



## RESEARCH ARTICLE

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# Utility of the end-of-season nitrate test for nitrogen sufficiency of irrigated maize under Mediterranean semi-arid conditions

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## Abstract

Calibration of decision tools to improve N fertilizer management is critical to increase its adoption by maize (*Zea mays* L.) growers. The objective of this study was to establish nitrate and total nitrogen concentrations in the basal maize stalks (BMS) at harvest to separate maize fields among three N availability categories (N-deficient, N-optimum, and N-excess) under Mediterranean irrigated semi-arid conditions. We analysed data from 26 irrigated maize trials conducted between 2001 and 2012. Trials included treatments receiving different N fertilizer rates and sources (mineral and organic), irrigation systems (flood, sprinkler) and soil types. The critical nitrate concentration in BMS to identify N-deficient plots ( $CNC_L$ ) is affected by the irrigation system. The  $CNC_L$  was lower under sprinkler irrigation (708 mg  $NO_3^-$ -N/kg) than under flood irrigation (2205 mg  $NO_3^-$ -N/kg), and the later presented a higher degree of uncertainty compared to sprinkler irrigated systems. The results showed the difficulty to identify the N-deficient plots with the BMS test and the higher sensibility of nitrate-N than total-N concentration in BMS to separate N-deficient from N-optimal plots. Under sprinkler irrigation, nitrate in  $BMS > 1500$  mg  $NO_3^-$ -N/kg had a 85% probability of having received an excess of N. Considering economic net returns to N fertilization, the range of nitrate concentration in BMS that maximized profit under sprinkler-irrigated conditions was established between 1100 and 1700 mg  $NO_3^-$ -N/kg. Results suggest that BMS test can be useful in detecting plots with an excess of N but considering irrigation efficiency is crucial for establishing successful CNC thresholds.

**Additional key words:** basal maize stalk; N-leaching; N fertilizer; sprinkler irrigation; flood irrigation

**Abbreviations used:** BMS (basal maize stalk); CI (confidence interval);  $CNC_L$  (critical nitrate concentration in BMS that separates N-deficient from non N-deficient plots);  $CNC_U$  (critical nitrate concentration in BMS that maximize the net-return of fertilizer);  $CTNC_L$  (critical total nitrogen concentration in BMS that separates N-deficient from non N-deficient plots); LRP (Linear Response and Plateau model);  $N_{opt}$  (minimal N fertilizer dose that maximize maize grain yield); SE (standard error)

**Citation:** Isla, R.; Salmerón, M.; Caveró, J.; Yagüe, M. R.; Quílez, D. (2015). Utility of the end-of-season nitrate test for nitrogen sufficiency of irrigated maize under Mediterranean semi-arid conditions. Spanish Journal of Agricultural Research, Volume 13, Issue 1, e09-002, 9 pages. <http://dx.doi.org/10.5424/sjar/2015131-6806>.

**Received:** 08 Sept 2014. **Accepted:** 03 Feb 2015

<http://dx.doi.org/10.5424/sjar/2015131-6806>

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**Funding:** This study was financed by the Ministerio Español de Ciencia e Innovación (Projects N° AGL2001-2214-C06-04, AGL2005-08020-C05-01, AGL2009-12897-C02-02), by the INIA (SC00-061-C02-1, RTA04-114-C03-01) and by FEDER funds.

**Competing interests:** The authors have declared that no competing interests exist.

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## Introduction

Maize (*Zea mays* L.) is one of the main crops grown in the irrigated areas of the Ebro river basin (Spain), with approximately 140,000 ha cropped each year (MAGRAMA, 2013). Grain yields typically range from 9 to 15 Mg/ha depending on year, soil type, or irrigation system. Maize has a high N fertilizer demand and is frequently over fertilized by farmers (Isidoro *et al.*, 2006; García-Garizabal *et al.*, 2012), especially when fertilizer prices are low, in order to ensure N suffi-

ciency levels. High nitrate concentrations have been measured in drainage return flows from maize fields and this crop has been recently recognized as one of the main contributors to nitrate pollution of surface and ground waters (Caveró *et al.*, 2003; Causapé *et al.*, 2004a; Isidoro *et al.*, 2006) in the Ebro river basin.

Several irrigated areas in the basin have been declared vulnerable to nitrate leaching by Regional governments following the EU Directive (EC, 1991). In those areas, action programs impose limitations to the amounts of N fertilizer that can be applied to crops,

which should be consistent with good agricultural practices and consider the specific characteristics of each farm such as realistic yield goals and N inputs from the soil and from irrigation water. In order to minimize N losses, several site characteristics should be taken into consideration: soil mineralization rate, irrigations system, doses of irrigation, previous crop and history of organic fertilization in the field. In most situations, the excess of N applied to maize fields cannot be assigned to a certain field due to the diffuse character of nitrate pollution associated to agricultural activities. It is necessary to develop decision tools for N management based on soil or plant N critical levels that can be easily implemented by growers in order to minimize N losses from maize fields while maintaining high yields.

Several plant tissue tests to improve N management in maize have been developed and evaluated in the US Corn Belt. Leaf N concentration at silking (Cerrato & Blackmer, 1991) or visual rating of firing in the lower part of the foliage (Binford & Blackmer, 1993) were considered not sensitive enough to indicate the N status of maize. The use of portable chlorophyll meters has been proposed (Blackmer & Sheppers, 1995; Piekielek *et al.*, 1995) and is currently used to determine the nutritional status of maize under experimental conditions. However, collecting chlorophyll meter readings in commercial fields during the growing period can present a practical limitation and it is usually refused by farmers because it requires an overfertilized area in the field as a control to compare with.

The end-of-season basal maize stalk (BMS) nitrate test (Binford *et al.*, 1990, 1992) is a tool to identify deficiencies as well as excess of N in maize that was proposed as feed-back information to optimize N management in the following seasons. This test provides an optimal range of BMS nitrate concentrations and does not require overfertilized areas in the fields. The lower limit of the critical nitrate concentration ( $CNC_L$ ) identifies N deficiency and the upper limit ( $CNC_U$ ) identifies N excess. Iowa State Extension Services (Blackmer & Mallarino, 1996) established the following nitrate-N concentrations in BMS to separate maize plots into four categories: <250 ppm (low N availability), 250-700 ppm (marginal N availability), 700-2000 ppm (optimal N availability), and >2000 ppm (excess N availability). According to Binford *et al.* (1992), plots with nitrate-N in BMS lower than 700 mg/kg indicate risk of economic penalties, whereas plots with more than 2000 mg/kg indicate N-excess and that maize was probably overfertilized.

