Strategies to Improve Nitrogen Use Efficiency in Winter Cereal Crops under Rainfed Conditions

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Nitrogen Management

ABSTRACT

Developing fertilizer strategies that increase nitrogen use efficiency (NUE) could reduce unnecessary input costs to farmers and environmental impact of N losses. Two field experiments were performed in northern Spain to evaluate alternative N fertilization strategies to improve NUE in winter cereal crops (wheat [*Triticum aestivum* L.] and barley [*Hordeum vulgare* L.]). The strategies were: (i) adjusting the fertilizer rate by soil mineral N before N application; (ii) splitting of N fertilizer application; and (iii) the use of fertilizer with a nitrification inhibitor (3,4-dimethylpyrazole phosphate, DMPP). The experiments were designed as a completely randomized block design with seven treatments and four replications. Treatments included a control, two single applications, one with DMPP, and four split N applications. Nitrogen balance was calculated according to the general equation of conservation of mass for any soil–crop system from which N-efficiency parameters were determined. Grain yield followed a quadratic-plus-plateau model with different optimum N rate depending on the year (71 and 98 kg N ha^{-1} in 2002–2003 and 2003–2004, respectively). Adjusting N fertilizer rate by soil mineral N before N application gave a maximum yield and a similar NUE to the optimum N rate predicted by the model. Neither applying N in two doses nor including a nitrification inhibitor with a single dose showed any advantages in terms of yield or N efficiency.

Cereal producers are under pressure to maintain profitability against a background of environmental constraints and high fertilizer costs. Mineral N fertilizer is regarded as a main contributor to water pollution by nitrates and to atmospheric pollution by nitrous oxides, but there is little likelihood that adequate food supplies can be maintained without fertilizers (Tilman et al., 2002). The development of cropping strategies that increase NUE could reduce unnecessary input costs to farmers and environmental impact of N losses while maintaining crop yield.

The concept of NUE, developed by Moll et al. (1982), provides a framework for evaluating variation of N use among genotypes as related to major physiological processes. It has the advantage that readily measured N accumulation data in various plant components are used to evaluate N use. However, differential response of cropping systems to applied N reveal that the evaluation of NUE needs to include efficiency factors related to soil processes (Huggins and Pan, 1993). Nitrogen budgets may be used to identify major soil–plant components of N efficiency and dominant processes of the N cycle under specified management practices (Meisinger and Randall, 1991). The combination of N balances and NUE offers a sound basis to adjust N fertilizer application to optimize N management for productivity and ecological sustainability of cropping systems.

A number of experiments in winter cereal have shown that adjusting fertilizer rate and splitting of N fertilizer application are strategies to improve NUE (Alcoz et al., 1993; Delogu et al., 1998; López-Bellido et al., 2005). Low fertilizer efficiencies have been attributed to excessive N applications, especially when residual or mineralized N was ignored. One of the most widely used approaches to adjust N applications to crop needs, is calculating fertilizer recommendations based on N requirements for a given crop corrected by the soil N supply (Blankenau et al., 2002). Provided that fertilizer N application is not excessive, the accumulation of soil mineral N and losses by leaching or denitrification can be minimized (Addiscott and Powlson, 1992).

Splitting of N fertilizer application has been suggested as a strategy to improve NUE in winter cereal, on the assumption that the timing of application has a significant effect on the N uptake by the crop (Dilz, 1988). Low efficiency attributed to N fertilizer application in autumn has been observed in a large number of studies, and justifies N applications in spring (Sowers et al., 1994; Strong, 1995), particularly in Mediterranean climates. Nitrogen fertilizer efficiency in wheat crops was greater when fertilizer was applied in spring before stem elongation rather than in autumn, before sowing (López-Bellido et al., 2005). However, supplying N in two or three applications in spring is a common fertilizer recommendation to increase NUE in temperate Europe (Limaux et al., 1999), despite inconsistent responses depending on weather condi-

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Abbreviations: ASN, ammonium sulfonitrate; GLAI, green leaf area index; N_a, organic nitrogen in aboveground plant parts; N_{bal}, nitrogen balance; N_f, nitrogen supplied by fertilizer; N_h, soil mineral nitrogen at harvest; NI, nitrification inhibitor; N_{ini}, soil mineral nitrogen at sowing; N_{lch}, nitrogen leached; N_m, mineral nitrogen supplied by soil; N_r, organic nitrogen in roots; NRF, nitrogen recovery fraction; NUE, nitrogen use efficiency; NU_pE, nitrogen uptake efficiency; NU_tE, nitrogen utilization efficiency.

Table 1. Soil properties at the beginning of the experiment.

Soil properties	Depth, cm		
	0–30	30–60	60–90
Sand, g kg^{-1}	140	130	120
Silt, g kg^{-1}	480	460	460
Clay, kg^{-1}	380	410	420
pH (1:2.5)	7.8	7.9	8
Organic C, g kg^{-1}	14.5	10.5	8.1
Total N, g kg^{-1}	1.6	1.1	0.9
C/N	9.1	9.5	9
Olsen P, mg kg^{-1}	19.1	7.4	3.5
Exchangeable K, mg kg^{-1}	350	273	246
EC (1:1), dS m^{-1}	0.37	0.3	0.27

Table 2. Fertilization treatments.

