



# Does the critical N dilution curve for maize crop vary across genotype x environment x management scenarios? - a Bayesian analysis

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## ARTICLE INFO

### Keywords:

Critical N dilution curve

Maize

Genotype-by-environment interactions

Uncertainty

## ABSTRACT

Improving the prediction of plant N status for field crops require robust estimation of their critical N dilution curves and their parameters. For maize (*Zea mays* L.), several studies have contributed to develop separate critical N curves for different genotype, environment, and management (GxExM) combinations, but so far no rigorous analysis has been formally conducted to assess if the use of different curves is fully supported by experimental data and to understand if a more universal curve could be used for defining the plant N status of this crop. This study presents a global synthesis of maize data related to plant biomass and plant N concentration (%N) with the main objective of implementing a Bayesian framework for fitting critical N curves ( $N_c = A_1 W^{-A_2}$ ) and testing the existence of differences in the main parameters of the curve ( $A_1$  and  $A_2$ ) across varying GxExM combinations. Five publications including trials with at least four sampling times for plant biomass were selected based on a systematic review of the literature. Biomass and %N pairs ( $n = 369$ ) measured during vegetative growth were extracted from the selected papers and included in a dataset covering four countries (China, US, Canada, and France) and twenty different GxExM combinations. Results of the Bayesian analysis revealed that the critical plant %N and biomass fitted for each GxExM combination presented negligible statistical differences for  $A_1$  but minor variation for  $A_2$  reflecting changes in N dilution pattern with biomass mostly when W was above  $5 \text{ Mg ha}^{-1}$  ( $\%N_c < 2\%$ ). A hypothesis of changes in plant canopy architecture, related to variations in GxM (e.g., plant density, row spacing, hybrids) related to variations in the N dilution curve was postulated and needs further evaluation. Refining the estimation of %N<sub>c</sub> will improve the assessment of plant N status.

## 1. Introduction

Nitrogen (N) is a key element for maize (*Zea mays* L.), with crop yield response depending on the total N supply, soil N plus external mineral N fertilizer rate. Past studies have focused on investigating the changes in plant N concentration (%N) decreasing as plant biomass (W) increases, with plant N content and crop mass following an allometric approach (Lemaire and Salette, 1984; Greenwood et al., 1990). The N dilution is mainly the result of two mechanisms: (i) non-uniform N content in leaf canopy linked to the light attenuation process (Sinclair and Horie, 1989; Field, 1983; Hirose and Werger, 1987; Drouet et al., 1999; 2004), and

(ii) a larger proportion of structural (rather than metabolic) and storage tissues as the plant aged (originally proposed by Caloin and Yu, 1984; and later by Charles-Edwards et al., 1987) characterized with lower target of plant %N.

Improving our understanding of the N dilution process can assist on the study of the temporal dynamic of N uptake in relation to plant growth, as already demonstrated by Lemaire and Salette (1984); Gastal and Lemaire (2002), and more recently reviewed by Lemaire et al. (2019). The minimum plant %N required for maximum growth has been coined by Lemaire et al. (2008) as critical %N ( $N_c$ ), establishing a plant N status of nutrient sufficiency or deficiency/luxury if the nutrient status

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departs from this %N at a comparable biomass level. The  $N_c$  concept has been utilized for plant N diagnosing purposes to calculate the critical N curve by relating in a mathematical expression the level of plant biomass and two parameters (herein termed as  $A_1$  and  $A_2$ ) predicted from the dilution model ( $N_c = A_1 W^{-A_2}$ ). The critical N curve has been evaluated in several crop species such as wheat (*Triticum aestivum* L.) (Justes et al., 1994), winter oilseed rape (*Brassica napus* L.) (Colnenne et al., 1998), maize (*Zea mays* L.) (Plénet and Lemaire, 2000), potato (*Solanum tuberosum* L.) (Bélanger et al., 2001), rice (*Oryza sativa* L.) (Ata-Ul-Karim et al., 2013), barley (*Hordeum vulgare* L.) (Zhao, 2014), tomato (*Solanum lycopersicum* L.) (Yang et al., 2015), and cotton (*Gossypium hirsutum*) (Ma et al., 2018) defining scenarios of luxury (N excess), sufficiency, and deficiency for plant N status.