The critical stalk nitrate concentration (CNC) seems to be remarkably constant across maize hybrids and environmental conditions in the US Corn Belt since it

was obtained by pooling data from different hybrids and across different soil conditions (Binford *et al.*, 1990). Moreover, other studies (Wilhelm *et al.*, 2000; Isla & Blackmer, 2007) demonstrated that the BMS nitrate test is robust to minor deviations in sampling procedure. However, a study performed in the North China Plains (Zhang *et al.*, 2013) suggests that the CNC of BMS nitrate test can differ across hybrids depending on their origin.

Most of the available information in relation to this test has been collected under the agronomic and environmental conditions of the US Corn Belt. Maize grown in the semiarid conditions of the Ebro valley is irrigated using either flood or, increasingly, sprinkler systems. Seasonal irrigation ranges from 700 to 1300 mm in sprinkler and flood irrigation systems, respectively. Moreover, average maize yields in the Ebro valley, especially under sprinkler irrigated conditions, are usually higher than under the rainfed conditions of the US Corn Belt area, suggesting higher N requirements and the need for specific BMS test threshold values for irrigated maize in semiarid conditions.

Binford *et al.* (1990) found similar relationships between Kjeldhal-N in BMS and yields in maize than using nitrate. However, they recommended measuring nitrate concentrations based on the higher simplicity and lower cost compared to Kjeldhal determinations. Nowadays, the determination of total N concentration in plant tissues by using dry combustion methods or by near infrared reflectance spectrometry is rapid and relatively inexpensive in comparison to the more time consuming methods of nitrate extraction and determination. The use of critical total N concentration (CTNC) instead of the CNC has the potential to simplify the BMS test under the hypothesis of the existence of a relationship between total N concentration in BMS and maize yield, but this relationship has received little attention.

Therefore, the objective of the study was to establish critical nitrate and total-N concentrations in the BMS to separate maize fields into three availability categories (N-deficient, N-optimal and N-excess) under semiarid irrigated conditions.

## Material and methods

### General description of the trials

Twenty six maize N-response trials were conducted between 2001 and 2012 in the semiarid conditions of the Middle Ebro valley (Spain). This area is characterized by a semiarid climate with an average rainfall and ETo (FAO Blaney-Criddle) of 350 and 1316 mm, respectively, and

an average air temperature of 14.8°C. Irrigation is needed in this area to grow summer crops such as alfalfa and maize and to significantly increase the yield of winter cereal crops. The field trials were performed under different soil types, irrigation systems, and agronomic practices to assess different aspects related to N management in maize such as N-doses, types of fertilizer, previous crops, or the use of winter cover crops. Table 1 presents the main characteristics of each field trial.

Different late-season maize hybrids (FAO 600 or FAO 700) were used at each trial, following the standard practices of the area. Maize was planted between mid-April to late May at a density of ~85,000 plants/ha with a row spacing of 0.75 m.

Experimental plots received mineral N fertilizer and/or pig slurry due to the frequent use of this organic fertilizer in the area. At four trials, mineral fertilizer was applied and legume and non-legume winter cover crops were incorporated into the soil as a green manure before maize sowing. At all trials, N was split between pre-planting and two side-dress applications when maize plants reached the six-leaves (V6) and a latter application between V12 and VT (tasseling), respectively. The

distribution of the total N between the three split applications varied with each trial although a higher percentage of the N (40-60%) was always applied at the first side-dress application (V6). The mineral N fertilizers used were urea before sowing, and ammonium nitrate at side-dressing. In plots receiving pig slurry, it was applied instead of urea before planting. The pig slurry application rate was based on the ammonium-N content in pig slurry determined in the field using a Quantofix® N volumeter (Piccinini & Bortone, 1991). Eighteen trials were sprinkler-irrigated and eight trials were flood-irrigated. The 589 experimental plots comprised in the present study represent the range of N availabilities, N fertilizer types, soil textures, and irrigation systems generally found in the Middle Ebro basin.

Maize grain yield at each experimental plot was determined by hand harvesting all the ears included in the central part of each plot, comprising an area ranging from 2 to 27 m<sup>2</sup>, depending on the plot size. Considering all trials, the averaged sampled area was 10 m<sup>2</sup>, and only in three trials the sampled area was 2 m<sup>2</sup>. The ears were threshed and the grain was weighed to determine yield that was expressed as Mg/ha of grain at 14% moisture.

**Table 1.** General description of the 26 field trials.

Trial	Year	USDA soil texture	No. of plots	No. N rates	N applied (kg N/ha)	Fertilization type <sup>a</sup>	Irrigation system <sup>b</sup>
1	2001	Sandy-loam	20	5	0-300	m	s
2	2002	Sandy-loam	18	5	0-300	m	s
3	2002	Silt loam	24	8	0-348	m, ps	f
4	2003	Sandy-loam	44	5	0-300	m	s
5	2003	Sandy-loam	48	16	0-350	m, ps	s
6	2003	Silt loam	24	8	0-400	m, ps	f
7	2004	Sandy-loam	20	5	0-300	m	s
8	2004	Sandy-loam	48	14	0-350	m,ps	s
9	2004	Sandy-loam	12	2	300-500	m	f
10	2004	Clay-loam	18	2	216-306	m	f
11	2004	Silt-loam	24	4	0-300	m	f
12	2007	Silt-loam	12	2	154-300	m+cc	f
13	2007	Silty-clay-loam	30	2	250-300	m+cc	s
14	2007	Loam	18	5	0-300	m	f
15	2008	Silt loam	12	2	159-300	m+cc	f
16	2008	Silty-clay-loam	31	3	0-300	m+cc	s
17	2008	Silt Loam	18	5	0-300	m	s
18	2010	Loam	21	9	0-400	m	s
19	2010	Loam	19	8	0-250	m	s
20	2010	Loam	21	11	0-400	m	s
21	2011	Silty-clay-loam	20	8	0-300	m	s
22	2011	Silty-clay-loam	20	8	0-300	m	s
23	2011	Silty-clay-loam	20	8	0-300	m	s
24	2012	Silty-clay-loam	20	7	0-300	m	s
25	2012	Silty-clay-loam	20	8	0-300	m	s
26	2012	Silty-clay-loam	20	8	0-300	m	s