Treatment†	Fertilizer‡	2002–2003			2003–2004		
		Total	First application	Second application	Total	First application	Second application
		Kg N ha^{-1}					
Control§		0	0	0	0	0	0
X-2A§	ASN	40	13	27	35	12	23
X-A§	ASN	80	26	54	70	23	47
X§¶	ASN	120	40	80	110	37	73
X+A§	ASN	160	53	107	140	48	97
NIT#	ENTEC	120	120	0	110	110	0
SG¶#	ASN	120	120	0	110	110	0

† X = reference rate [crop N uptake– soil mineral N (0–60)]; A = arbitrary number to establish different treatments.

‡ ASN = ammonium sulfonitrate (26% N, 32% S); ENTEC = ammonium sulfonitrate + DMPP (3'4 dimethyl pirazol phosphate).

§ Treatments to evaluate fertilizer rates.

¶ Treatments to evaluate fertilizer splitting.

Treatments to evaluate the use of a fertilizer with a nitrification inhibitor (DMPP).

tions (Alcoz et al., 1993). In addition, some studies under Mediterranean conditions have shown that splitting N fertilizer application did not decrease losses by leaching because fertilizer is applied in late winter or early spring when drainage becomes negligible (Arregui and Quemada, 2006). There is, therefore, a need to clarify the conditions of splitting N application in spring that increase NUE in winter cereal under Mediterranean conditions.

A further possible means of increasing NUE is the addition of a nitrification inhibitor (NI) to the fertilizer, particularly in the case of large applications. Since the 1960s, the fertilizer industry has developed compounds that delay bacterial oxidation of NH_4^+ to NO_2^- (first step of nitrification) to diminish nitrate losses and to increase N-fertilizer efficiency (Prasad and Power, 1995). Recently, Zerulla et al. (2001) proposed DMPP as a new NI, effective when added to granulated N fertilizers at a rate of 0.5 to 1.5 kg ha^{-1}, and able to diminish N_2O emissions to the atmosphere (Irigoyen et al., 2003). Pasda et al. (2001) observed that, in most cases, N uptake and yield increased in winter wheat fertilized with ammonium sulfonitrate (ASN) plus DMPP with respect to ASN application. Nevertheless, Barth et al. (2001) recommended further field studies before drawing conclusions.

The objective of this work was to evaluate various N fertilization strategies to improve the efficient use of available N in rainfed cereal crops under Mediterranean conditions, through the study of crop response, N balance and NUE. The strategies were: (i) adjusting N fertilizer rate by soil mineral N before fertilizer application; (ii) splitting of N fertilizer; and (iii) the use of a fertilizer with the nitrification inhibitor, DMPP.

MATERIALS AND METHODS

Field Experiment

Field experiments were performed at the Experimental Field of Universidad Pública de Navarra (Pamplona, Navarra, Spain) in northern Spain for 2 yr from July 2002 to July 2004. The soil is representative of a large area of rainfed cropping and was classified as Calcixerollic Xerochrept (Soil Survey Staff, 1992). These soils have silty clay loam texture in the upper 0.6 m, are highly calcareous, and have moderate organic matter content in the upper layers. The relevant soil characteristics at the study site are presented in Table 1. During 5 yr before the beginning of the experiment the field had received inorganic N fertilizer following the agricultural practices of the area but no organic amendments. Wheat (cv. Soissons) was planted on 28 Oct. 2002 and barley (cv. Puffin) on 6 Nov. 2003, with a target density of 400 plants m^{-2}. Timing of selected growth stages were recorded weekly, following a decimal code (Zadoks et al., 1974).

The experiment was designed as a randomized complete block with four replications. Plot size was 8 by 5 m. Seven treatments were applied including a control: two single applications of N fertilizer (X kg N ha^{-1}) one without (SG) and one with a nitrification inhibitor (NIT), and four split N applications with total applications of X-2A, X-A, X and X+A (Table 2). The "A" term was an arbitrary number to establish different treatments. The reference N fertilization (X) was calculated by correcting crop N requirement by mineral N content in the upper 0.6 m of soil just before the first fertilizer application. Crop N requirement was estimated as the product of the expected yield (6 Mg ha^{-1} for each crop) and a N extraction coefficient. Nitrogen extraction coefficients were 30 kg N Mg^{-1} grain for wheat (Gooding and Davies, 1997) and 26 kg N Mg^{-1} grain for barley (Delogu et al., 1998). Soil mineral N content in the upper 0.6 m before the first fertilizer application was not significantly different among treatments and the mean values (60 kg N ha^{-1} the first year and 50 kg N ha^{-1} the second) were adopted for calculations. In split treatments, fertilizer was applied as ammonium sulfonitrate (ASN) (ASN 26% N:19.5% NH_4^+–N and 6.5% NO_3^-–N) divided into two applications to match crop N demand (Delogu et al., 1998): one-third was hand broadcast to plots at mid-tillering (GS-25), and two-thirds at the beginning of stem elongation (GS-30). In treatments SG and NIT, N fertilizer was hand broadcast to plots in a single application at GS-25 as ASN in the first case, and by application of ENTEC (BASF, Ludwigshafen, Germany), which contains ASN and 1% of 3,4-dimethylpirazol phosphate relative to NH_4^+–N, in the second. Mid-tillering occurred on 18 Feb. 2003 and on 29 Jan. 2004; Stem elongation commenced on 25Mar. 2003 and on 17 Mar. 2004. All plots were fertilized before sowing the first year with 35 kg P ha^{-1} and 50 kg K ha^{-1}, mixed in the upper 0.25 m soil layer. Weed control was performed with 3125 g ha^{-1} of Diflufenican plus 3125 g ha^{-1}of MCPA in 2002–2003, and 126 g ha^{-1} of Diflufenican plus 1350 g ha^{-1}of Isoproturon in 2003–2004, respectively. On 4 July 2003 and 2 July 2004 a 1.6-m-wide central section was harvested from each plot (12.8 m^2 per plot) and yield was expressed per hectare at 88% dry weight. Cut straw was baled and remaining stubble was incorporated with a moldboard plow.

