a rising trend was observed in the total N uptake when increasing the N$_{\text{rate}}$ (Figure 2).

3.3. Soil NO$_3^-$-N content

Residual soil NO$_3^-$-N content after the barley and maize harvests in the studied depths (0-30, 30-60, 60-82 and 0-82 cm) were affected by the GS and by maize N fertilization (except in the top 30 cm after the barley harvest) (Figure 3). However, barley N fertilization did not affect the amount of the residual N content in the soil after the harvest of either barley or maize. A rising tendency in residual soil NO$_3^-$-N was observed when increasing the N$_{\text{rate}}$ applied in maize (Figure 3a and Figure 3b). Consequently with the higher N rates applied in maize (up to 300 kg N ha$^{-1}$) than barley (100 kg N ha$^{-1}$), higher residual soil NO$_3^-$-N contents were determined after the maize harvest than the barley harvest.
After the maize harvest, most of the residual soil $\text{NO}_3^-$-N was in the first 30 cm of soil (Figure 3b) for all the N treatments. However, after the barley harvest, most of the residual soil $\text{NO}_3^-$-N was between 60 and 82 cm of depth (Figure 3a). The N mineralized from the OM was estimated as 25 kg N ha$^{-1}$ for barley and 169 kg N ha$^{-1}$ for maize on average for the 3 GS. It ranged from 15 to 47 kg N ha$^{-1}$ for barley and from 145 to 191 kg N ha$^{-1}$ for maize depending on the GS.

### 3.4. N efficiencies

The overall year NUE, the NRE and the ANR were significantly affected by maize N fertilization and GS (Table 3). Consistently, N efficiencies were significantly higher with lower N$_{\text{rate}}$ applied (Table 3).

The highest NUE per GS, were determined for the non-fertilized treatment (1.06 kg kg$^{-1}$) and for the annual N$_{\text{rate}}$ of 100 kg ha$^{-1}$ (0.96 and 0.95 kg kg$^{-1}$). The NRE and ANR for either barley, maize or both crops together showed similar tendencies than NUE. For instance, ANR was up to 70% in maize and 80% in both crops together with applications of 100 kg N ha$^{-1}$ yr$^{-1}$. However, with applications of 300 kg N ha$^{-1}$ yr$^{-1}$ the corresponding values were, respectively, 30% and 60%. Barley NRE and ANR determined in the fertilized barley were higher when the N residual from maize was higher.

### 3.5. Soil Organic Carbon

The SOC in the first 30 cm of soil did not change over the N treatments analysed in the study (Figure 4). An overall average of 56.9 Mg of C ha$^{-1}$ was determined in the experimental field during the three GS. Growing season 2 showed the higher average SOC values (58.5 Mg of C ha$^{-1}$) and was different from GS 3 (55.4 Mg of C ha$^{-1}$). The
control N treatment (0 kg N ha\(^{-1}\) yr\(^{-1}\)) in the GS 3 was different to the barley 0 N barley + maize 200 N treatment in the GS 2. No differences were observed between the rest of the treatments.

3.6. Economic return

Maize N fertilization and the GS affected the ER of either barley, maize or both crops. Nevertheless, barley N fertilization affected the ER of the barley and both crops together, but not the maize ER (Figure 5). Annual N\(_{\text{rate}}\) of 200 kg N ha\(^{-1}\) or above, obtained the maximum ER (above 2,700 € ha\(^{-1}\)), independently of the N distribution between the crops. Barley obtained the highest ER when it was fertilized (100 kg N ha\(^{-1}\)) or when it did not receive N fertilizer but the previous maize N\(_{\text{rate}}\) was 300 kg N ha\(^{-1}\) (813-984 € ha\(^{-1}\)). Maize ER differences were found between non-N-fertilized (0 kg N ha\(^{-1}\)) and N-fertilized maize (100 kg N ha\(^{-1}\) or above), but not between the different N\(_{\text{rate}}\) applied (100, 200 and 300 kg N ha\(^{-1}\)). The barley ER in the non-N-fertilized treatment showed a rising tendency when increasing the N\(_{\text{rate}}\) of the previous maize. In that case, the higher the N\(_{\text{rate}}\) applied in maize, the higher the ER obtained in the barley. The economic optimum N rate (EONR) was estimated as 215.1 kg N ha\(^{-1}\) (Figure 6).