<sup>a</sup> m, mineral; ps, pig slurry; m+cc, mineral fertilizer plus cover crop incorporated as green manure. N-applied with pig slurry was calculated taken into account slurry composition (measured at each experiment) plus an estimation of N mineralized from previous applications. <sup>b</sup> f, flood irrigation; s, sprinkler irrigation

## Basal maize stalks sampling and analysis

A few days before maize harvest, the BMS were collected following the standard procedure proposed by Binford *et al.* (1990). Briefly, a total of 15 to 20 BMS from 15 to 35 cm above the soil surface were collected from each experimental plot. Leaves were removed from the stalks. The stalks were oven dried to a constant weight at 65°C, ground in a mill and sieved to 0.5 mm. A subsample of 2.5 g of finely ground BMS was extracted with 50 mL of a solution of 2 N KCl, shaken for 30 min and filtered using a cellulose Whatman paper N°1. The determination of nitrate was made using a selective electrode (Wilhelm *et al.*, 2000) or by a colorimetric method using a continuous flow analyzer (AA3, Bran+Luebbe, Norderstedt, Germany). Total-N concentration of BMS was analysed by dry-combustion (LECO FP-528 or TruSpec CN, LECO, St. Joseph, MI, USA). The nitrate and total-N in BMS were expressed as mg N/kg of dry weight.

## Statistical analysis

### Determination of the optimal N rate

Statistical analyses were performed using the SAS/STAT (v.9.1, SAS Inst. Inc., Cary, NC, USA). Grain yield response to N applied was fitted to linear response and plateau (LRP) models (Eq. [1]) by using the NLIN procedure:

$$\begin{aligned} Y &= a + bX & X \leq C \\ Y &= P = a + bC & X > C \end{aligned} \quad [1]$$

where Y is the average grain yield in each N-fertilizer treatment, X is the N applied by mineral fertilizer or pig slurry, and C is the optimal N rate ( $N_{opt}$ , Fig. 1) defined as the minimum N rate to achieve the predicted maximum yield (P). The plateau yield is presented by P or predicted maximum yield. The LRP model was preferred to the quadratic-plateau model because of its simplicity and because in most of the trials the quadratic coefficient was not significant ( $p > 0.05$ ).

LRP models were fit to data from the 15 trials where more than three N rates were applied. Relative yields for each plot were obtained by dividing the grain yield of each plot by the predicted maximum yield at each trial (P, [Eq. 1]). In non-responsive trials (*i.e.*, trials with no significant relationship between N applied and yield) and in trials with less than four N treatments, relative yields were obtained by dividing the actual

grain yield at each plot by the average yield of the three highest yielding plots within each trial.

### Lower critical N concentrations ( $CNC_L$ and $CTNC_L$ ) in BMS

Two different approaches were used to determine the critical nitrate-N ( $CNC_L$ ) and total-N ( $CTNC_L$ ) concentrations in BMS that separates deficit and optimal N availability categories. In the first approach, LRP models were fit to the relationships between relative maize yield and nitrate and total-N in BMS. The  $CNC_L$  and  $CTNC_L$  were defined as the minimum nitrate-N and total-N concentrations in BMS that maximized the relative yields, respectively. In the second approach, the Cate-Nelson analysis (Cate & Nelson, 1971) was used to estimate the  $CNC_L$  and  $CTNC_L$  due to the difficulty of the LRP model to describe the sharp increase of yield in the N-deficient plots (Binford *et al.*, 1990).

The  $CNC_L$  and the  $CTNC_L$  were calculated for each trial where the relationship between relative maize yield and BMS nitrate or total-N concentrations was significant ( $p < 0.05$ ), and also for the pooled data across all trials. The  $CNC_L$  and the  $CTNC_L$  were further estimated separately for the different fertilizer types (organic vs mineral) and for the different irrigation systems (flood vs sprinkler).

To determine the success of the  $CNC_L$  in identifying stalk N concentrations that are adequate to obtain maximum yields, plots with nitrate-N in BMS below and over the  $CNC_L$  were defined as “N-deficient” and “non N-deficient”, respectively. The percentage of “N-deficient” and “non N-deficient” plots was compared to the percentage of plots yielding less and more than 95% of the maximum yield.

### Upper critical N concentration ( $CNC_U$ ) in BMS

The net return of fertilizer was calculated for each plot as the difference between the gross income from the maize grain considering two price scenarios (150 and 200 €/Mg) and the cost from the N fertilizer considering two price scenarios (0.50 and 0.70 €/kg N). To remove part of the variability due the high number of plots, the BMS nitrate data were ranked from lower to higher values and reclassified in groups containing 20 plots each. The averaged nitrate and total-N in BMS and net-return to fertilizer from each group was used in the analysis instead of the individual plot values.