Yield Response

The yield response to N fertilization for each crop was fitted to a quadratic-plus-plateau model (Cerrato and Blackmer, 1990; Makowski et al., 1999; Alivelu et al., 2006) defined by Eq. [1] and [2]:

$$Y = a + bN + cN^2 \qquad \text{if } N < N_{op} \qquad [1]$$

$$Y = M \qquad \text{if } N \geq N_{op} \qquad [2]$$

where Y is grain yield (Mg ha^{-1} at 88% dry wt), N is the fertilizer dose (kg N ha^{-1}), a is grain yield predicted for the Control plot, b and c are linear and quadratic coefficients, respectively, and N_{op} is the intersection of the two functions is the smallest N fertilizer dose required to reach M, the plateau yield.

Crop Nitrogen Uptake

Crop N uptake of aboveground plant parts (N_{up}) was determined before fertilizer application and at harvest, in all treatments. Aboveground crop biomass was determined from two 0.28 m^2 samples from each plot oven dried to constant weight at 65°C. Subsamples of the dry material were analyzed for N concentration by Kjeldahl's method (Horwitz, 2000). Aboveground crop N (N_a) was calculated as the product of dry biomass and N concentration. Nitrogen in roots at harvest (N_r) was estimated as 25% of N_a (Rroço and Mengel, 2000), and added to N_a to obtain crop nitrogen uptake (N_{up}).

Green Leaf Area Index

Green leaf area index (GLAI) was determined at GS-30, GS-37, and GS-65 from plants taken from one 0.28 m^{-2} sample per plot of Control, X, and X+A treatments. Plants were cut at ground level, the green leaves removed and transported to the laboratory to determine their area using a portable ADC AM300 (ADC Bioscientific Ltd., Hoddesdon, UK). Total green area was expressed as a fraction of unit land area to give GLAI.

Soil Mineral Nitrogen

Soil mineral nitrogen (N_{min})content was determined in soil samples taken at sowing, before first fertilizer application (26 Jan. 2003 and 12 Jan. 2004) and at harvest. Soil samples were taken at 0.3 m intervals to a depth of 0.9, using Eijelkamp cylinder augers (Eijelkamp, Giesbeek, the Netherlands) (2.5 cm inside diam., 15 cm long). Three soil samples were taken per plot and per depth each time that the experiment was sampled. The samples were extracted with 1 M KCl, (20 g of soil: 100 mL of KCl), centrifuged, decanted, and a subsample of the supernatant volume was stored in a freezer until later analysis. Nitrate concentration in the extracts was determined by spectrophotometry, after reduction with a cadmium column (Keeney and Nelson, 1982), ammonium was measured using the method of Solorzano (1969).

Nitrogen Balance

The calculation of the simplified N balance was performed according to the general conservation of mass equation for any soil–crop system (Meisinger and Randall, 1991):

$$N_{bal}\ (\text{kg N ha}^{-1}) = N_{input}\ (\text{kg N ha}^{-1}) - N_{output}\ (\text{kg N ha}^{-1}) \qquad [3]$$

where N_{input} is the sum of the soil mineral N (0–90 cm) at sowing (N_{ini}), the mineral N provided by the fertilizer (N_f), and the soil N mineralization during the cropping period (0–90 cm) (N_m). N_{output} is the sum of the crop N uptake at harvest (N_{up}), the N leached (N_{lch}), and the soil mineral N (0–90 cm) at harvest (N_h). The N_m was estimated in the control plots (0 kg N ha^{-1}) by applying Eq. [3], where N_{ini}, N_{up}, and N_{lch} were measured directly.

The use of Control plots to estimate N_m assumes that (i) applied N does not influence gains or losses of available N from other soil N pools and (ii) other inorganic N inputs are minimal and included in N_m (Huggins and Pan, 1993). Soil mineral N and nitrate leaching data collected in the same plots and during the same years, were previously published (Arregui and Quemada, 2006) and were used in the present article to complete the N balance.

