

# Site-Specific Covariates Affecting Yield Response to Nitrogen of Late-Sown Maize in Central Argentina

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## ABSTRACT

Optimizing fertilizer rates is a common problem in modern agriculture. Frequently used response models ignore basic statistical assumptions and do not allow quantifying the effects of variables influencing yield response to fertilizer, generating uncertainty in fertilizer rate recommendations. We used linear mixed-effects models to explore maize (*Zea mays* L.) yield response to applied nitrogen (N) in late sowings, and we tested different predictors for explaining yield responses across sites. Data included yield response trials to applied N at 17 different environments (combination site × year) with four to five N rates replicated twice in each trial. The best model (Model A) that included significant effect of N rate applied, sowing date, and soil N-NO<sub>3</sub> at sowing described grain yield variations with high accuracy ( $R^2 = 0.93$ ). Another best model (Model B) showed that soil type as additional variable affected significantly yield response to applied N. The final model indicated that the overall response across sites was characterized by a linear coefficient of 67 kg grain ha<sup>-1</sup> per additional kg N ha<sup>-1</sup> applied and a quadratic coefficient of -0.37 kg grain ha<sup>-1</sup> per additional kg N ha<sup>-1</sup> applied. Across all sites, soil N-NO<sub>3</sub> at sowing (explored range from 34 to 356 kg N ha<sup>-1</sup>) explained 46% of the variability in the linear yield response to applied N. We proposed a method and generated statistical models with site specific covariates that can help optimize farmers' decisions on the use of optimal N fertilizer rates.

## Core Ideas

- We used linear mixed effects models to explore maize yield response to applied N.
- Final models accurately described the observed data ( $R^2 = 0.93$ ).
- Best models indicated that yield response to applied N depended on soil N and soil type.
- Information is useful to optimize management decisions on N fertilizer rates.
- Resulting models are better than traditional ones based on ordinary least squares.

OPTIMIZING FERTILIZER rates is a common agricultural objective, currently aggravated by the negative environmental effects of excess fertilizer. The present challenge to increase yields while reducing the environmental impacts of agriculture (Foley et al., 2011) demands adequate response models for fertilizer applications. This is particularly true for N, a major plant nutrient.

The most widely used method for estimating N fertilizer needs is based on conducting so-called yield response trials. This method consists on applying a wide range of N rates in individual plots, measuring the yield at each applied N rate, and fitting a response model. Commonly used response models include a quadratic response, Mitscherlich curve, linear or quadratic responses followed by a plateau at high fertilizer rates, a logistic response, among others (Cerrato and Blackmer, 1990). All too often, individual plot yield data versus fertilizer rates from different sites and years are pooled together. The simple approach is to fit a response function to all the data using ordinary least squares (Monbiela et al., 1981; Sain and Jauregui, 1993; Pagani et al., 2008; Salvagiotti et al., 2011; Diaz Valdez et al., 2014). This provides a simple model for determining fertilizer rates at regional level.

Mentioned models, although widely used for their simplicity, have several limitations. When databases include large spatial and temporal variability, correlation coefficients are often low (Kim et al., 2008). This can be attributed to large variability in soil N supply, variable N losses related to mechanisms like leaching, volatilization, or denitrification, or yield limitations due to other factors like water availability, among others (Kyvergya et al., 2013). Typically, the easiest solution is to subjectively remove extreme data or extreme response trials, reducing the possibility to find any rational explanation for those particular cases. This increases the uncertainty to N recommendations.

These models also have important statistical limitations, as they ignore the correlations that probably exist between the responses of different plots at the same site or within the same year (Wallach, 1995). Ignoring assumptions might result in inefficient parameter estimators (Zuur et al., 2009). Mixed-effects models (also called hierarchical or multilevel models) are statistical tools to deal with these common limitations. However,

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**Abbreviations:** AIC, Akaike's information criterion; PCV, proportional change in variance; REML, restricted maximum likelihood.

they have been rarely used for optimizing fertilizer rates (Wallach, 1995; Kyveryga et al., 2013). They allow including predictors or covariables to explain the variability at different grouping levels (Gelman and Hill, 2007; Qian et al., 2010). The resulting fertilizer recommendations will then depend on these predictors. Other advantages of mixed-effects modeling include the ability to assume some effects to be random or fixed, based on the particular interest of the analysis (Smith et al., 2005).

Argentina is an important maize (*Zea mays* L.) producer, and is currently facing a relevant change in its production system. The planting date at the central region has extended later in the growing season. While traditional recommended sowing date is during September to early October, at present another recommended sowing date is during December. A longer fallow period allows more water and N accumulation at planting. Late-sown maize (December) locates the critical flowering period for yield definition (Andrade et al., 1999) under conditions of higher probability of rainfall and less evaporative demand compared to earlier traditional sowings. Although yield potential at these late sowing dates is lower than earlier ones (Mercuau and Otegui, 2014), farmers are obtaining acceptable yields with higher yield stability. There are also commercial benefits related to lower fertilizer needs. Late sowing has become a valid alternative for maize producers to reduce risk. At present 45 to 65% of the total maize produced in Argentina is considered late sowing (PAS, 2015).

Recently Gambin et al. (2016) described the relative importance of genotype, management, and environmental variables affecting grain yield of late sown maize using linear mixed-effects models. The study demonstrated that management variables related to genotype selection, N availability, and stand density are relevant when optimizing its crop management. Although the information is valuable to estimate general fertilizer needs, analyzed data involved simultaneous variation with different management variables, and thus have relevant limitations for using as a model to assist N recommendations. Particularly, the experimental design was not focused on fertilizer crop yield response, meaning that differences in yield response to applied N across trials could not be explored. If differences exist, as expected, defining which predictors (mineral soil N, soil type, rainfall, etc.) at the field level can help explain yield response differences is critical. This will allow developing a reasonable model to assist N recommendations rates.

In the present study our objective was to explore yield responses to applied N in late sown maize using linear mixed-effects models. Data included individual response plots to different levels of applied N at 17 different environments (combination of site-year). We started testing models considering applied N as an individual level predictor to explore yield response to applied N across sites. We later tested different predictors at the site level (soil N-NO<sub>3</sub> at sowing, organic matter, soil phosphorus (P), soil type, rainfall during the crop cycle, initial soil water content, presence of an influential water table, and sowing date) to help explain yield variations and response differences to applied N among sites. We hypothesize that yield response to applied N varies with sites, and that some of these predictors will help explain site-to-site differences. As the influence of initial soil N-NO<sub>3</sub> on fertilizer response is widely known in this and others crops (González Montaner

et al., 1997; Makowski et al., 2001; Salvagiotti et al., 2011), we expected the yield response to applied N will be negatively influenced in those sites with higher levels of soil N-NO<sub>3</sub> at sowing. In addition, as the presence of a water table showed negative effects on yield at these environments (Gambin et al., 2016), we hypothesize that the presence of a water table will have a negative influence on the crop yield response to applied N.