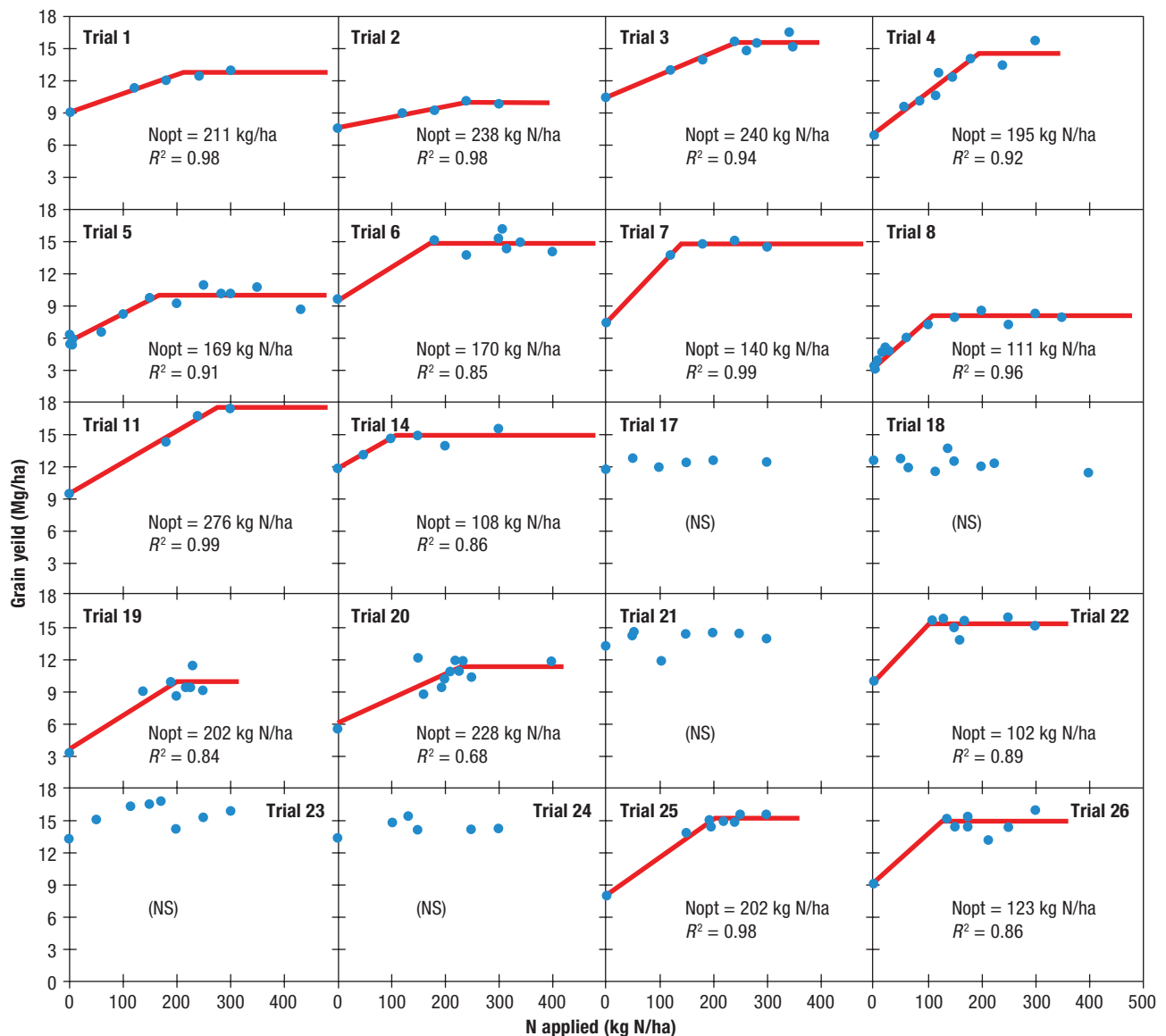
An alternative methodology is proposed to fine-tune the lower and upper CNC's and CTNC's in maize stalks. Each plot in the study was classified as N-deficient (if

received less than  $N_{opt}-25$  kg N/ha), N-optimum (if received between  $N_{opt}-25$  kg N/ha and  $N_{opt} + 50$  kg N/ha), and N-excess (if received more than  $N_{opt} + 50$  kg N/ha). For each of the 3 N-sufficiency categories, the frequency distributions of the nitrate-N and total-N in the BMS were calculated. Moreover, the intervals of nitrate-N and total-N in BMS that include the 75% of the plots in each category (plots compressed between percentiles 12 and 88) were obtained using the UNIVARIATE procedure of SAS software using the option PCTLDEF=5 with averaging to calculate the percentiles. For each of the three categories, the average relative yield, nitrate-N in BMS and total-N in BMS were also calculated. For the sprinkler irrigated plots, the percentage of plots of each category within different established intervals of nitrate-N in BMS were calculated.

## Results and discussion

### Relationships between N applied and grain yield

Maximum observed yields in the field trials (9 to 17 Mg/ha) were representative of the values usually found in the Ebro basin region. In 5 out of the 20 trials with more than 3 N rates, maize yield was not responsive to N fertilization (Fig. 1). In the responsive trials, the LRP model fit well to the data ( $R^2$  ranged from 0.68 to 0.99) and the optimal N rates ( $N_{opt}$ ) ranged from 108 to 276 kg N/ha. The high variability in maize grain yield response to N fertilization among different trials was probably associated to the irrigation efficiency variabil-



**Figure 1.** Maize grain yield (Mg/ha) response to N applied (kg N/ha) in twenty trials with more than three N rates. The linear response plateau (LRP) model was fit to the data and the minimum amount of N fertilizer that maximized yield ( $N_{opt}$ ) is presented when a significant response was found.

ity, the effect of precedent crops, and the differences in soil N supply. This variability is in agreement with the results obtained by Berenguer *et al.* (2009) in the same agricultural area. In our trials, maize yields in the non N-fertilized treatments ranged from 3.5 to 11.8 Mg/ha, indicating significant differences in soil N availability across the different trials. There was no significant relationship ( $p>0.05$ ) between maximum grain yields and  $N_{opt}$  across trials. However, the lowest yielding trial (trial 8, about 8 Mg/ha) was the trial with the second lowest  $N_{opt}$  (111 kg N/ha) and the highest productive trial (trial 11, about 17.5 Mg/ha) showed the highest  $N_{opt}$  (276 kg N/ha). Yield goal is used to guide many maize fertilizer N recommendations although it is a poor predictor of  $N_{opt}$  (Lory & Scharf, 2003). Thus, expected grain yield alone is not adequate to determine  $N_{opt}$  because of site and growing season specific factors. This can be especially true under the irrigated maize conditions of Spain, where medium to long season maize hybrids are grown and yields are quite steady for a given environment (soil and climatic conditions) and in the absence of a significant soil or water stress.

### Relationships between nitrate-N and total-N in BMS and yield

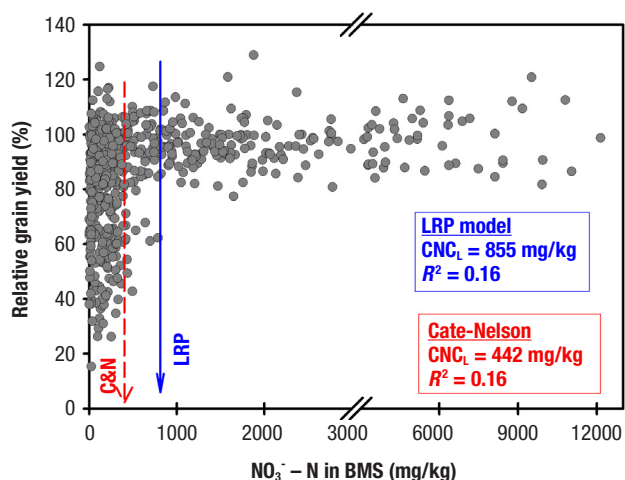
On average across the 26 trials, nitrate concentrations in BMS explained about 48% of the variability in grain yield when the LRP model was fit in each individual trial. Only in 12 of 26 trials, significant ( $p<0.05$ )  $CNC_L$  were obtained. The  $CNC_L$  ranged from 26 mg  $NO_3^-$ -N/kg in trial 25 to 3393 mg  $NO_3^-$ -N/kg in

trial 3, suggesting that  $CNC_L$  is significantly affected by environmental factors. Significant and similar variation among trials in the estimation of  $CNC$  of maize was also found in previous studies by Binford *et al.* (1990, 1992).