For maize, several investigations have re-adjusted (calibrated) the critical N curve and implemented the N nutrition index (NNI) concept, expressed as the ratio of the actual plant %N and the  $N_c$  at a similar biomass level, for diagnosing N status on this crop (e.g., Plénet and Lemaire, 2000; Ziadi et al., 2008; Chen et al., 2010; Hermann and Taube, 2014; Li et al., 2012; Zhao et al., 2018; Du et al., 2020). However, at the present time a synthesis review testing if the parameters of the N dilution equation change with variations in genotype by environment by management (G x E x M) combinations is not yet available. Thus, the ability to test the universality of the critical N curve ( $A_1$  and  $A_2$ ) is critical to comprehend if a more generic crop N diagnostic tool can be used for maize crop under varying GxExM scenarios. Many studies have established site-specific dilution curves, but it is difficult to know whether these differences reflect real effects of G, E or M, or rather correspond to estimation errors resulting from the variability of the observations and designs of experiments used. To know this, it is necessary to conduct a rigorous uncertainty analysis of the dilution curves estimated from the available data.

In order to formally analyze the uncertainty in the plant %N-biomass curves, a new Bayesian statistical framework has been tested for several field crops including maize (Makowski et al., 2020). In addition, this new approach permits to estimate all the parameters of the critical N curve without requiring the classification of the data for non-N- versus N-limited, also to fit a dilution curve in one step with plant %N and biomass as main inputs. The results of this fitting procedure are expressed through probability distribution that can be conveniently used to compare different curves for varying GxExM scenarios.

Here, we analyze a large maize dataset including % N-biomass observations collected during the vegetative growth of the crop (roughly until silking time). More specifically, the aim of this study was to: (i) build a dataset for maize plant %N-biomass dilution process during vegetative growth, and (ii) evaluate the uncertainty of the critical N dilution curves estimated for a large range of genotype x environment x management scenarios (e.g., hybrids, management, and environments), and assess the statistical significance of their differences.

## 2. Materials and method

### 2.1. Data acquisition

We conducted a literature search using the Web of Science database for articles reporting critical N dilution curves for maize without restriction in the year of publication or country. The structure of the search included the terms “N dilution curve”, “nitrogen dilution curve”, “maize”, “corn” to retrieve articles published before February 3, 2020. When screening publications, the selection criteria (similar as previously established in past review papers, e.g., Ciampitti and Vyn, 2012) included (i) experiments carried under field conditions, (ii) number of times during the season with data on both plant %N-biomass (at least with four sampling times during vegetative growth), (iii) dates of measure when plant biomass was 1 Mg ha<sup>-1</sup> until silking stage, (iv) different N rate levels including an unfertilized or low-N fertilized (e.g., <30 kg N ha<sup>-1</sup>) treatment, (v) hybrid name and management information was

provided, for example, studies presenting several hybrids as average were not considered, and (vi) was written in English language. The screening process yielded five studies, namely Plénet and Lemaire (2000); Chen et al. (2013); Ziadi et al. (2008); Ciampitti et al. (2013), and Zhao et al. (2018). Specific data collected from each study were year of experiment, plant biomass, plant %N, fertilizer N rate level, genotype name (individual distinction between nine hybrids), sampling time (or phenology), sowing date, plant density, harvest time, and other management information (further details can be revised in Table 1). The total number of unique GxExM combinations is equal to twenty sites. The plant biomass data ranged from roughly 1 (early growth stages) to about 12.9 Mg ha<sup>-1</sup> (until silking time). Similarly, plant %N ranged from 3.44 % (early growth stages) to 0.61 % (at silking time). The total number of pairs for plant %N-biomass for maize was 369 from all five studies (combination of number of treatments x sites x sampling dates x hybrid x management factors). Geographically, the dataset spanned from Europe (France), Asia (China), and North America (US and Canada), and included maize hybrids mainly produced during the last two decades [except for Plénet and Lemaire (2000) were cv Volga-Pioneer, from before 1990 were used].