## MATERIALS AND METHODS

### Study System

Experiments were located at different sites around the central temperate production area in Argentina during three growing seasons (2012–2013, 2013–2014, and 2014–2015, hereafter called 2013, 2014, and 2015). Sites are described in Table 1, indicated by the closest city name and harvest year with suffixes (\_13, \_14, and \_15, for 2013, 2014, and 2015, respectively). Sites included one location in 2013, nine locations in 2014, and seven locations in 2015. The term “location” is a loose spatial reference as, in different seasons, the location (summarized by the town name) may actually be different paddocks, farms, and/or soil types subject to different management practices. The term “site” will be used herein to define the combination of a particular experiment in a given year (Table 1). All fields were managed under no-tillage for a minimum of 8 yr. All fields belong to farmers grouped within AAPRESID, the Argentinian Association of No-Tillage Farmers ([www.aapresid.org.ar](http://www.aapresid.org.ar); verified 3 Jan. 2017). The predominant landscape is comprised of flat to gently rolling continental dunes (Hall et al., 1992).

Each individual experiment was managed by the farmer in terms of hybrid, stand density, row spacing, and P management, and cropped using available technology (planter, harvesting). Sites are representative of the maize production system in the region of interest. Selected hybrids were commercial hybrids recommended for late sowings in the region of interest by different seeds companies (relative maturity around 120 to 123 d). All experiments were rainfed, and weeds and insects were chemically controlled using standard practices for the region. Soils are predominantly deep sandy loams (typic hapludoll, entic hapludoll, and haplustoll) and shallower clay soils (aquic argiudoll and argialboll) (Soil Survey Staff 2014). Soil types represent the ones commonly used for maize production in the region (types I, II, and III) (Klingebiel and Montgomery, 1961). The predecessor crop was soybean (*Glycine max* L.) in most sites, with the exception of LLA\_15 and LPI\_15, where the previous crops were vicia (*Vicia faba* L.) and maize, respectively. Individual experiments were entirely fitted within a field portion having uniform soil characteristics based on soil taxonomy maps and similar management of previous crops.

Each site had a randomized complete block design with two adjacent replicates, except one with three replicates (ALB\_14). Plot size was around 50 m wide, and always longer than 100 m, but specific size depended on the specific site. Plot size was ultimately determined to be operatively efficient at the farmer scale considering that fertilizer applications were done using available commercial farmer technology.

Inter-row spacing was 0.52 m, except at ALB\_14 and NJU\_14 where inter-row spacing was 0.70 m. Four fertilizer treatments were randomly assigned to individual plots: (i) control, with no

Table 1. List of sites and their environmental and management variables.

| Site ID | Soil N-NO <sub>3</sub><br>kg ha <sup>-1</sup> | Soil OM†<br>% | Soil P<br>mg kg <sup>-1</sup> | Soil classification | Soil type | Soil AWC‡<br>mm | Water table<br>0 or 1§ | Rainfall¶<br>mm | Sowing date | Stand density<br>plant m <sup>-2</sup> | Hybrid    |
|---------|---|---------------|-------------------------------|---------------------|-----------|-----------------|------------------------|-----------------|-------------|--|-----------|
| LAB_13  | 75  | 2.07          | 19                            | Udic Haplustoll     | llc       | 292             | 1                      | 337             | 13 Dec.     | 7.5                                    | DK7210    |
| ALB_14  | 125   | 3.96          | 7                             | Typic Argiudoll     | llw       | 320             | 1                      | 578             | 17 Dec.     | 3.8                                    | DK7210    |
| COL_14  | 112   | 2.70          | 42                            | Aquic Argiudoll     | llep      | 317             | 1                      | 461             | 7 Jan.      | 6.8                                    | DM2771    |
| COL_15  | 64  | 2.80          | 10                            | Aquic Argiudoll     | llep      | 317             | 0                      | 415             | 6 Jan.      | 6.9                                    | DK7210    |
| COR_15  | 176   | 3.32          | 60                            | Typic Hasplustoll   | lllc      | 292             | 0                      | 619             | 3 Jan.      | 6.0                                    | PROAVE467 |
| GAL_15  | 156   | 2.90          | 27                            | Typic Argiudoll     | l         | 375             | 1                      | 476             | 24 Dec.     | 7.0                                    | ACA468    |
| GOD_14  | 150   | 2.41          | 16                            | Vertic Argiudoll    | llws      | 317             | 1                      | 780             | 12 Dec.     | 7.3                                    | AX852     |
| GOD_15  | 117   | 2.41          | 17                            | Vertic Argiudoll    | lls       | 312             | 0                      | 398             | 6 Jan.      | 7.3                                    | AX7822    |
| LLA_15  | 34  | 1.70          | 15                            | Entic Hapludoll     | lllc      | 172             | 0                      | 635             | 12 Dec.     | 4.4                                    | DK7210    |
| LPI_14  | 109   | 1.70          | 31                            | Molic Argiudoll     | llep      | 266             | 1                      | 625             | 19 Dec.     | 6.5                                    | AX878     |
| LPI_15  | 214   | 2.44          | 15                            | Molic Argiudoll     | llep      | 266             | 0                      | 682             | 3 Jan.      | 6.7                                    | DK7310    |
| MJU_14  | 151   | 2.63          | 68                            | Typic Argiudoll     | llc       | 375             | 0                      | 462             | 2 Dec.      | 6.5                                    | DK7010    |
| NJU_14  | 171   | 2.60          | 7                             | Entic Hapludoll     | ll        | 292             | 0                      | 493             | 7 Dec.      | 6.1                                    | DM2738    |
| NOE_14  | 356   | 2.56          | 47                            | Typic Argiudoll     | llc       | 375             | 1                      | 373             | 14 Dec.     | 5.5                                    | P31Y05    |
| RDO_14  | 130   | 1.80          | 47                            | Typic Haplustoll    | lllc      | 108             | 0                      | 481             | 19 Dec.     | 4.5                                    | DK7210    |
| RSE_15  | 38  | 1.86          | 16                            | Typic Haplustoll    | lllc      | 154             | 0                      | 721             | 16 Dec.     | 5.6                                    | DK7210    |
| SUR_14  | 162   | 2.87          | 62                            | Typic Argiudoll     | l         | 375             | 1                      | 527             | 3 Dec.      | 6.5                                    | DOW505    |

† OM, organic matter.

‡ AWC, available water content at sowing (0–2 m depth).

§ Presence (1) or absence (0) of water table at planting (less than 2 m) depth.