4. Discussion

4.1. Grain and biomass yield

The increase of grain and biomass yields when incrementing the N\(_{\text{rate}}\) demonstrated the large effect of N in cereal yields (Shanahan et al., 2008). Total annual grain yield (20 Mg ha\(^{-1}\)) and biomass yield (35 Mg ha\(^{-1}\)) (Table 2) were slightly higher than those previously reported in other double-annual cropping system areas (Zhao et al., 2006), or under humid (non-irrigated) Mediterranean conditions (Ovejero et al., 2016;
These results suggest the high yield potential of a double-annual cropping strategy under irrigated Mediterranean conditions. Iguácel et al. (2010) and Yagüe and Quílez (2013) have respectively reported total annual grain yields of 17.5 and 14.9 Mg ha\(^{-1}\), and Grignani et al., (2007) reported biomass yields of 23-26 Mg ha\(^{-1}\) under irrigated Mediterranean conditions.

Mono-cropping strategies in the Ebro Valley, usually irrigated maize, yield less than the total annual yields obtained in the present study (Berenguer et al., 2009; Biau et al., 2012; Isla et al., 2015; Maresma et al., 2016; Martínez et al., 2017; Yagüe and Quílez, 2013). Previous studies in different areas have quantified an increase of 25-50% total dry matter of the double-annual cropping strategies compared to mono-cropping systems (Crookston et al., 1978; Heggenstaller et al., 2008; Lloveras, 1987b; Raphalen, 1980).

Moreover, under irrigated Mediterranean conditions grain and biomass yields seem to be more stable among GS in double-annual cropping systems than in mono-cropping systems (Berenguer et al., 2008, 2009; Biau et al., 2012; Cela et al., 2013). Therefore, the stability of the yield among years could contribute to have more stable N recommendations, compared with monocrop conditions.

In our study, the application of the same total amount of N fertilizer annually achieved similar grain and biomass yields, independently of which crop received the N application. The optimum total N\(_\text{rate}\) to achieve maximum grain and biomass yields was 232.5 and 240.5 kg N ha\(^{-1}\) yr\(^{-1}\), respectively (Figure 1). These results were similar to previous monocropped maize fertilization studies under irrigated Mediterranean environments. Maresma et al., (2016), Maresma et al., (2018) and Yagüe and Quílez (2015) reported optimum N\(_\text{rate}\) of 240, 217 and 300 kg N ha\(^{-1}\), respectively, but with lower grain yields achieved (17.6, 19.4, and 18 Mg ha\(^{-1}\), respectively).
A significant fraction of the N applied to each crop (barley or maize) that was not taken up by the crop, was available for the following one. This could be observed mainly in the non-fertilized barley, where the effect of maize residual N was evident in barley yields. There was a rising tendency of barley grain and biomass yields when increasing the N rate applied in maize (Table 2). However, the N residual effect of fertilized barley in maize yields was not as evident as the N residual effect of maize in barley. Probably, the higher OM mineralization during summer (Magdoff et al., 1984), provided a high amount of N to maize that masked the possible effect of the barley residual N on maize yields. In addition, the N applied in sidedress (February) may be leached if the barley did not use it during its GS. In our study, there was not N sequestration of the barley stover (Salmerón et al., 2011) because it was removed in the experiment.

4.2. Biomass and grain N content and total N uptake

Crop N uptake was mostly determined by the yields because the differences of N content were less evident than the grain or biomass yield differences among the different N treatments.

The N content of both crops was similar to that reported by other authors (Berenguer et al., 2008; Delogu et al., 1998; Perramon et al., 2016; Salmerón et al., 2011). The non-N-fertilized maize had the lowest maize N content (grain and biomass) independently of the barley N fertilization, reflecting high N deficit when maize was not fertilized with N. The maize N biomass content in the non-N-fertilized maize was lower than the other N treatments (Table 2), fact that could be probably occasioned by the deficient content of N in the plant and accentuated translocation of N from plant to grain in order to mitigate the N deficit (Cliquet et al., 1990).
Total annual N\textsubscript{rate} of 200 kg N ha\textsuperscript{-1} or above, presented similar N grain and biomass contents. Nevertheless, a rising tendency in the N uptake was identified when increasing the total annual N\textsubscript{rate} applied (Figure 2). The maximum annual N uptake determined in this study (398.7 kg N ha\textsuperscript{-1}) was higher than that reported in other double-annual cropping studies (Grignani et al., 2007; Ovejero et al., 2016; Perramon et al., 2016). However, N uptake by each crop was in agreement with the reported proportions of total N uptake by the winter crop and summer crop in these previous works, which were 35% and 65%, respectively (Grignani et al., 2007; Perramon et al., 2016).