Nitrogen efficiency parameters were determined according to Moll et al. (1982), Huggins and Pan (1993), Delogu et al. (1998), and López-Bellido et al. (2005). Nitrogen use efficiency was the ratio of grain yield biomass (kg ha^{-1}) to N_{input} (kg N ha^{-1}). Nitrogen uptake efficiency (NU_pE) was the ratio of N_{up} (kg N ha^{-1}) to N_{input}. N apparent recovery fraction (NRF) was the ratio of ($N_{upi} - N_{up0}$) to N_{fi}, being N_{upi} crop N uptake of treatment i, N_{up0} crop N uptake of control, and N_{fi} fertilizer–N applied in treatment i. Nitrogen harvest index (NHI) was the ratio of N in grain to N_{up}.

Statistical Analysis

The N fertilizer treatments were applied to the same plots for two consecutive years. Because of that, results from the second year combined the residual with the yearly effect of the N fertilizer treatment. Although the analysis is unable to separate between residual and yearly effects, it can evaluate the cumulative treatment effect. The soil mineral N content in the upper 0.6 m before the first fertilizer application was initially analyzed as a split-split plot, with time of sampling as main plot and N fertilizer treatment and block as successive plots. Because block had no significant effect and its interactions were not significant ($P > 0.05$), the data were pooled as replicates and reanalyzed as a split plot with time of sampling as main plot and treatment as a subplot. A Fisher LSD test ($\alpha = 0.05$) was used to test differences between treatments means. Crop effect was not considered as a factor, so data of each year were analyzed independently. The quadratic-plus-plateau model was fitted to yield and aboveground biomass at harvest from all replications using a nonlinear regression procedure. The statistical analyses were made using the SPSS software program (SPSS, Chicago, IL) (SPSS, 2005).

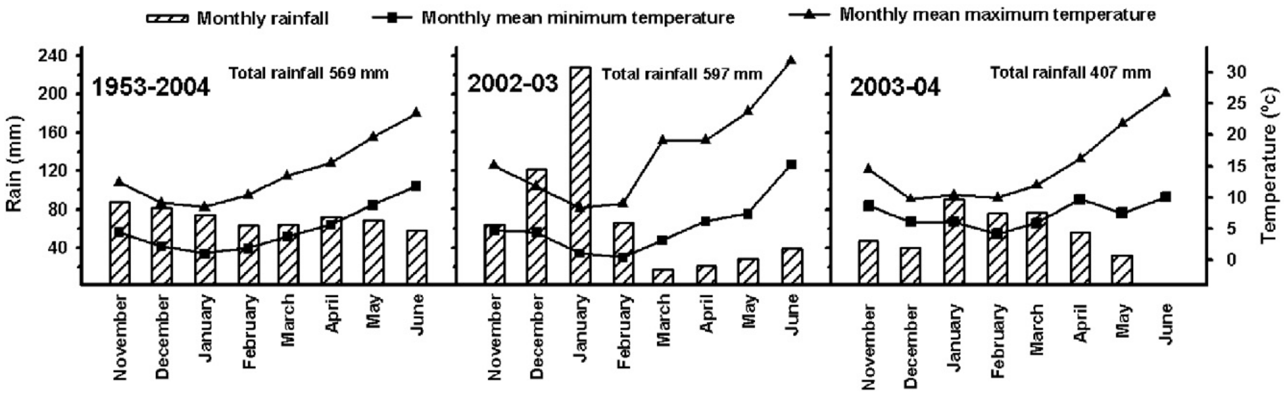

Fig. 1. Monthly temperature and rainfall, during 1953 to 2004 and during the cropping period of each year of experiment.

RESULTS

Climate

Figure 1 shows the time trend of mean maximum and mean minimum monthly temperatures, recorded during the two crop seasons of the experiment, together with accumulated monthly rainfall. Temperatures and rainfall of the last 50 yr (1953–2004) are included for reference.

The first season, with 597 mm of accumulated rainfall, was wetter than the second (407 mm) and the historical average (569 mm). It was also a season with high rainfall during the period from sowing to first N application (November–February; 421 mm), followed by a dry Spring with temperatures slightly above the average (Fig. 1). By contrast, rainfall in the second year was uniformly distributed throughout the crop season, with abundant rain events during the period from first N application to harvest (February–June; 232 mm)

The soil water content was suitable for sowing operations in the first season (Arregui and Quemada, 2006), but was wetter, around field capacity in the second with resultant difficulties in sowing and problems for crop establishment.

Mean temperatures from sowing to first N application were very similar in both study years, and varied between –1 and 14°C in 2002–2003 and between 0 and 13°C in 2003–2004. During the grain-filling period (May–June), in 2002–2003 the monthly mean temperature was 15°C in May and 23°C in June, coinciding with low soil water content (Fig. 1; Arregui and Quemada, 2006). In 2003–2004, mean temperatures were lower (8°C in May and 20°C in June) and the soil was wetter.