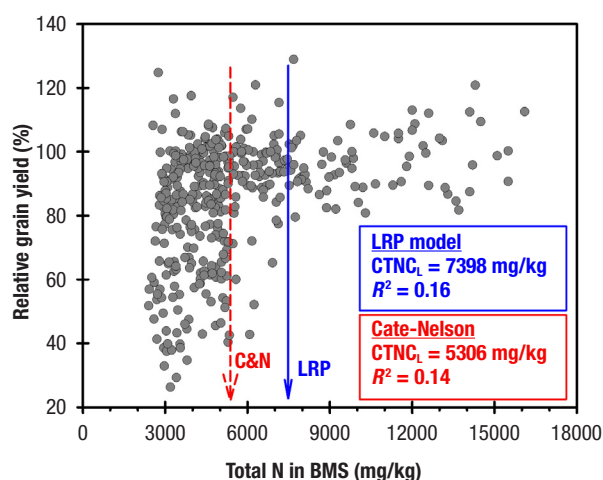
The estimated  $CNC_L$  when the 26 trials were pooled using relative yields (Fig. 2) was 855 mg  $NO_3^-$ -N/kg (95% CI=600-1110; SE=130), much higher than the value obtained by Binford *et al.* (1992) of 250 mg  $NO_3^-$ -N/kg for the Corn Belt conditions of USA. In our conditions, the  $NO_3^-$ -N in BMS from the pooled data only explained 16% of the variability in relative grain yield, much lower than the 68% reported by Binford *et al.* (1992). The low coefficient of determination could be partially due to the difficulty of the LRP model to describe a sharp increase of relative yield in the N-deficient plots as the BMS nitrate concentrations increased. This problem, already mentioned by Binford *et al.* (1990), tends to overestimate the real  $CNC_L$ .

The  $CNC_L$  estimated by the Cate-Nelson procedure was 442 mg  $NO_3^-$ -N/kg. This value was lower than the value obtained using the LRP model, although the two methodologies explained the same amount of variability ( $R^2=0.16$ ) of the relative grain yields. The difference in  $CNC_L$  obtained with LRP and the Cate-Nelson approaches is in agreement with the results obtained by Binford *et al.* (1990, 1992).

The relationship between relative maize yields and the total-N concentration in BMS when pooling the data of 26 experiments is shown in Fig. 3. The  $CTNC_L$  obtained with the LRP model was established at 7398 mg N/kg (95% CI=6111-8686; SE=655). Again, the ability of the LRP model to describe the relationship was quite low since only a 16% of the total variability



**Figure 2.** Relationship between relative grain yield and nitrate concentration in the basal maize stalks (BMS) for all the experimental plots ( $n=587$ ). The lower critical nitrate concentrations ( $CNC_L$ ) obtained with the linear response plateau (LRP) models and the Cate-Nelson procedure are presented.



**Figure 3.** Relationship between relative maize grain yield and total-N concentration in the basal maize stalks (BMS) in all the experimental plots. The lower critical total-N concentrations ( $CTNC_L$ ) obtained with the linear response plateau (LRP) models and the Cate-Nelson procedure are presented.

in relative yields was explained by the total-N concentrations in BMS. The  $CTNC_L$  obtained with the Cate-Nelson procedure was lower (5306 mg/kg) than that obtained with the LRP model, but the percentage of variability explained was similar (14%).

The  $CNC_L$  estimated with the LRP model successfully classified 60% of the plots as high-yielding or low-yielding plots (Table 2). Thirty-one percent of the plots were wrongly classified by excess (high-yielding plots classified as N-deficient plots), and 9% of the plots were wrongly classified by defect (low-yielding plots classified as non N-deficient plots).

Similar successful rates were obtained with the Cate-Nelson approach (Table 2). When using the LRP model, the percentage of N-deficient plots that was misclassified as non N-deficient was lower than when the Cate-Nelson procedure was considered. This suggests that the  $CNC_L$  obtained with the LRP is more conservative for maize growers when used as a guideline for N fertilizer recommendations than the obtained with the Cate-Nelson approach.

The low percentage of variability in relative yield explained by the nitrate concentration in the BMS, together with the high uncertainty associated with the  $CNC_L$  and  $CTNC_L$  estimates, raise the question of the practical usefulness of the test as a good indicator of the crop N status. The low coefficients of determination of the relationship between relative yield and BMS nitrate and total-N concentrations could be due to the variability in management practices across the 26 trials analysed in this study. These included different fertilizer types (organic, mineral, or combination of both), and irrigation systems (flood and sprinkler irrigation). The estimated  $CNC_L$  did not differ ( $p < 0.05$ ) when data were segregated between mineral ( $CNC_L = 725$  mg  $NO_3^-$ -N/kg,  $SE = 141$ ) and organic ( $CNC_L = 895$  mg  $NO_3^-$ -N/kg,  $SE = 136$ ) fertilized plots. This finding contrasts with the results obtained by Kyveryga & Blackmer (2012) who found higher nitrate concentrations in BMS when

manure was used as a fertilizer. In our study the organic fertilizer used was pig slurry which presents a relatively low residual effect because of its low organic matter content compared to other organic manures.

The irrigation system significantly ( $p < 0.05$ ) affected the  $CNC_L$  estimated with the LRP model (Table 3). Under flood-irrigated plots, the  $CNC_L$  (2205 mg  $NO_3^-$ -N/kg) was higher than under sprinkler irrigated plots (708 mg  $NO_3^-$ -N/kg). Similarly, the standard error of the  $CNC_L$  under flood irrigation was almost one order of magnitude higher than under sprinkler irrigation. Both  $CNC_L$  for sprinkler and flood irrigation are still higher than the threshold obtained by Binford *et al.* (1990) of 250 mg  $NO_3^-$ -N/kg under rain-feed conditions in the USA Corn Belt region. The estimated  $CTNC_L$  was similar (Table 3) for flood and sprinkler irrigation systems although a higher uncertainty ( $SE = 1461$  mg N/kg) of the  $CTNC_L$  was observed under flood-irrigated conditions than under sprinkler irrigation ( $SE = 525$  mg N/kg).