¶ Rainfall during the crop cycle (from planting to physiological maturity).

applied N, (ii) dose 50, with an application rate of 50 kg N ha<sup>-1</sup>, (iii) dose 100, with an application rate of 100 kg N ha<sup>-1</sup>, and (iv) dose 130, with an application rate of 130 kg N ha<sup>-1</sup>. Nitrogen fertilizer was always applied as urea (46:0:0 N–P–K), and incorporated between sowing and V6 (Ritchie and Hanway, 1982). In twelve sites a minimum amount of N was applied at planting with P fertilizer (average 11 kg N ha<sup>-1</sup>) as MAP (11:52:0).

At each site, soil samples until 60-cm depth were taken before sowing for determining initial soil properties. Twenty soil samples were collected across the experimental field, from which a compound sample was used for laboratory determinations. Soil test included organic matter (0–20 cm), P amount (mg kg<sup>-1</sup>, 0–20 cm), and N-NO<sub>3</sub> (0–60 cm) determinations. Organic matter was determined by semi-micro Walkley and Black technique (Walkley and Black, 1934), and P and N-NO<sub>3</sub> were determined by spectrophotometry.

Soil available water content was determined at each site to 2-m depth. Soil water content was determined by the gravimetric method (Black, 1965). Depth of water table was indicated when present at sowing, until 2-m depth. Rainfall during the crop cycle was recorded at each site.

Harvest was done with a commercial combine harvester, and the yield of each replicate plot was obtained weighting tractor trailer grain tanks with sensors. Grain yield data is presented with 14% moisture concentration. Sites showed no major incidences regarding weeds, lodging, or diseases. The first winter killing frost was always latter than physiological maturity.

### Statistical Analysis

Data were modeled using linear mixed-effects models (lme4 package, lmer function) (Bates et al., 2015) in R version 3.3.2 (R Core Team 2016). The analysis was done in two steps. First, we fitted random intercept and random intercept and slope models with an individual level predictor (applied N)

to explore site-to-site variations in yield response to applied N. Second, we fitted random intercept and slope models with predictors at a higher grouping level (site) to explore if different predictors helped understand variations in yield response to applied N, and provide a final model to assist N fertilization rates. When fitting these models, we tested both a linear and a curvilinear yield response model to applied N using non-transformed or log-transformed variables. The curvilinear response was explored by fitting a quadratic function. The model improved (had a lower Akaike's information criterion [AIC]) when considering a curvilinear response curve, and therefore we present models with the linear coefficient  $\beta_1$  and the quadratic coefficient  $\beta_2$  from the quadratic function.

### Random Intercept, and Random Intercept and Slope Models

For exploring site-to-site variations in yield response to applied N, two models were fitted. The first and simplest model considered the variation among the intercept for the different sites and blocks nested within site, and a fixed effect that estimated the yield response to applied N for the population of sites (Model 1). Yield at each site and applied N level is modeled as:

$$\gamma_{ij} = \beta_{0j} + \beta_1 N_{ij} + \beta_2 N_{ij}^2 + \sigma_{ij} \quad [1]$$

where  $\gamma_{ij}$  is the yield at  $i$  level of applied N at site  $j$ ;  $\beta_{0j}$  is the intercept variation due to site  $j$ ;  $\beta_1$  is the linear coefficient, and  $\beta_2$  is the quadratic coefficient;  $N_{ij}$  is the  $i$  level of applied N at site  $j$ ; and  $\sigma_{ij}$  is the error term. Random term  $\beta_{0j}$  is assumed normally distributed, with a mean of zero and constant variance [ $\beta_{0j} \sim N(0; \sigma_{\beta_0}^2)$ ]. Fixed effects are represented by the intercept, the linear coefficient, and the quadratic coefficient, indicating the effects across the population of sites.

The second model is similar to the first one, but considered an applied N by site interaction term to explore site-to-site differences in yield response to applied N (Model 2). Yield at each site and applied N level is modeled as:

$$\gamma_{ij} = \beta_{0j} + \beta_{1j}N_{ij} + \beta_{2j}N_{ij}^2 + \sigma_{ij} \quad [2]$$

where all parameters are the same as Eq. [1], but now  $\beta_1$  and  $\beta_2$  are allowed to vary for each site (note the subscript  $j$  on both parameters in Eq. [2]). Random terms  $\beta_{0j}$ ,  $\beta_{1j}$ , and  $\beta_{2j}$  are assumed normally distributed with a mean of zero and constant variance of  $\sigma_{\beta_0}^2$ ,  $\sigma_{\beta_1}^2$ ,  $\sigma_{\beta_2}^2$ , respectively). As in Eq. [1], fixed effects are represented by the intercept, the linear coefficient, and the quadratic coefficient, indicating the effects across the population of sites. Models were fitted using estimates of restricted maximum likelihood (REML) and compared by the log-likelihood ratio test.

### Random Intercept and Slope Models with Predictors at the Site Level

Different predictors at the site level were explored to help explain site-to-site differences in yield and yield response to applied N. The inclusion of predictors was based on several aspects, including data availability and enough variation across sites. The following predictors were explored:

- Soil N-NO<sub>3</sub> at sowing (kg ha<sup>-1</sup>, 0–60 cm depth) as a quantitative variable.
- Soil organic matter (% , 0–20 cm depth) as a quantitative variable.
- Soil P (mg kg<sup>-1</sup>, 0–20 cm depth) as a quantitative variable.
- Soil type as a categorical variable with three levels (Type I, II, and III). Soil classification was also explored as a categorical variable based on great group (Argiudoll, Hapludoll, and Haplustoll).
- Soil available water content at sowing (mm, 0–2 m depth) as a quantitative variable.
- Rainfall during the crop cycle (mm) as a quantitative variable, from planting to physiological maturity.
- Water table at planting as a nominal variable (two levels: 0, absence; 1, presence at less than 2 m depth).
- Planting date as a quantitative variable (d, after 1 November).

To determine the inclusion of a predictor into the model, we explored the correlation between each predictor and parameters describing yield variation across sites ( $\beta_{0j}$ ) and the linear coefficient of the yield response to applied N obtained in Model 2 ( $\beta_{1j}$ ). Data exploration suggested the explanatory variables in the fixed component that were most likely to contribute to the optimal model were sowing date, soil N-NO<sub>3</sub>, and soil type (Supplemental Fig. S1). Sowing date was negatively correlated ( $p < 0.05$ ) to yield variations across sites ( $\beta_{0j}$ ), while soil N-NO<sub>3</sub> and soil type were both negatively correlated ( $p < 0.05$ ) to yield response to applied N ( $\beta_{1j}$ ) (Supplemental Fig. S1). No apparent correlation was found between  $\beta_{0j}$  and  $\beta_{1j}$  with other predictors, nor  $\beta_{2j}$  and predictors (not shown). The final model was obtained following the top-down strategy of model selection process (Zuur et al., 2009), which involves starting with the model including all potential variables influencing