### 2.2. Model and statistical analysis

Our model is the Bayesian hierarchical model described by Makowski et al. (2020). This model considers that the response of biomass to N content follows a linear-plus-plateau function, as commonly considered in many studies. The variability of the parameters of the linear-plus-plateau function across dates of observation is described by probability distributions estimated using a Bayesian method. The parameters of the critical N dilution curve are then derived directly from the fitted probability distributions. The model includes three levels. The first level of the model describes the biomass response to N content for a given date of measurement based on a linear-plus-plateau function. Each date of measurement corresponds to a specific crop growth stage in a given year at which biomass and N content are measured for different N fertilizer levels. The second level of the model describes the variability of the parameters of the linear-plus-plateau function across observation dates using probability distributions, and computes the critical N dilution curve. The third level describes prior knowledge about parameter values. In order to limit the influence of priors on the results, we used here the weakly informative priors defined by Makowski et al. (2020).

With the classical approach initially described in Justes et al. (1994) for wheat and used by Plénet and Lemaire (2000) for maize, the  $N_c$  data point used for determination of the critical N dilution curve were considered as without uncertainty. The uncertainty of the fitted curve was only due to the regression error that did not allow a rigorous comparison across different conditions. With this new approach the whole uncertainty can be estimated. Therefore, compared to more traditional approaches commonly used to fit N dilution curves (as previously mentioned), this Bayesian method does not require the classification of nitrogen-limited data against non-nitrogen-limited data and does not necessitate the preliminary identification of critical N concentrations. Another advantage of this method is that it facilitates the analysis of the uncertainty of the fitted critical N curves. The estimation results are expressed by probability distributions from which the uncertainty of any quantity of interest can easily be analyzed. In particular, our approach allows us to calculate the credibility intervals of the N critical curves and their parameters.

The statistical model was fitted with a Markov chain Monte Carlo algorithm (MCMC) implemented with the R package rjags (Plummer, 2017). The algorithm was first run with three chains of 50,000 iterations each. After checking for convergence, the algorithm was run again during 100,000 additional iterations which were then used to compute the median and 95 % credibility intervals for the parameters of the N dilution curve. This procedure was applied both for each hybrid