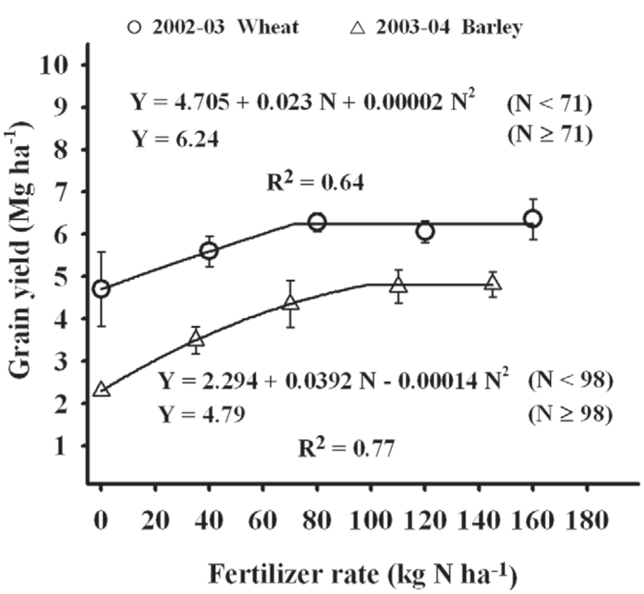

Fig. 2. Quadratic plus plateau model fitting to grain yield (88% dry wt.) in 2002–2003 and 2003–2004. Fertilizer rates correspond to Control, X-2A, X-A, X, and X+A treatments. Each point is the mean value of four replications and bars show the standard error of the mean. Model fitting was performed with all replications but only means plus standard error are reported to improve readability.

Effect of Fertilizer Rate Adjustment

Crop Response

The quadratic-plus-plateau model described yield responses well with determination coefficients (R^2) of 0.64 and 0.77 for the first and second crop seasons, respectively (Fig. 2). According to the model, the maximum yield was 6.24 Mg ha^{-1} in 2002–2003 and 4.79 Mg ha^{-1} in 2003–2004. The optimum fertilizer dose (N_{op}) lay between treatments X-2A and X-A (71 kg N ha^{-1}) in 2002–2003 and between X-A and X (98 kg N ha^{-1}) in 2003–2004. The reference dose (X) obtained after the adjustment by N_{min} at first N application was higher than N_{op} in both seasons and produced maximum yields. There was no yield increase with the highest dose (X+A) in relation to X (Fig. 2).

The production of aboveground biomass at harvest responded similarly to yield (Table 3), with a range showing a positive response to N fertilizer application followed by one with no response. The quadratic-plus-plateau model fitted the data significantly ($P < 0.05$), with R^2 = 0.57 in the first crop season and R^2 = 0.77 in the second. According to the model, the maximum production of aboveground biomass was 11.9 Mg ha^{-1} in 2002–2003 and 9.1 Mg ha^{-1} in 2003–2004. The N optimum doses were very similar to those obtained for yield.

There were no differences between the maximum and the reference doses at harvest (Table 3). Most of the N extracted by crop (N_a) (70–80%) was in grains (Table 3). In both seasons and the three growth stages sampled, the control treatment developed smaller GLAI than fertilized treatments. No differences were observed, however, in GLAI between treatments X+A and X (Fig. 3).

Nitrogen Balance and Nitrogen Efficiency

In both seasons the difference between N inputs and outputs (N_{bal}) was positive (Table 4). The N_{bal} corresponding to X+A treatment in 2003–2004 (136 kg N ha^{-1}) was higher than for X dose (82 kg N ha^{-1}) ($P < 0.05$). Most of N_{bal} originated from the first N application to harvest, the period when total fertilizer was applied (Table 5). Small N losses by leaching in this period were observed in both seasons.

The NUE and NU_pE values obtained for X-2A, X-A, and X treatments were statistically similar while there was a decrease of both efficiencies for X+A treatment (Table 6). Therefore, no differences in NUE values between X and N_{op} were found and they varied from 15 to 16 kg grain dry wt kg^{-1} N in the first season to 16 to 17 in the second one (Table 6). There were also no differences between NU_pE for X and N_{op} doses, corresponding to 0.51 and 0.39 in the first and second year, respectively.

Fertilizer efficiency (NRF) varied from 0.50 to 0.85 in 2002–2003 and from 0.29 to 0.46 in 2003–2004 (Table 6). The NRF for N_{op} ranged between 0.64 of X-A and 0.85 of X-2A in the first season and between 0.44 of X and 0.46 of X-A in the second one. No differences were observed between NRF of the N_{op} and X doses. It is noteworthy that the greatest standard error was associated with NRF values.

Effect of Fertilizer Splitting and the Use of a Nitrification Inhibitor (DMPP)

The single application of fertilizer caused neither a significant decrease in yield, biomass, N_a nor N_{gr} in comparison with the split doses (Table 3). The inclusion of DMPP (NIT) did not elicit a different response to conventional fertilizer in a single (SG) or split (X) application. No differences were observed in NUE, NU_pE, and NRF for NIT, SG, and X treatments irrespective of the crop season (Table 6).