The higher  $CNC_L$  obtained under irrigated semiarid conditions compared to the  $CNC_L$  proposed by Binford *et al.* (1990, 1992) for the Corn Belt region of the USA, could be related to the high N rates generally applied in irrigated maize in the study area. A recent study (Zhang *et al.*, 2013) also suggests the necessity to adjust the  $CNC$  when using varieties not used in the USA.

The irrigation system has a significant impact on irrigation efficiency and consequently on crop N use efficiency. In the Ebro river basin, lower nitrate losses have been found in sprinkler-irrigated areas (Cavero *et al.*, 2003) than in flood-irrigated areas (Isidoro *et al.*, 2006). Under similar fertilization practices (dose and splitting) maize grown under flood irrigation should have less available N than under the more efficient sprinkler irrigation systems due to higher N leaching, especially at the end of the growing period. The higher  $CNC_L$  obtained under flood irrigation (Table 3) sug-

**Table 2.** Percentage of success and failure of the lower critical nitrate concentrations ( $CNC_L$ ) and critical total N concentration ( $CTNC_L$ ) estimates, using the LRP model and the Cate-Nelson procedure, to classify maize plots as low-yielding (relative grain yield < 95%) or maximum yielding (relative grain yield > 95%).

	$CNC_L$		$CTNC_L$	
	LRP model 855 mg/kg	Cate-Nelson 442 mg/kg	LRP model 7398 mg/kg	Cate-Nelson 5306 mg/kg
Success (%)	60	64	66	67
Failure (%)	40	36	34	33
by excess <sup>a</sup>	31	23	28	20
by defect <sup>b</sup>	9	13	6	13

<sup>a</sup> % of high-yielding plots classified as N-deficient. <sup>b</sup> % of low-yielding plots classified as non N-deficient

**Table 3.** Effect of irrigation system on the lower critical nitrate-N concentration ( $CNC_L$ ) and critical total-N concentration ( $CTNC_L$ ) of basal maize stalks (BMS) estimated with the Linear response plateau (LRP) model. The standard error (SE) and the 95% confidence interval of the estimated values are also presented.

	Irrigation system	
	Flood	Sprinkler
<b>BMS nitrate-N concentration</b>		
Number of plots	142	445
$CNC_L$ , mg $NO_3^-$ -N/kg	2205	708
SE, mg $NO_3^-$ -N/kg	854	115
95% CI	517-3893	481-934
$R^2$	0.15	0.17
<b>BMS total-N concentration</b>		
Number of plots	130	263
$CTNC_L$ , mg N/kg	7166	6477
SE, mg N/kg	1461	525
95% CI	4276-10057	5442-7512
$R^2$	0.15	0.19

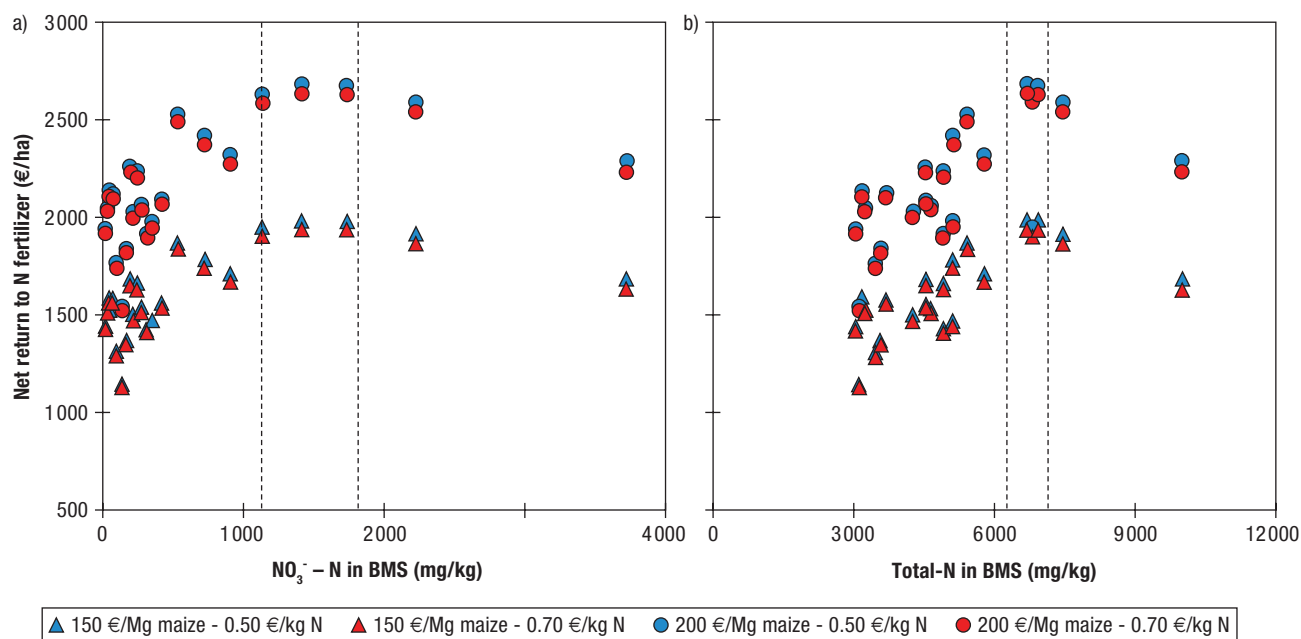
gests a lower N efficiency in the flood irrigated plots than in the sprinkler irrigated plots, and the necessity of higher N fertilization rates to fully cover maize N requirements and reach maximum yields.

The practical consideration is that it is not possible to provide a single recommendation of  $CNC_L$  in BMS under different irrigation systems with significant differences in irrigation efficiency. Moreover, the variability

of  $CNC_L$  was much higher for flood than for sprinkler conditions as reflected by the confidence interval range (Table 3). The high variability in flood irrigation systems is related to the high variability of irrigation efficiency and makes it difficult to establish a unique  $CNC_L$  for this irrigation that can be applied across different yield conditions. Considering these results, we suggest that  $CNC_L$  in flood irrigation systems should be established separately for different levels of irrigation efficiency, as irrigation efficiency is affected by soil characteristics and irrigation management practices and therefore is highly variable. Sprinkler irrigation systems present higher and less variable irrigation efficiency than flood irrigation systems and the variability of  $CNC_L$  was much lower allowing a more confident estimation of  $CNC_L$ .