variations among sites and variations in the yield response to applied N (usually called “the beyond optimal model”), finding the optimal random structure based on REML estimations and finally finding the optimal fixed structure based on ML estimations. Last, the final model is presented using REML estimations. The “beyond optimal model” was:

$$\gamma_{ij} = \beta_{0j} + \beta_{1j}N_{ij} + \beta_{2j}N_{ij}^2 + \sigma_{ij} \quad [3]$$

where

$$\beta_{0j} \sim N(\mu_{\beta_0j}; \sigma_{\beta_0}^2) \text{ and } \mu_{\beta_0j} = \alpha_0 + \alpha_1 \text{Sowing date}_j \quad [4]$$

$$\beta_{1j} \sim N(\mu_{\beta_1j}; \sigma_{\beta_1}^2) \text{ and } \mu_{\beta_1j} = \gamma_0 + \gamma_1 \text{Soil N}_j + \gamma_2 \text{Soil type}_j \quad [5]$$

$$\beta_{2j} \sim N(\mu_{\beta_2j}; \sigma_{\beta_2}^2) \text{ and } \mu_{\beta_2j} = \delta_0 + \delta_1 \text{Soil N}_j + \delta_2 \text{Soil type}_j \quad [6]$$

Note that Eq. [3] is the same as Eq. [2], but now  $\beta_{0j}$ ,  $\beta_{1j}$ , and  $\beta_{2j}$  are dependent on predictors at the grouping level (site) with consequences to the coefficient estimates.  $\beta_{0j}$  depends on a constant fixed term ( $\alpha_0$ ) and the fixed effect of sowing date ( $\alpha_1$ ) (Eq. [4]).  $\beta_{1j}$  depends on a constant fixed term ( $\gamma_0$ ), the fixed effect of soil N-NO<sub>3</sub> at sowing ( $\gamma_1$ ), and the fixed effect of soil type ( $\gamma_2$ ) (Eq. [5]). Similarly,  $\beta_{2j}$  depends on a constant fixed term  $\delta_0$ , the fixed effect of soil N-NO<sub>3</sub> at sowing ( $\delta_1$ ), and the fixed effect of soil type ( $\delta_2$ ) (Eq. [6]).

The final model was obtained following the multi-model inference based on the information-theoretic approach (Burnham and Anderson, 2002, 2004). This approach does not accept the notion that there is a simple “true model” in biological sciences. Selection of a best approximating model represents the inference from the data and tells us what “effects” (represented by parameters) can be supported by data (Burnham and Anderson, 2002). We used AIC to select the best fitting models for combinations of the four fixed-effect predictor variables (Aho et al., 2014). Based on the context and our objectives, AIC is the appropriate tool for model selection when compared to others like BIC or hypothesis testing (Aho et al., 2014; Burnham and Anderson, 2002, 2014; Burnham et al., 2011). Because models have different fixed effects (but with the same random structure), ML estimation was used, and not REML. We checked the Gaussian and homoscedasticity assumptions (Zuur et al., 2009) for the standardized model residuals with graphical analysis. There was no covariance among random effects.

The proportional change in variance (PCV) was calculated following Merlo et al. (2005). The PCV monitors changes specific to a variance component, that is, how the inclusion of additional predictor(s) reduce or increase the variance component at particular levels. Proportional change in variance is calculated as follows:

$$\text{PCV} = (V_{N-1} - V_{N-2}) / V_{N-1} \quad [7]$$

where  $V_{N-1}$  is the variance in the model without predictors and  $V_{N-2}$  is the variance in the final model with predictors. A positive value indicates a reduction in the variation among

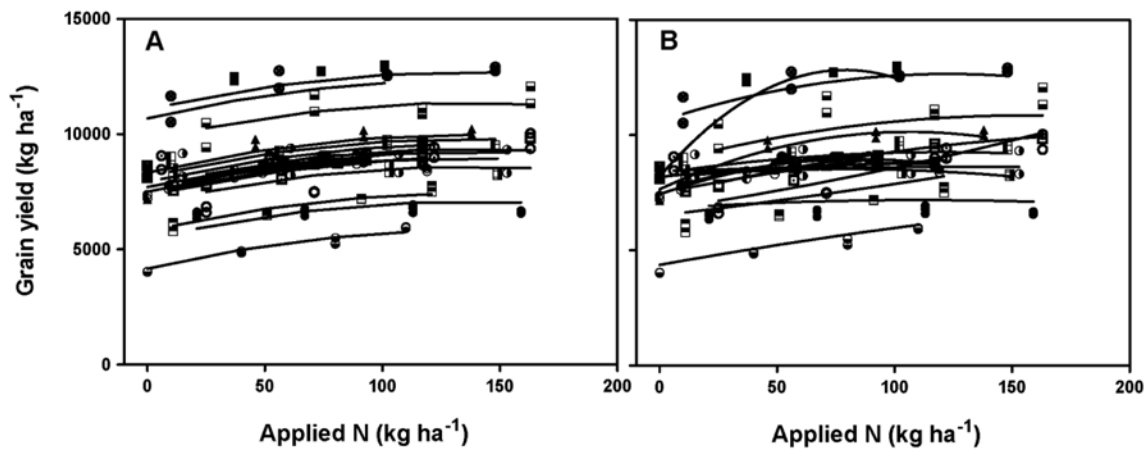


Fig. 1. Observed (symbols) and fitted values obtained by mixed-effects modeling (lines). Different symbols indicate different sites. (A) Random intercept model in which a site effect is included in the model as a variation around the intercept. (B) Random intercept and slope model in which a site effect is included as a variation around the intercept and an applied N by site interaction effect is included as a variation around the slope. For clarification, fitted values for the population (fixed components) are not shown.

groups (e.g., sites) given by the incorporation of predictors. The model without predictors is similar to Eq. [2], but with a single fixed effect represented by the model intercept.

The  $R^2$  of adjusted models were obtained following the methodology described in Nakagawa and Schielzeth (2013) for generalized linear mixed models. Both marginal and conditional  $R^2$  were calculated. Marginal  $R^2$  ( $R^2_m$ ) represents the variance explained by fixed factors and is given by:

$$R^2_m = \frac{\sigma_f^2}{\sigma_f^2 + \sum_{l=1}^u \sigma_l^2 + \sigma_\epsilon^2} \quad [8]$$

where  $\sigma_f^2$  is the variance calculated from the fixed effect components of the linear mixed model,  $\sigma_l^2$  is the variance component of the  $l$ th random factor, and  $\sigma_\epsilon^2$  is the residual variance. Equation [8] can be modified to express conditional  $R^2$  ( $R^2_c$ ) as:

$$R^2_c = \frac{\sigma_f^2 + \sum_{l=1}^u \sigma_l^2}{\sigma_f^2 + \sum_{l=1}^u \sigma_l^2 + \sigma_\epsilon^2} \quad [9]$$

which represents the variance explained by the entire model (fixed and random factors) (Nakagawa and Schielzeth, 2013).