DISCUSSION

Effect of Fertilizer Rate Adjustment

The response of yield to N fertilizer application corresponded to the quadratic-plus-plateau model proposed by Cerrato and Blackmer (1990) and Makowski et al. (1999). This model allowed calculation of an optimum dose (N_{op}) above which no yield increase was recorded. Since N_{op} was lower than the reference dose (X) used in the calculations for the remaining treatments, we assured that the experiment comprises an array of doses with a positive crop response to N fertilization.

The NUE value for N_{op} and X can be considered similar since there were no significant differences between NUE values for X and X-A in either crop season. Hence, a maximum yield and a similar efficiency to N_{op} proposed by the model was achieved by applying the N dose calculated for crop requirement corrected by N_{min} content at first N application. This method of N dose adjustment was proposed in the 1970s (Dahnke and Vasey, 1973; Wehrmann and Scharpf, 1979) and is still successfully used in areas of temperate climate (Blankenau et al., 2002). In mediterranean Europe, the method has been applied in some horticultural crops (Vázquez et al., 2006) but there are no reports of use in winter cereals. In agreement with Németh (1996) and Álvarez et al. (2004), the increment of fertilizer dose above X, produced a decrease in both NUE and NRF. That is, over fertilization did not enhance yield but rather increased N_{bal}.

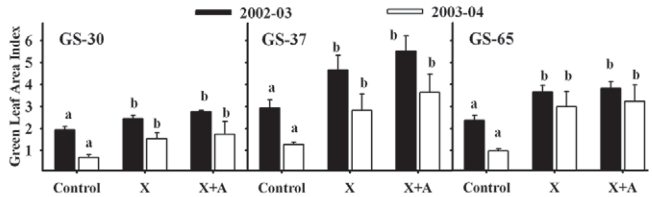

Fig. 3. Treatment influence on green leaf area index, in three growth stages and each year. Within a growth stage and year, data following common letters are not significantly different according to Fisher LSD at a 0.05 probability level. Bars indicate standard error of the mean.

In comparison to previous work performed in similar conditions, the lower NUE in our crop system was mainly due to a lower NU_pE. Here, nitrogen utilization efficiency (NU_tE) for wheat was 30 to 31 (kg grain kg^{-1} N), very similar to that obtained by López-Bellido et al. (2005) for soft wheat in Andalucía (South Spain) but NU_pE at 0.51 in 2002–2003 and 0.39 in 2003–2004 were lower than the 0.60 to 0.70 obtained by López-Bellido et al. (2005).

The main cause of low NU_pE in the 2002–2003 season was nitrate leaching during the period from sowing to first N application. This was identified by Arregui and Quemada (2006) who described this period as the most risky for nitrate leaching in this area. The effect was not repeated the 2003–2004 season, however, because of smaller N_{ini} and less rainfall during this period, confirming the significance of both factors.

In the 2003–2004 crop season, the low values of NU_pE could be attributed to N losses from the fertilizer (NRF = 44–46%). These losses occurred mainly during the period from first N application to harvest, when N_{bal} reached values between 63 and 105 kg N ha^{-1}. Considering that the soil is

Table 3. Yield, aboveground biomass (DM_a), nitrogen in aboveground biomass (N_a) and grain nitrogen (N_{gr}) at harvest, for all treatments and years.

	2002–2003				2003–2004			
Treatment†	Yield	DM_a	N_a	N_{gr}	Yield	DM_a	N_a	N_{gr}
	t ha^{-1} 88% dm	t ha^{-1} dm	kg N ha^{-1}		t ha^{-1} 88% dm	t ha^{-1} dm	kg N ha^{-1}	
Control	4.71a‡	9.08 a	104 a	80 a	2.29 a	4.06 a	45 a	34 a
X-2A	5.5 b	10.44 b	131 b	98 b	3.49 b	6.09 b	54 b	44 b
X-A	6.28 c	11.98 c	145 bc	108 bc	4.35 c	7.59 c	70 c	57 c
X	6.06 c	11.47 c	162 c	117 c	4.47 c	9.25 d	86 d	67 d
X+A	6.36 c	12.20 c	168 c	120 c	4.82 c	8.98 d	89 d	70 d
NIT	6.20 c	12.46 c	172 c	118 c	5.09 c	8.93 d	78 d	63 d
SG	6.32 c	12.90 c	171 c	118 c	4.92 c	9.55 d	81 d	64 d

† X = reference rate [crop N uptake– soil mineral N (0–60)]; A = arbitrary number to establish different treatments; NIT = reference rate with a nitrification inhibitor; SG = single application of N reference rate.

‡ Within each column, data following common letters are not significantly different according to Fisher LSD at a 0.05 probability level.

Table 4. Nitrogen balance of main treatments from sowing to harvest in each of two successive years.