4.3. Soil NO\textsubscript{3}\textsuperscript{-}-N content

The soil NO\textsubscript{3}\textsuperscript{-}-N content after the barley or maize harvest showed high variation depending on maize N treatments. However, barley N fertilization did not have an effect on the residual soil NO\textsubscript{3}\textsuperscript{-}-N after either barley or maize harvest. Higher soil NO\textsubscript{3}\textsuperscript{-}-N concentrations were determined in the topsoil layer (0-30 cm) than in deeper layers after the maize harvest. This suggests that the following barley could use a major part of the maize residual N. The faster the subsequent barley is established, the lower the probability of losing the residual NO\textsubscript{3}\textsuperscript{-}-N from maize.

In double-annual cropping systems, the residual soil NO\textsubscript{3}\textsuperscript{-}-N from previous crops may not be lost, and could potentially be taken up by the next crop thereby partially avoiding N leaching of nitrates (Heggenstaller et al., 2008; Ovejero et al., 2016). Winter crops mitigate N runoff and leaching after maize caused by winter and early spring rains (Gabriel and Quemada, 2011; Hirel et al., 2011; Salmerón et al., 2011). Grignani et al. (2007), Perramon et al. (2016) and Ovejero et al. (2016) found an increase in winter crop yields after high N\textsubscript{rate} applied to maize in double-annual cropping systems.
Though the highest amount of residual N after maize harvest was determined in the topsoil layer (0-30 cm), the residual N after the barley harvest was more concentrated in the deepest soil layer (60-82 cm). This fact suggests that the residual N from maize that was not taken up by the barley was leached to deeper layers and was more likely to be lost. When maize roots reach to explore these layers (60-82 cm), the NO$_3^-$-N probably would be leached out of the system. The residual N content in the top-layer (0-30 cm) after the barley was around 15 kg N ha$^{-1}$ (Figure 3), which was lower than the calculated N mineralized from OM during the maize GS that around 170 kg N ha$^{-1}$. This fact could explain why there was not effect of the barley N fertilization in maize.

Traditional applications of 100 and 300 kg N ha$^{-1}$ to barley and maize, respectively (Isidoro et al., 2006; Sisquella et al., 2004), seem to be excessive for our double-annual cropping system and could contribute to polluting the agro-ecosystem environment. Our results showed that with annual N$_{rate}$ of 200-300 kg N ha$^{-1}$ the build-up of soil NO$_3^-$-N was prevented maintaining the yield potential. However, the high N mineralization of the OM supposed a relevant contribution to the fertilization of the crops (around 190 kg N ha$^{-1}$). Without these high levels of N mineralization, the N requirement could be similar to previous studies (Isidoro et al., 2006; Sisquella et al., 2004) but it also entails a reduction of the global N efficiency of both crops.

4.4. N efficiencies

The N efficiencies decreased as the N$_{rate}$ increased, agreeing with the trend reported by Fageria and Baligar (2005) for cereal crops. The increase of NUE contribute to mitigating N leaching while maintaining or increasing yields. The N efficiencies calculated in this study were similar or higher than those reported in similar conditions in mono-cropping cereals (Berenguer et al., 2009; Martínez et al., 2016; López-Bellido et
al., 2005; Bosch-Serra et al., 2015), or in double-cropping systems (Ovejero et al., 2016) fertilized with organic or inorganic N applications. Quemada et al. (2013) and Heggenstaller et al. (2008) concluded that replacing a fallow with a non-legume cover crop reduced N leaching by 50% and 34%, respectively. Therefore, the establishment of two crops in the same year could help promote high efficiency of residual N (Yagüe and Quílez, 2013).

Our results showed not only a high efficiency to the N_rate applied to barley, but also a high recovery of the residual N from the previous maize. Barley yielded up to 6.7 Mg ha\(^{-1}\) (extracting around 150 kg N ha\(^{-1}\)) with applications of 100 kg N ha\(^{-1}\). The total annual N mineralized estimated in the non-N fertilized treatment in our study was around 190 kg N ha\(^{-1}\) yr\(^{-1}\) (with a soil OM of 19.4 g kg\(^{-1}\)). Higher annual N_rate produced a reduction of maize ANR whereas increased barley ANR. This fact made a compensation between the ANR of barley and maize, and then, no significant differences were found between N treatments in the annual ANR.