## RESULTS

### Management and Environmental Variations across Sites

Management and environmental variables showed ample variation across sites (Table 1). Soil N-NO<sub>3</sub> at sowing varied importantly, from 34 to 356 kg ha<sup>-1</sup>, in agreement to previously reported values for late sowings in our region (Gambin et al., 2016). Soil organic matter ranged from 1.7 to 3.9%. Soil P ranged from 7 to 68 mg kg<sup>-1</sup>. Soil type ranged from Type I to III, indicating productive soils. The same was evident based on soil classification. Soil available water content at sowing showed a smaller variation across sites, mainly because water content was near field capacity in most cases. In only three sites (LLA\_15, RDO\_14 and RSE\_15), soil available water content was below

field capacity (74, 35, and 70% of field capacity, respectively). A water table was detected in most sites at less than 2-m depth. Rainfall during the crop cycle varied across sites, from 337 to 780 mm (Table 1). Sowing dates ranged from December 2 to January 7, and stand density ranged from 4.4 to 7.5 plants m<sup>-2</sup>.

### Random Intercept, and Random Intercept and Slope Models

Our first goal was to determine if yield response to applied N varied across sites, as a previous step before exploring management or environmental variables affecting any differential response. This simplest model (usually called “random intercept model”, Model 1) is depicted in Fig. 1A. Note that the model considers an intercept variation due to each site, but the slope of the yield response to applied N is the same for all sites (no applied N by site interaction). The second model we tested is the usually called “random intercept and slope model”, which incorporates an applied N by site interaction term to model site-to-site variations in yield response to N applied (Model 2). This model is depicted in Fig. 1B, showing variations in both intercepts and slopes.

The likelihood ratio test indicated that the model with an applied N by site interaction term is better ( $p < 0.001$ ). The AIC of the model having the interaction was lower than the model without interaction (2223 vs. 2251, respectively). The same was evident when considering the residuals, they were lower in the model having an applied N by site interaction term (264 kg ha<sup>-1</sup>) than the model without this interaction (580 kg ha<sup>-1</sup>).

### Random Intercept and Slope Models with Predictors at the Site Level

In the previous section, we confirmed site-to-site variations in yield and in yield response to applied N. We were now interested in determining explanatory variables explaining these variations. Data exploration suggested that explanatory variables in the fixed component that were most likely to contribute to the optimal model were sowing date, soil N-NO<sub>3</sub>, and soil type (Supplemental Fig. S1). These three variables were considered further.

There were two best fitting models based on AIC (Table 2). The best fitting one (Model A) indicated that the yield depended

Table 2. Akaike's information criterion (AIC) for mixed effects models of the potential effect of soil N-NO<sub>3</sub> and soil type on grain yield response to applied N in late-sown maize. The best five models are shown (from a total of 10 models). Columns are the different predictor variables. The inclusion of variables in a particular model is indicated (+).  $R^2_m$  is the variance explained by fixed factors, and  $R^2_c$  is the variance explained by the entire model.

| Model | Predictors  |           |                                    |                       | $R^2_m$ | $R^2_c$ | AIC  | $\Delta AIC$ † |
|-------|-------------|-----------|------------------------------------|-----------------------|---------|---------|------|----------------|
|       | Sowing date | Applied N | Applied N × soil N-NO <sub>3</sub> | Applied N × soil type |         |         |      |                |
| A     | +           | +         | +                                  |                       | 0.35    | 0.93    | 2198 | 0.0            |
| B     | +           | +         | +                                  | +                     | 0.33    | 0.94    | 2198 | 0.2            |
| C     | +           | +         |                                    | +                     | 0.28    | 0.94    | 2207 | 9              |
| D     |             | +         | +                                  |                       | 0.12    | 0.91    | 2211 | 13             |
| E     |             |           |                                    |                       | –       | –       | 2216 | 17             |

† The  $\Delta$  column indicates the difference between a model's AIC and that of the best-fitting model.

Table 3. Variance components (VC) of random effects, and estimates ( $\pm$  standard error [SE]) of fixed effects for different models applied to data. The model without fixed effects is only represented by a fixed intercept. Models A and B are the best fitting models based on the Akaike's information criterion (see Table 2). All models have the same random structure.

| Effect                             | Parameter†   | Model without fixed effects | Model A            | Model B            |
|------------------------------------|--------------|-----------------------------|--------------------|--------------------|
| Random effects                     |              |                             |                    |                    |
| VC                                 |              |                             |                    |                    |
| Site                               | $\beta_{0j}$ | 1,131,162                   | 999,000            | 1,020,350          |
| Applied N × site                   | $\beta_{1j}$ | 17,024                      | 9,214              | 2,847              |
|                                    | $\beta_{2j}$ | 0.5                         | 0.4                | 0.1                |
| Residual                           |              | 85,758                      | 91,090             | 69,510             |
| PCV <sub>site</sub> ‡              |              |                             | 12                 | 10                 |
| PCV <sub>applied N × site</sub>    | $\beta_{1j}$ |                             | 46                 | 83                 |
|                                    | $\beta_{2j}$ |                             | 11                 | 73                 |
| PCV <sub>residual</sub>            |              |                             | –6                 | 19                 |
| Fixed effects                      |              |                             |                    |                    |
| Estimate and SE                    |              |                             |                    |                    |
| Intercept                          | $\alpha_0$   | 8,073 $\pm$ 383             | 11,680 $\pm$ 1,012 | 11,390 $\pm$ 1,059 |
| Sowing date                        | $\alpha_1$   | –                           | –76 $\pm$ 19       | –74 $\pm$ 20       |
| Applied N                          | $\gamma_0$   | –                           | 67 $\pm$ 47        | 66 $\pm$ 47        |
|                                    | $\delta_0$   | –                           | –0.37 $\pm$ 0.32   | –0.35 $\pm$ 0.32   |
| Applied N × soil N-NO <sub>3</sub> | $\gamma_1$   | –                           | –0.30 $\pm$ 0.30   | –0.21 $\pm$ 0.18   |
|                                    | $\delta_1$   | –                           | 0.002 $\pm$ 0.002  | 0.001 $\pm$ 0.001  |
| Applied N × soil type§             | $\gamma_2$   | II                          | –                  | –13 $\pm$ 40       |
|                                    | $\delta_2$   | II                          | –                  | 0.09 $\pm$ 0.28    |
|                                    | $\gamma_2$   | III                         | –                  | –14 $\pm$ 44       |
|                                    | $\delta_2$   | III                         | –                  | 0.06 $\pm$ 0.31    |

† Parameters of the quadratic function describing the yield response to applied N (see Eq. [3] to [6]).

‡ PCV, proportional change in variance.