### Nitrate and total-N concentrations in BMS and economic net return

In the sprinkler irrigated fields, the relationship between nitrate concentration in BMS and net return to N fertilization shows a peak of maximum return at a BMS nitrate concentration between 1100 and 1700 mg  $NO_3^-$ -N/kg ( $CNC_U$ ) (Fig. 4), irrespective of the different grain and fertilizer prices analysed, which is within the interval (700-2000 mg  $NO_3^-$ -N/kg) proposed by Blackmer & Mallarino (1996). Similarly, total-N



**Figure 4.** Relationships between the net returns to N fertilizer (€/ha) and the nitrate concentration (a) and total-N concentration (b) in the basal maize stalks (BMS) for the sprinkler-irrigated plots. The X-Y plots are presented for different scenarios of maize grain prices (150 and 200 €/Mg) and N fertilizer prices (0.50 and 0.70 €/kg). Each value is moving average of 20-plots ordered by their nitrate (a) or total-N concentrations (b) in the BMS. Vertical dashed lines indicate the range of nitrate-N ( $CNC_U$ ) and total-N ( $CTNC_U$ ) in BMS that maximized the net return to N fertilizer.

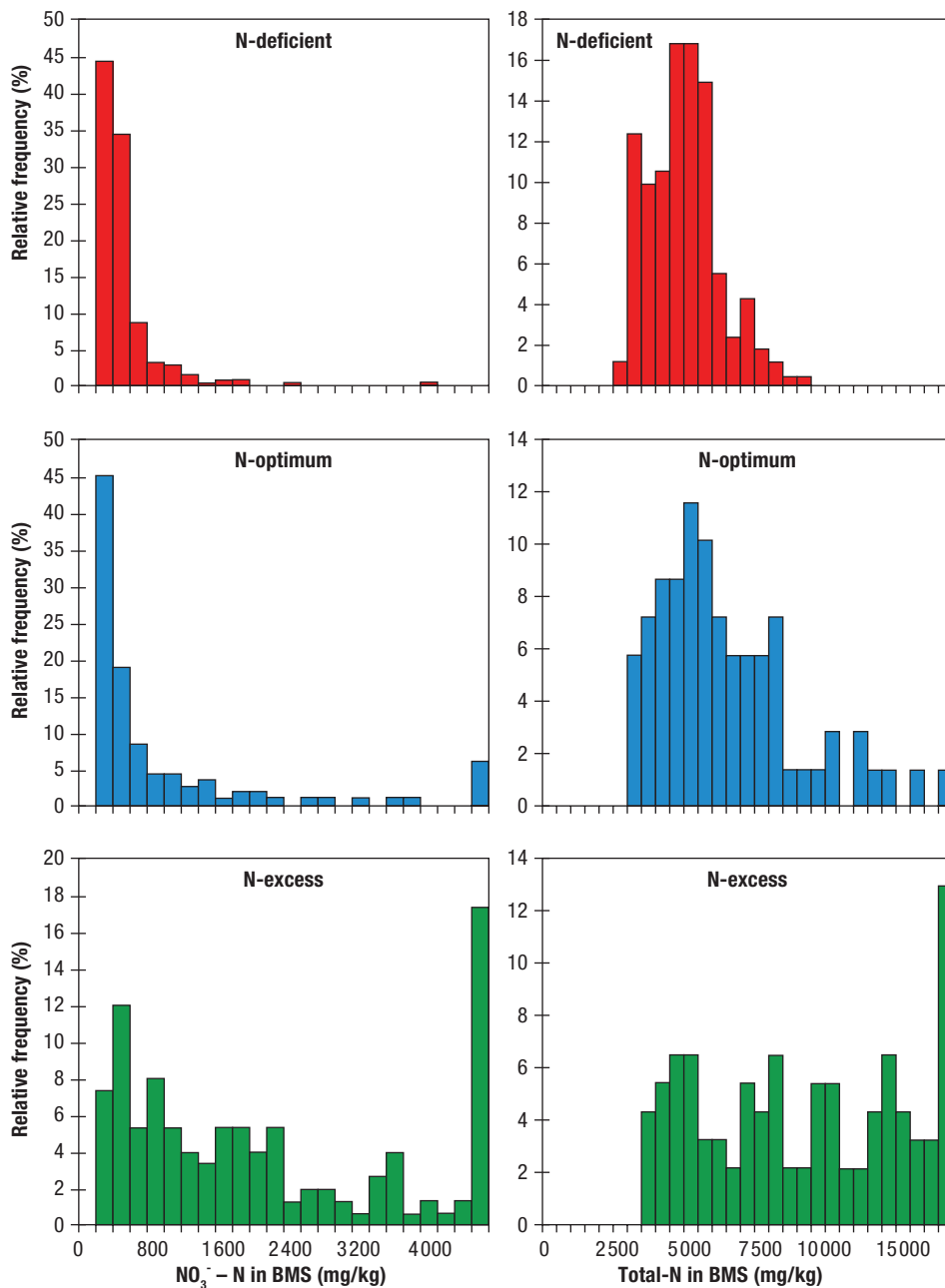


concentrations in BMS between 6000-7000 mg N/kg maximized the net return of N fertilizer ( $CTNC_U$ ) (Fig. 4).

The lower end of the  $CNC_U$  interval (1100 mg  $NO_3^-$ -N/kg) is clearly higher than the  $CNC_L$  obtained with the LRP approach in the sprinkler irrigated plots (708 mg/kg). This indicates that some of the plots with stalk nitrate concentrations below 1100  $NO_3^-$ -N mg/kg have a significant risk of economic penalties associated with N deficiencies. The use of  $CNC_U$  interval (1100-1700 mg  $NO_3^-$ -N/kg) to optimize the economic net return

can lead to an N overfertilization in some plots, which shows the difficulty to make compatible the best management of N fertilizer from an environmental point of view with the maximum benefit to the farmers.

The  $CNC_U$  for flood irrigated plots should be established for different levels of irrigation efficiency and no attempt to establish these values have been made in this work due to lack of information about irrigation efficiencies in the different field trials and the significantly lower number of plots under flood (142) than under sprinkler (445) irrigation.



**Figure 5.** Relative frequency histograms of nitrate (left side) and total-N concentration (right side) in basal maize stalks (BMS) for the three N-sufficiency categories (deficient, optimum, and excess) considering all the trials of the study.