Balance component	Control	X†	X + A	NIT	SG
	kg N ha^{-1}				
	2002–2003				
N_f‡	0	120	160	120	120
N_{ini}§	252	252	252	252	252
N inputs¶	278 a#	398 b	438 c	398 b	398 b
N_{up}††	130 a	203 b	210 b	215 b	214 b
N_{lch}‡‡	73 a	74 a	74 a	76 a	74 a
N_h§§	76 a	99 b	96 b	95 b	91 b
N outputs	278 a	376 b	380 b	385 b	379 b
N_{bal}¶¶	*0 a*	*22 ab*	*58 b*	*13 ab*	*19 ab*
	2003–2004				
N_f	0	110	145	110	110
N_{ini}	116 a	145 b	170 c	156 b	151 b
N inputs	133 a	272 b	332 c	282 b	278 b
N_{up}	56 a	107 b	111 b	98 b	101 b
N_{lch}	6 a	7 a	13 b	12 b	16 b
N_h	70 a	77 a	71 a	69 a	71 a
N outputs	133 a	190 b	195 b	178 ab	187 b
N_{bal}	*0 a*	*82 b*	*136 c*	*104 b*	*91 b*

† X = reference rate [crop N uptake – soil mineral N (0–60)]; A = arbitrary number to establish different treatments; NIT = reference rate with a nitrification inhibitor; SG = single application of N reference rate.

‡ N_f = N supplied by fertilizer.

§ N_{ini} = N_{min} at sowing. Same value for all treatments at the beginning of the experiment in 2002–2003.

¶ N inputs included soil N mineralization estimated from control plots (26 kg N ha^{-1} and 17 kg N ha^{-1} in 2002–2003 and 2003–2004, respectively).

Within each row, data following common letters are not significantly different according to Fisher LSD at a 0.05 probability level.

†† N_{up} = crop nitrogen uptake at harvest (aboveground crop nitrogen plus N in roots).

‡‡ N_{lch} = N leached (data from Arregui and Quemada, 2006).

§§ N_h = N_{min} at harvest.

¶¶ N_{bal} = Difference between N inputs and N outputs.

Table 5. Nitrogen balance of main treatments (0–90 cm) from first N application to harvest in each of two successive years.

Balance component	Control	X†	X + A	NIT	SG
	kg N ha^{-1}				
	2002–2003				
N_f‡	0	120	160	120	120
N_{pres}§	145	145	145	145	145
N inputs¶	187 a#	307 b	347 b	307 b	307 b
N_{up}††	107 a	180 b	187 b	192 b	191 b
N_{lch}‡‡	4 a	5 a	5 a	7 a	5 a
N_h§§	76 a	99 b	96 ab	95 ab	91 b
N outputs	187 a	284 b	288 b	294 b	287 b
N_{bal}¶¶	*0 a*	*23 a*	*59 a*	*13 a*	*20 a*
	2003–2004				
N_f	0	110	145	110	110
N_{pres}	62 a	74 b	77 b	84 b	78 b
N inputs	117 a	239 b	277 c	249 b	243 b
N_{up}	44 a	94 b	95 b	81 b	97 b
N_{lch}	3 a	5 ab	6 b	7 bc	9 c
N_h	70 a	77 a	71 a	69 a	71 a
N outputs	117 a	176 c	172 c	157 b	177 c
N_{bal}	*0 a*	*63 b*	*105 c*	*92 c*	*66 b*

† X = reference rate [crop N uptake –soil mineral N (0–60)] ; A = arbitrary number to establish different treatments; NIT = reference rate with a nitrification inhibitor; SG = single application of N reference rate.

‡ N_f = N supplied by fertilizer.

§ N_{pres} = Soil mineral N (0–90 cm) before first N application. Same value for all treatments in 2002–2003.

¶ N inputs included soil N mineralization estimated from control plots (42 kg N ha^{-1} and 55 kg N ha^{-1} in 2002–2003 and 2003–2004, respectively).

Within each row, data following common letters are not significantly different according to Fisher LSD at a 0.05 probability level.

†† N_{up} = crop nitrogen uptake at harvest (aboveground crop nitrogen plus N in roots).

‡‡ N_{lch} = N leached (data from Arregui and Quemada, 2006).

§§ N_h = N_{min} at harvest.

¶¶ N_{bal} = Difference between N inputs and N outputs.

alkaline and rainfall was <5 mm in the week after first N application, the losses can be attributed to ammonia volatilization. According to Meisinger and Randall (1991), these circumstances may produce N fertilizer losses up to 60% in the ammonia form. Additional losses were likely by denitrification with abundant rainfall (40 mm) in the week following the second N application in 2003–2004.

Effect of Fertilizer Splitting

No improvement in the crop response was achieved by split applications of fertilizer. This is in contrast with other reports in the literature depending on the area of study. For instance, Dilz (1988) established advantages of split applications in terms of yield, N extraction, and efficiency, although the benefits were greatest in the wettest areas or periods. According to our results, the dose splitting did not produce a significant improvement in NRF, irrespective of the crop season.

In practice, the effects of split doses are not easily predictable because they can be biased by: (i) the number of applications, their timing, and quantities (Mahler et al., 1994), (ii) the weather conditions during the season that influence leaching, volatilization, and crop growth (Alcoz et al., 1993), and (iii) the N_{min} amount present in the soil at the time fertilizer application (Sowers et al., 1994).

Various studies in winter cereal crops have demonstrated greater NUE in spring than in autumn applications (Sowers et al., 1994; López-Bellido et al., 2005) but there is a lack of information on the effects of splitting applications in spring. In the present experiment, all treatments received fertilizer from GS-25, when losses due to leaching were small (Arregui and Quemada, 2006) and N absorption by the crop was high. These conditions favored greater NRF when N was applied in a single application compared with split applications.