§ Soil type is a categorical variables with three levels (Type I, II, and III); the baseline soil is Type I.

on sowing date, applied N, and on the applied N by soil N-NO<sub>3</sub> interaction term that explained the differential yield response across sites (Table 2). The second best model (Model B) was similar to Model A, but included another interaction term (applied N by soil type). Interaction terms indicated that the differential response to applied N across sites depended on soil N-NO<sub>3</sub> at sowing and soil type. These models were better than a model without predictors (Model E; Table 2). This is evidenced when comparing the AIC value of the model with no predictors with the best models (Table 2). Variance explained by the fixed factors ( $R^2_m$ ) was 0.35 and 0.33 for Model A and B, respectively. Variance explained by the entire model ( $R^2_c$ ) was 0.93 and 0.94 for Model A and B, respectively, indicating that they appropriately and accurately described observed yield data.

In the model without fixed effects, site-to-site variation in yield had the greatest contribution to the total variance (90%). This is an expected result given the important variation in environment and management variables across sites (Table 1). This is also evident in the intercept variation of both models in Fig. 1. Final models indicated that part of yield variations among sites were due to

sowing date. Sowing date explained 10 to 12% of the site-to-site yield variation, as evidenced by the PCV for site (Table 3).

Variation in yield response to applied N across sites (i.e., applied N by site interaction) represented 1.3% of the total variance in the model without fixed effects (Table 3). After considering fixed predictors, Model A indicated that part of the variation in yield response to applied N among sites was due to site differences in soil N-NO<sub>3</sub> at sowing (PCV was 46 and 11% for  $\beta_{1j}$  and  $\beta_{2j}$ , respectively; Table 3). For Model B, which incorporates more predictors (soil N-NO<sub>3</sub> and soil type), the PCV was higher (83 and 73% for  $\beta_{1j}$  and  $\beta_{2j}$ , respectively; Table 3). Distribution of random effects of the final Models A and B are depicted in Supplemental Fig. S2.

We further examined the regression coefficient estimates for the final models. This allows quantifying the specific influence of each predictor variable on grain yield response to N fertilizer rate. Sowing date had a negative effect over grain yield, being quite similar for both models (–76 and –74 kg ha<sup>–1</sup> d<sup>–1</sup> of delay in sowing date after November 1). Applied N showed a positive decelerating effect on grain yield (Table 3; Fig. 2).

The linear coefficient  $\gamma_0$  was 67 and 66 kg grain ha<sup>-1</sup> per additional kg N ha<sup>-1</sup> applied for Models A and B, respectively. The quadratic coefficient  $\delta_0$  was -0.37 and -0.35 kg grain ha<sup>-1</sup> per additional kg N ha<sup>-1</sup> applied for Models A and B, respectively. The reduction in yield response to applied N at increasing levels of soil N-NO<sub>3</sub> at sowing was higher in Model A than Model B (approximately 3 or 2 kg grain ha<sup>-1</sup> per applied kg N ha<sup>-1</sup> for every 10 kg ha<sup>-1</sup> of soil N-NO<sub>3</sub> at sowing for Models A and B, respectively; Table 3). Model B included an additional reduction in response to applied N given by soil type. Relative to soil of Type I, this reduction was 13 and 14 kg grain ha<sup>-1</sup> per applied kg N ha<sup>-1</sup> for Types II and III, respectively. Both final models correctly described yield responses to applied N across sites (Fig. 3).

Figure 4 provides a graphical representation of the final models. Lines represent the expected yield response for different rates of applied N after considering different levels of the soil N-NO<sub>3</sub> at sowing. Response to applied N (Fig. 4, y-axis) was calculated as the first derivative of the quadratic equation for yield versus applied N (Eq. [5] and [6]). Based on Model A, the impact of soil N-NO<sub>3</sub> at sowing was important in reducing the yield response to applied N, independently of soil type (Fig. 4A). Based on Model B, the impact of soil N-NO<sub>3</sub> at sowing was less important, and there was an additional reduction in the yield response associated with soil type (Fig. 4B–D). The yield response to applied N was higher in soil of Type I and lower in soil of Types II and III (Fig. 4B–D).

Information provided in Fig. 4 can be used for N fertilizer recommendations by equalizing the yield response to applied N rates (Fig. 4, y-axis) with relative prices of fertilizer and grain. For example, considering an expected relative price of 15, Model A indicates that yield response to applied N is always lower than this value if soil N-NO<sub>3</sub> at sowing is 190 kg ha<sup>-1</sup> (Fig. 4A). This indicates there is no economic benefit for N fertilizer applications above this value. However, if soil N-NO<sub>3</sub> at sowing is 130 or 50 kg ha<sup>-1</sup>, the yield response to applied N is higher than 15 for N rates approximately  $\leq 70$  kg ha<sup>-1</sup>, being the yield response higher at reducing levels of soil N-NO<sub>3</sub> at sowing. Yield response for applied N levels higher than 80 kg ha<sup>-1</sup> becomes negligible at any level of soil N-NO<sub>3</sub> at sowing (Fig. 4A). Model B indicated that, for soils of Type I, economic benefit of N application was approximately 70 kg ha<sup>-1</sup> at 50 kg ha<sup>-1</sup> of soil N-NO<sub>3</sub> at sowing or 40 kg ha<sup>-1</sup> when soil N-NO<sub>3</sub> at sowing was 190 kg ha<sup>-1</sup> (Fig. 4B). For relative less productive soils (soil of Type III), economic benefit of N application is approximately 50 kg ha<sup>-1</sup> for 50 kg ha<sup>-1</sup> of N-NO<sub>3</sub> at sowing, and there is no economic benefit of applying N at high levels (190 kg ha<sup>-1</sup>) of soil N at sowing (Fig. 4D).

## DISCUSSION

We provided reasonable and parsimonious models that reduce uncertainty to farmers and advisers when deciding N fertilizer rates in late planted maize at our region. The models were satisfactory in describing the spatial and temporal variation in maize grain yield to applied N ( $R^2 = 0.93$ ) and have several important attributes. The models: (i) consider the hierarchical data structure, a common characteristic of data used for developing fertilizer recommendation models, (ii) consider that the yield response to applied N varies across sites, and (iii) predict that the yield response to applied N depends on some environmental variables at the field level, in our case soil N-NO<sub>3</sub> at sowing and soil type.

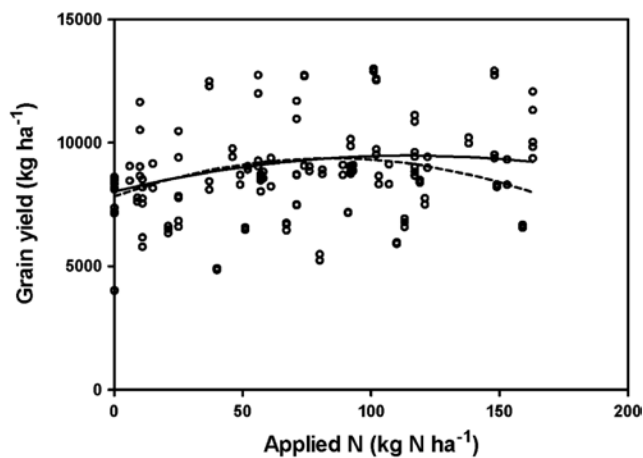


Fig. 2. Grain yield versus applied N for the entire data set. Black lines reflect the final models for the population of sites (full line, Model A; dashed line, Model B). Parameters of the final models are represented by the fixed effects shown in Table 3.