In the double-annual system barley-maize, annual applications of 200 kg N ha\(^{-1}\) could trigger a high risk of soil N mining (NUE \(\leq 0.9\)); which happens when the N removal with the harvested crop tends to exceed the N input (EUNEP, 2015). EUNEP (2015) defined the range of NUE from 0.7 to 0.9 as desirable for agriculture production because it entails a balance N fertilization and guarantees the sustainability of the system. The annual N_rate of 300 and 400 kg N ha\(^{-1}\) obtained values of NUE of 0.73-0.75 and 0.67, respectively. Therefore, the 300 kg N ha\(^{-1}\) yr\(^{-1}\) seems to be appropriate to maintain the yields and the sustainability of the system. A decreasing trend was observed for the NUE when increasing the N_rate, confirming higher N losses when fertilizer management is not optimized according to crop N requirements (Quemada et al., 2013).
4.5. Soil Organic Carbon

Previous studies have described the evolution of the SOC in short periods of time (3-4 years) in high-yielding environments (Bertora et al., 2009; Biau et al., 2013, Krueger et al., 2012). Generally, the incorporation of the crop residues after harvest have contributed to increase SOC (Bertora et al., 2009; Fuentes et al., 2009; Grignani et al., 2007; Krueger et al., 2012). However, in high yielding environments (>20 Mg of grain ha⁻¹) Biau et al., (2013) found that stover incorporation had minimal impact on C and N storage in the short term (3-years study), though stover removal slightly reduced SOC.

Probably, in high yielding environments stover incorporation is common and the SOC level requires the high C inputs of the stover to be maintained.

Double-annual cropping system present a significant challenge for the maintenance of soil fertility and could potentially lead to SOC reductions if crop residues are not retained in fields due to the increased extractions of the system (Heggenstaller et al., 2008). In our study there were not significant variations in the SOC, even in the N treatments with the highest N deficit for the crop (0 kg N ha⁻¹ yr⁻¹ applied) the SOC level was maintained in the 3-year period of the experiment. Probably, the stable SOC content during the experiments could be the result of long-term equilibrium between OM inputs and mineralisation. Indeed, not only was the maize’s stover incorporated during the experiment, but also the farmer had incorporated crop’s residues after harvest for the past ten years.

Therefore, the experimented N fertilization strategies in the double-annual cropping system (barley-maize) could be sustainable in a short-term period, but further research is needed to guarantee the long-term sustainability, especially, when exists a risk of soil N mining (NUE > 0.9).
4.6. Economic return

Determination of the ER is important because the optimum ER is consistent with good environmental stewardship and could be used as a tool to determine crop N requirements (Sripada et al., 2008). As the N fertilizer to maize price ratio is positively correlated with NUE; increased N fertilizer to maize price ratios lead farmers to apply lower N rates and consequently obtain higher NUE (Zhang et al., 2015).

In our study, non-significant differences in the ER were detected with annual N\textsubscript{rate} of 200, 300 or 400 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Figure 5). However, the price ratio (N:Maize) used in our this study was classified close to the optimum prices for farmers in the study of Sripada et al. (2008), who tested historical price ratios (N:Maize) that ranged from 4:1 to 14:1. It is evident that at higher N:Cereal price ratios (worse price relation for farmers), the ER of the lower N\textsubscript{rate} will be less reduced than ER of the high N rates. Therefore, the N fertilizer strategy of 400 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} seemed to be not economically justified and could reduce crop profitability.

In our study, the low N:Cereal price ratio contributed to reducing differences between the EONR (215.1 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) (Figure 6) and the N\textsubscript{rate} to achieve maximum yields (232.5 kg N ha\textsuperscript{-1} yr\textsuperscript{-1}) (Figure 1). Fact that demonstrated the large effect of the grain yield in the ER at the studied N:Cereal price ratios.

5. Conclusions

The double-annual cropping system (barley-maize) showed high grain and biomass yield potential as well as stability under irrigated Mediterranean environments. The total annual sum of grain or biomass yields in the barley-maize system could be up to 20 and 35 Mg ha\textsuperscript{-1} yr\textsuperscript{-1} of grain and biomass, respectively. Our study showed that the extended duration of the cropping season in double-cropping systems contributed to
mitigate the potential for NO$_3^-$-N leaching of the residual N after harvest. In a double-
annual rotation, the following crop could use the residual N of the previous crop,
enhancing the NUE of the cropping system. Under irrigated Mediterranean environments,
barley was efficient in the uptake of maize residual N, but maize was not affected by the
barley residual N.

The determined EONR entailed a high NUE, suggesting some risk of soil N
mining. Indeed, even in the N treatments with the highest N deficit (0 kg N ha$^{-1}$ yr$^{-1}$
applied), the yields were maintained in a three-year period without decreasing SOC
levels. Thus, the sustainability of the different N treatments where exists a risk of soil N
mining (NUE > 0.9) should be tested over a long-term period. Further research is needed
to fine-tune the N fertilization strategy of double-annual cropping system (barley-maize).

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of Crop and Forest Science, University of Lleida) for his help with the statistical analyses,
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staff of the Field Crops Laboratory of the University of Lleida for their help in processing
the data.