## Nitrate in BMS and N sufficiency

The frequency distribution of nitrate and total-N concentrations in BMS in each of the three N sufficiency categories is presented in Fig. 5. In the N-optimum category, the nitrate in BMS was positively skewed and followed a lognormal distribution (Kolmogorov-Smirnov test,  $p > 0.1$ ). The shape of the frequency of distribution suggests that the nitrate concentration in BMS is more accurate at identifying N-excess plots than N-deficient plots. In other words, it is difficult to separate N-deficient from N-optimum plots due to the significant overlapping between these two categories. This is valid for both nitrate and total-N in BMS.

Table 4 presents the average relative yield, nitrate, and total-N concentration in BMS within each category. As expected, grain yield in the N-excess plots was not significantly higher than at the N-optimum plots, but the N-deficient plots yielded on average 30% less than the optimum and N-excess plots. The effect of the irrigation system is evident, being the average nitrate concentrations in BMS in the N-optimum plots significantly higher under flood irrigation (average=3086 mg NO<sub>3</sub><sup>-</sup>-N/kg) than under sprinkler irrigation (460 mg NO<sub>3</sub><sup>-</sup>-N/kg).

Table 4 also presents the lower and upper limits of the intervals that include 75% of the plots with nitrate and total-N BMS concentrations within the three above-mentioned categories for sprinkler and flood irrigated fields. Under sprinkler irrigated conditions, a higher overlapping among the N sufficiency categories was observed for total-N concentrations than for nitrate concentrations, consistent with the information provided in Fig. 5. In addition, a higher overlapping among

**Table 5.** Probability of the sprinkler-irrigated plots to fall within the different N-sufficiency categories (deficient, optimum, and excess) depending on the nitrate-N concentration in the basal maize stalk (BMS).

BMS nitrate-N (mg/kg)	N-Deficient (%)	N-Optimum (%)	N-Excess (%)
0-250	57	34	9
250-500	67	18	15
500-750	24	24	52
750-1000	24	29	48
1000-1500	19	30	52
1500-2000	13	17	71
2000-2500	0	17	83
< 500	60	29	11
> 1500	4	10	85
> 2500	0	3	97

the N sufficiency categories was observed between the nitrate and total-N in BMS under flood-irrigated plots than under sprinkler irrigated plots. This higher overlapping could be associated to the lower number of experimental plots under flood than under sprinkler irrigation and the expected higher variability of the irrigation efficiency under flood-irrigated plots as already explained previously.

Table 5 presents the estimated probability to belong to each N-sufficiency category depending on the value of nitrate-N in BMS. Nitrate in BMS > 1500 mg NO<sub>3</sub><sup>-</sup>-N/kg presents a high probability (85%) of belonging to the N-excess group but only a 5% probability of being N-deficient. The separation between N-optimum and N-deficient plots is more difficult and no clear cut-off can be obtained. Thereby, plots with nitrate in BMS lower than 500 mg NO<sub>3</sub><sup>-</sup>-N/kg present a 60%

**Table 4.** Average relative maize yield, nitrate-N concentration, and total-N concentration in the basal maize stalks (BMS) for the 3 different N-sufficiency categories under sprinkler and flood irrigation systems. Means followed by the same letter were not significantly different at  $p = 0.05$  (Tukey test). The interval of nitrate-N and total-N concentrations that includes the central 75% of the experimental plots (percentile 12 to percentile 88) for each N-sufficiency class are presented between brackets. N-deficient plots received less than  $N_{opt}-25$  kg N/ha, N-optimum plots received between  $N_{opt}-25$  and  $N_{opt}+50$  kg N/ha, and N-excess plots received more than  $N_{opt}+50$  kg N/ha.  $N_{opt}$ =optimal N rate for maximum yields.

N-sufficiency category	n	Relative grain yield (%)	BMS (mg/kg)	
			Nitrate-N	Total-N
Sprinkler-irrigated plots				
Deficient	173	69.7b	278b [1, 331]	4623c [2400, 5200]
Optimum	102	96.8a	460b [36, 1178]	5624b [3930, 7670]
Excess	121	99.0a	1741a [649, 6968]	7982a [5265, 14900]
Flood-irrigated plots				
Deficient	27	78.4b	596b [4, 640]	3970c [2590, 4500]
Optimum	21	99.6a	3086a [17, 8424]	6983b [3175, 11600]
Excess	30	99.5a	4930a [1205, 12142]	10698a [7930, 16100]

probability of being N-deficient, although a high percentage of these plots were still classified as N-excess (11%) or N-optimum (29%).

Soil available N is related to irrigation efficiency at the plot level, affecting nitrate losses and N availability to the crop. In sprinkler irrigated systems, irrigation efficiency is high and has low variability (Tedeschi *et al.*, 2001; Cavero *et al.*, 2003), whilst in surface irrigated systems irrigation efficiency is generally low and highly variable depending on soil type and soil management practices (Causapé *et al.*, 2004b). Therefore, depending on the criteria used, the limits to separate N-deficient and N-excess from N-optimum plots were different. The variability can be in part due to the fact that similar level of nitrogen stress at different maize growth stages can produce different nitrate and total-N concentrations in BMS at harvest time. Nitrogen deficits during vegetative stages of maize development (V4 to V12 stages) can affect significantly yield, although nitrate concentrations in BMS can be increased with later supply of nitrogen to the crop due to N fertilizer applications or mineralisation from organic fertilizers.

In summary, the different  $CNC_L$  in BMS obtained when using different statistical approaches indicate the difficulty to establish a unique critical  $CNC_L$  to identify N-deficient plots with high confidence under irrigated semiarid conditions. The response of relative maize yield to nitrate concentration in BMS was different when maize was grown under different irrigation systems, with higher nitrate concentrations and  $CNC_L$  under flood than under sprinkler irrigated plots for the same level of N availability. For surface irrigated plots, the high uncertainty observed in the obtained  $CNC_L$  precludes its use to guide N fertilization. For sprinkler irrigated plots, nitrate in BMS that maximized economic net return ( $CNC_U$ ) ranged between 1100 to 1700 mg  $NO_3^-$ -N/kg, which also implies some degree of over-fertilization. Our results also suggest better separation between the different sufficiency categories using nitrate than total-N concentration in BMS. The separation between N-optimum and N-deficient plots is more difficult than between optimum and N-excess plots. Therefore, under semiarid sprinkler irrigated conditions, the BMS test is more robust to detect N-excess than N-deficiency.

## Acknowledgements

To the personnel of Soils and Irrigation Department of CITA for their field and laboratory assistance. Special thanks to an anonymous reviewer that significantly improved the quality of our manuscript.

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