Evidence of using these types of models for fertilizer management recommendations are rare, whereas the more frequent approach is to fit a response function to all data using ordinary least squares (Monbiela et al., 1981; Sain and Jauregui, 1993; Pagani et al., 2008; Salvagiotti et al., 2011; Diaz Valdez et al., 2014). Linear mixed-effects models (also called hierarchical or multilevel models) are a powerful tool (Wallach, 1995; Zuur et al., 2009; Qian et al., 2010), and their use to explore the influence of management and environmental variables on crop yield at a regional level is increasing. We also recently used these models to assist in crop management options for late sown maize (Gambin et al., 2016).

As noted by Gelman and Hill (2007), a multilevel model can be considered a method for compromising two extremes models: ignoring the variation between sites-years (complete pooling) or estimating separate models within each site-year (no pooling). Generating separate models for each combination of site and year can provide models with high accuracy, but are not practical when the objective is to develop a model for N fertilizer management recommendations with regional application. On the other hand, applying a complete pooling model to data, which is the typical situation, gave us a model with  $R^2 = 0.08$ , which is considerably lower than the mixed-effect model proposed here. This complete pooling model also indicates that yield is maximized at a higher level of applied N compared to the proposed models. Model parameters indicated that yield is maximized at 150 kg of applied N ha<sup>-1</sup> based on a quadratic model fit to all data using ordinary least squares, while final models proposed here indicated that yield is maximized at approximately 80 to 120 kg of applied N ha<sup>-1</sup> (response to applied N equal to zero in Fig. 4) depending on the model and based on intermediate expected levels of soil N-NO<sub>3</sub> at sowing. This suggests that farmers will be applying N in excess if they use extremely simplistic approaches.

Although the benefits of linear mixed-effect models for fertilizer recommendations have been recognized more than twenty years ago (Wallach, 1995), their use has been limited. Examples using these models for N recommendation rates were explored for winter wheat in France (Makowski and Wallach, 2001; Makowski et al., 2001). Authors demonstrated the importance of random parameter models to be consistent with the type of data, and highlighted the possibility of extending the model by

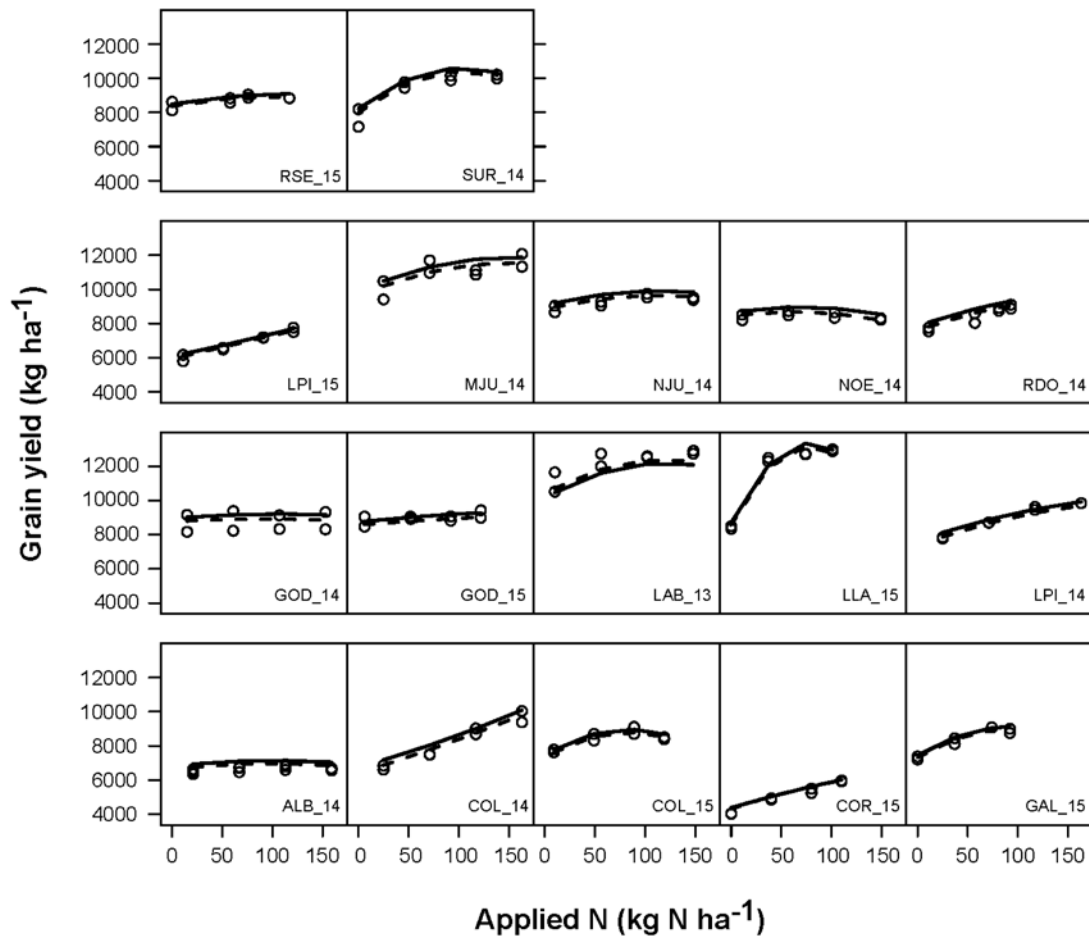


Fig. 3. Grain yield response to applied N for each site. Black lines reflect the final models (full line, Model A; dashed line, Model B). Fixed components of the models are shown in Fig. 2, while Fig. 3 shows the site effect as a variation around the intercept ( $\beta_{0j}$ ) and the N applied by site interaction effect as a variation around the slope ( $\beta_{1j}$  and  $\beta_{2j}$ ).

including site-year characteristics. In their case, they showed how the inclusion of one particular predictor (end of winter mineral soil N) improved their prediction accuracy (Makowski and Wallach, 2001). Although in the present study we explored several others predictors, results are consistent with those found in these previous studies, showing the importance of initial soil N-NO<sub>3</sub> to assist fertilizer recommendations. This is also consistent with models based on total nutrient approach which combine soil N and fertilizer N as a single measure of total nutrient (González Montaner et al., 1997; Salvagiotti et al., 2011). The alternative approach used here (the covariance approach; Sain and Jauregui, 1993) has practical advantages, including a variable yield response to applied N based on soil N-NO<sub>3</sub> (not possible when considering the total nutrient approach) or less dependence on soil tests by assuming some expected soil level.