Since fertilizer splitting imposes greater labor and fuel costs, these results indicate that it is not possible to justify split applications for greater yield or NUE when N fertilizer application is itself adjusted to crop requirements in time and amount. In practice, however, splitting can offer the important opportunity of reducing total dose if the second application depends on the crop needs on each season (Arregui et al., 2006). The use of diagnostic methods based on soil or plant analysis may allow adjustment, including elimination, of the second dose. In our assay, for example, fertilizer dose was corrected for N_{min} in both single and split applications, because soil analyses were available before fertilization. It is likely, however, that farmers will not have N_{min} data available before the first N application and so can only apply corrections in the case of split doses. Late applications can be used to achieve various objectives, including improvement in grain quality as well as yield (Arregui et al., 2006).

Effect of the Use of a Fertilizer with a Nitrification Inhibitor (DMPP)

In this study, inclusion of the nitrification inhibitor showed no advantage in any variable studied, including NUE, in comparison with the single application, irrespective of the season and crop. This is consistent with reports in the literature and the conditions under which N inhibitors are most likely to achieve positive responses. In various studies with wheat, Prasad and Power (1995) some, but not all, show positive responses while in others (Pasda et al., 2001) the yield advantage was small, averaging just 5%. In general, nitrification inhibitors have been shown to increase yield when the fertilizer is applied in conditions that favor nitrate losses by leaching, that is, in periods of high rainfall or irrigation, or in sandy or highly permeable soils (Barth et al., 2001). In the present experiment, fertilizer was applied, not only when the leaching was low and also when crop demand was high (Arregui and Quemada, 2006). These provide a combination of conditions that does not favor advantage from the inclusion of nitrification inhibitors.

The results from the conventional fertilizer (SG) and the fertilizer with DMPP (NIT) in 2003–2004 season, support the above mentioned observation about the importance of losses by volatilization in our experiment. The N absorbed by crop was higher in SG than in NIT in the period from N application to harvest. At the same time, N_{bal} was higher in NIT treatment, reaching during this period values similar to those of X+A treatment. The higher N_{bal} in NIT treatment was probably related to an enhancement of ammonia volatilization, due to higher ammonium content in soil compared to SG treatment. These results corroborate the DMPP nitrification inhibitory capability already observed in previous research (Arregui and Quemada, 2006), but also show that this process causes an increase in volatilization losses rather than differences in yield between SG and NIT. This is a relevant constraint for the application of fertilizers with inhibitors in conditions that favor volatilization and show the need to study in depth this issue.

The use of fertilizer with nitrification inhibitor (DMPP) did not result in any improvement in the remaining components of N balance. The main cause could be similar to that previously mentioned in the case of dose splitting and repeated throughout this work. The fertilizer dose applied was adjusted when the crop was actively extracting N and from then onward the leaching risk decreased greatly. Since there was no influence over the balance components, the use of fertilizer with DMPP did not affect NUE. Nevertheless, a complete factorial of N rate and timing, as well as different rates of NIT, would tell us much more information about their effectiveness.

Table 6. Effect of various N rates and fertilization strategies on nitrogen use efficiency (NUE), nitrogen uptake efficiency (NU_pE), and nitrogen recovery fraction (NRF) in 2002–2003 and 2003–2004.

Efficiency parameters	Control	X-2A†	X-A	X	X + A	NIT	SG
				2002–2003			
NUE, kg kg^{-1}	15 a‡	15 a	16 a	14 a	13 a	14 a	14 a
NU_pE	0.47 a	0.51 a	0.51 a	0.51 a	0.48 a	0.54 a	0.54 a
NRF		0.85 b	0.64 ab	0.61 ab	0.50 a	0.71 ab	0.70 ab
				2003–2004			
NUE, kg kg^{-1}	15 ab	16 b	17 b	16 b	13 a	16 b	16 b
NUpE	0.43 b	0.34 a	0.39 a	0.39 a	0.34 a	0.35 a	0.36 a
NRF		0.29 a	0.44 a	0.46 a	0.38 a	0.37 a	0.40 a

† X = reference rate [crop N uptake –soil mineral N (0–60)] ; A = arbitrary number to establish different treatments; NIT = reference rate with a nitrification inhibitor; SG = single application of N reference rate.

‡ Within each row, data following common letters are not significantly different according to Fisher LSD at a 0.05 probability level.

CONCLUSIONS

Two winter cereal crops in rainfed Mediterranean conditions achieved maximum yield and a similar N use efficiency to the optimum dose, calculated by a quadratic-plus-plateau model, when fertilizer dose was adjusted by N_{min} (0–60 cm) at the first N application. In these conditions, neither applying N in two doses nor including a nitrification inhibitor (DMPP) with a single dose showed any advantages in terms of yield or N efficiency.

The results reveal that low N efficiency in this system was mainly due to a low efficiency in N uptake by the crop. They further suggest the importance of fertilizer loss by ammonium volatilization and the need to study this issue in depth.

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