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Figure 1. Response curves to total N applied (barley + maize) during the year (average of three growing seasons), on total grain and biomass. CNR: Critical fertilization N rate to achieve maximum yields. Barley N rate indicates if 0 or 100 kg N ha$^{-1}$ were applied to barley. Error bars indicate the standard error of the mean.
Figure 2. Barley, maize and the sum of both crops (both) N uptake averaged in three consecutive growing seasons (2013-2016) for studied N rates. Tukey’s HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. Abbreviations: N fertilizer treatment, †N; Barley N treatment, N_b; Maize N treatment, N_m; Growing season; GS.

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<td>Season (GS)</td>
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<td>GS x N_b</td>
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<tr>
<td>GS x N_m</td>
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<tr>
<td>GS x N_b x N_m</td>
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<tr>
<td>Error b</td>
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</table>

NS: No significance, * significant at 0.05 level, ** significant at 0.01 level
**Figure 3.** Residual soil NO$_3^-$-N in three consecutive layers (0-30, 30-60 and 60-82 cm depth) after **a)** barley and **b)** maize harvests, averaged in three consecutive growing seasons (2013-2016) for studied N rates. Tukey’s HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. Abbreviations: N fertilizer treatment, $^*$N; Barley N treatment, $N_b$; Maize N treatment, $N_m$; Growing season; GS.
Figure 4. Soil organic carbon (SOC) per growing season (after maize harvest) among the different N rates tested in the study (2013-2016). Error bars indicate the standard error of the mean. Abbreviations: N fertilizer treatment, †N; Barley N treatment, Nb; Maize N treatment, Nm; Growing season; GS. Tukey’s HSD test: The SOC value of the 0 Nb and 200 Nm in the 2nd GS is different from the 0 Nb and 0 Nm of the 3rd GS, all the rest differences are not significant at a p-value of <0.05.
Figure 5. Barley, maize and the sum of both crops economic return (ER) averaged in three consecutive growing seasons (2013-2016) for studied N rates. Tukey’s HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. Abbreviations: N fertilizer treatment, $^\dagger$N; Barley N treatment, $N_b$; Maize N treatment, $N_m$; Growing season; GS.

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<td>$N_b$ x $N_m$</td>
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<td>Error a</td>
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<tr>
<td>Season (GS)</td>
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<tr>
<td>GS x $N_b$</td>
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<tr>
<td>GS x $N_m$</td>
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<tr>
<td>GS x $N_b$ x $N_m$</td>
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<td>Error b</td>
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NS: No significance, * significant at 0.05 level, ** significant at 0.01 level
Figure 6. Economic return response curve to total N applied (barley + maize) during the whole year (average of three growing seasons). EONR: Economic optimum nitrogen rate. The N:cereal prices ratios were determined at 5.6:1 and 5.3:1 for barley and maize, respectively. Barley N rate indicates if 0 or 100 kg N ha$^{-1}$ were applied to barley. Error bars indicate the standard error of the mean.
Table 1

Chemical and physical soil properties at the beginning of the experiment (2013).

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<td></td>
<td>0-30 cm</td>
<td>30-60 cm</td>
<td>60-82 cm</td>
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<td>Sand, %</td>
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<td>Silt, %</td>
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<td>Clay, %</td>
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<td>P (Olsen), mg kg⁻¹</td>
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<tr>
<td>K (NH₄Ac), mg kg⁻¹</td>
<td>241</td>
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<td>59</td>
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Table 2

Grain and biomass yields, grain and biomass N contents averaged in three consecutive growing seasons (2013-2016) for studied N rates. Tukey’s HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Abbreviations: N fertilizer treatment, †N; Barley N treatment, N_b; Maize N treatment, N_m; Growing season; GS.

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<th>Biomass yield (Mg ha^{-1})</th>
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<th>N biomass (g kg^{-1})</th>
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<td>9.41 ab</td>
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ANOVA

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NS No significance  * Significant at p-value < 0.05  ** Significant at p-value < 0.01
Table 3

Nitrogen use efficiency (NUE), N recovery efficiency (NRE) and apparent N recovery (ANR) averaged in three consecutive growing seasons (2013-2016) for studied N rates. Tukey’s HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Abbreviations: N fertilizer treatment, †N; Barley N treatment, Nb; Maize N treatment,Nm; Growing season; GS.

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<th>ANR (kg kg(^{-1}))</th>
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**Error a: NS = Non-significant, * = p < 0.05, ** = p < 0.01**
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NS No significance  * Significant at p-value < 0.05  ** Significant at p-value < 0.01