An alternative model indicated that more integrative variables like soil type influence yield response to applied N. Isolated soil characteristics like organic matter or soil P showed no apparent relationship with yield response to applied N. This is clear evidence of the complex reality between the addition of fertilizer to the soil and yield as a black box in which some known and many unknown processes are integrated (Sain and Jauregui, 1993). In this sense, Nelson et al. (1985) concluded that no single model can be recommended for all situations, and that the researcher can only hope that the best model has

some agronomic rationale. This is in accordance with our model selection strategy. This approach does not accept the notion that there is a simple “true model” in the biological sciences. Selection of a best approximating model represents the inference from available data and tells us what effects can be supported by data (Burnham and Anderson, 2002, 2004). The world view perspective of model selection based on AIC considered that “all models are wrong, but some are useful” (Aho et al., 2014).

Yield response to applied N was not influenced by other environmental predictors like rainfall during the crop cycle, initial soil water content, and presence of an influencing water table, or by a management predictor like sowing date. The lack of interaction with soil P could be expected, given that most sites evidenced soil P values above the threshold below which soil P is considered limiting in our region (12 to 14 mg kg<sup>-1</sup>; Rubio et al., 2016). Under conditions with reduced soil P, the yield response to applied N is expected to be lower (Rubio et al., 2016). However, we were not able to explore this hypothesis. Because late sown maize is particularly sown in medium to poor environments, the interaction with other nutrients is an important aspect that deserves further attention.

The lack of interaction with other explored variables might be related to the limited number of sites and/or predictor variation. In turn, the fact that several sites showed little or no yield response to applied N (Fig. 3) reduces the ability to find



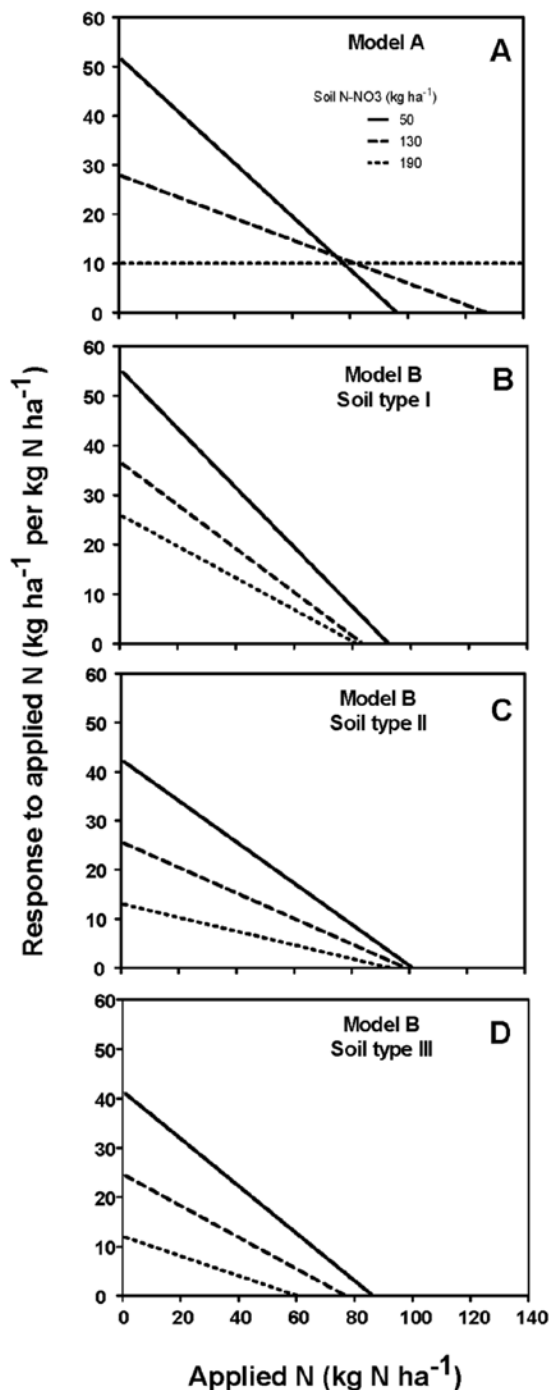


Fig. 4. Expected yield response to applied N for different rates of applied N. Response to applied N (y-axis) was calculated as the first derivative of the quadratic equation for yield versus applied N (Eq. [5] and [6]) for Model A (Panel A) and Model B (Panels B–D). In Model B, the yield response to applied N varied with soil type (Panel B, Type I; Panel C, Type II; and Panel D, Type III). Each line indicates the predicted yield response to applied N for different levels of soil N-NO<sub>3</sub> at sowing. Soil N-NO<sub>3</sub> levels are quantile 10 (50 kg ha<sup>-1</sup>), 50 (130 kg ha<sup>-1</sup>), and 90 (190 kg ha<sup>-1</sup>) based on observed data.

predictors explaining differential responses. Other variables that could not be explored here but are known to influence grain yield response to N (such as stand density, Duncan, 1954; Carlone and Russell, 1987; or genotype, Gambin et al., 2016) are of special interest for future analysis. The importance of the size of the data set on the value of including an explanatory variable has been

demonstrated (Makowski et al., 2001). Although our study is limited in the data set size (number of site-year combinations), it is a valid exercise showing a statistical approach that leads to better models than the ones currently used.

Finally, yield variations among sites were highly important independently of the levels of applied N or soil N-NO<sub>3</sub> at sowing. Final models indicated that sowing date explained part of this variability, being lower at delayed sowing dates from early December to early January. Similar results were found by Mercuro and Otegui (2014) using a simulation model, highlighting the importance of this management variable. None of the other explored variables showed apparent association with yield. Other management variables not explored (genotype and stand density) are known to have a relevant influence on yield in late maize (Gambin et al., 2016), and could be behind yield variations not captured by present models.

## CONCLUSIONS

We described reasonable and parsimonious models that reduce the uncertainty to farmers and advisors when deciding N fertilizer rates for late sown maize in central Argentina. Models satisfactorily described the spatial and temporal variation in maize grain yield to applied N ( $R^2 = 0.93$ ). Yield response to applied N depended on soil N-NO<sub>3</sub> at sowing and soil type.

The type of analysis we conducted shows several important attributes in that it (i) considers the dependence or hierarchical data structure, (ii) considers that the yield response to applied N varies with environments, and (iii) uses predictors or explanatory variables at the environment level to estimate the magnitude of the yield response to applied N. As a consequence, the resulting models are better suited for predictions than traditional ones based on ordinary least squares estimates.

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## SUPPLEMENTAL MATERIAL

Supplemental material is available with the online version of this article.

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