**Table 1**  
Characteristics of the experimental sites used in the study.

Study	Location	Year	Genotypes	N rate (kg ha <sup>-1</sup> )	Sowing date	Targeted Plant density (plants ha <sup>-1</sup> )	Row spacing (m)	Sampling dates (days after planting)	Soil type (texture)	Reference
1	Onard, France	1992	Pioneer Volga	30–80- 130–230-280	April 25, 1992	90,000	0.80	30,38,45,51,58,65,73, and 81	Sandy soil	<a href="#">Plénet and Lemaire (2000)</a>
2	St-Louis, Canada	2004	Pioneer 39D82	20–50- 100–150- 200–250	May 15, 2004	93,300	0.75	52,59, 66, 73, 79, and 86	Typic Humaquepts	<a href="#">Zaidi et al. (2008)</a>
3	St-Louis, Canada	2005	Pioneer 39D82	20–50- 100–150- 200–250	May 17, 2005	93,300	0.75	50,57,64, and 71	Typic Humaquepts	<a href="#">Zaidi et al. (2008)</a>
4	St-Basile, Canada	2004	Pioneer 39W54	20–50- 100–150- 200–250	May 21, 2004	93,300	0.75	70,76,82,91, and 96	Typic Haplorthods	<a href="#">Zaidi et al. (2008)</a>
5	L'Acadie, Canada	2004	Pioneer 38A24	20–73- 125–178	May 18, 2004	93,300	0.75	49,57,61,73, and 78	Typic Humaquepts	<a href="#">Zaidi et al. (2008)</a>
6	L'Acadie, Canada	2005	DKC-4627 BT	30–83- 135–188	May 12, 2005	93,300	0.75	56,63,70, and 77	Dystic Eutrodepts	<a href="#">Zaidi et al. (2008)</a>
7	Ste-Catherine, Canada	2005	Pioneer 39W54	20–50- 100–150- 200–250	May 6, 2005	93,300	0.75	66, 73, and 80	Typic Haplorthods	<a href="#">Zaidi et al. (2008)</a>
8	Yucheng, China	2011	Zhengdan 958	0–70- 140–210-280	June 21, 2011	67,500	0.60	15,25,32,40,45,54,61, and 69	Loam	<a href="#">Chen et al. (2013)</a>
9	Wanatah, US	2010	2M750	0–112-224	April 19, 2010	79,000	0.76	45,66,77, and 86	Typic Argiaquoll	<a href="#">Ciampitti et al. (2013)</a>
10	Wanatah, US	2010	2T789	0–112-224	April 19, 2010	79,000	0.76	45,66,77, and 86	Typic Argiaquoll	<a href="#">Ciampitti et al. (2013)</a>
11	Wanatah, US	2011	2M750	0–112-224	May 20, 2011	79,000	0.76	28,48,59, and 68	Typic Argiaquoll	<a href="#">Ciampitti et al. (2013)</a>
12	Wanatah, US	2011	2T789	0–112-224	May 20, 2011	79,000	0.76	28,48,59, and 68	Typic Argiaquoll	<a href="#">Ciampitti et al. (2013)</a>
13	West Lafayette, US	2010	2M750	0–112-224	April 9, 2010	79,000	0.76	46,67,81,89	Typic Endoaquoll	<a href="#">Ciampitti et al. (2013)</a>
14	West Lafayette, US	2010	2T789	0–112-224	April 9, 2010	79,000	0.76	46,67,81, and 89	Typic Endoaquoll	<a href="#">Ciampitti et al. (2013)</a>
15	West Lafayette, US	2011	2M750	0–112-224	May 9, 2011	79,000	0.76	28,51,59, and 73	Typic Endoaquoll	<a href="#">Ciampitti et al. (2013)</a>
16	West Lafayette, US	2011	2T789	0–112-224	May 9, 2011	79,000	0.76	28,51,59, and 73	Typic Endoaquoll	<a href="#">Ciampitti et al. (2013)</a>
17	Xinxiang, China	2015	Zhengdan 958	0–75- 150–225-300	June 8, 2015	70,000	0.60	30, 42, 54, 63, and 75	Light loam	<a href="#">Zhao et al. (2018)</a>
18	Xinxiang, China	2015	Denghai 605	0–75- 150–225-300	June 8, 2015	60,000	0.60	30, 42, 54, 63, and 75	Light loam	<a href="#">Zhao et al. (2018)</a>
19	Xinxiang, China	2016	Zhengdan 958	0–90-180–270	June 6, 2016	75,000	0.60	27, 40, 53, 64, and 74	Light loam	<a href="#">Zhao et al. (2018)</a>
20	Xinxiang, China	2016	Denghai 605	0–90-180–270	June 6, 2016	80,000	0.60	27, 40, 53, 64, and 74	Light loam	<a href="#">Zhao et al. (2018)</a>

separately and for all hybrids globally, leading to a series hybrid-specific parameter estimates and to global parameter estimates. Significant differences in parameter estimates  $A_1$  and  $A_2$  across genotypes were determined by evaluating whether the 95 % credibility interval of the differences [derived from the samples produced by the Markov chain Monte Carlo (MCMC) algorithm] included zero.

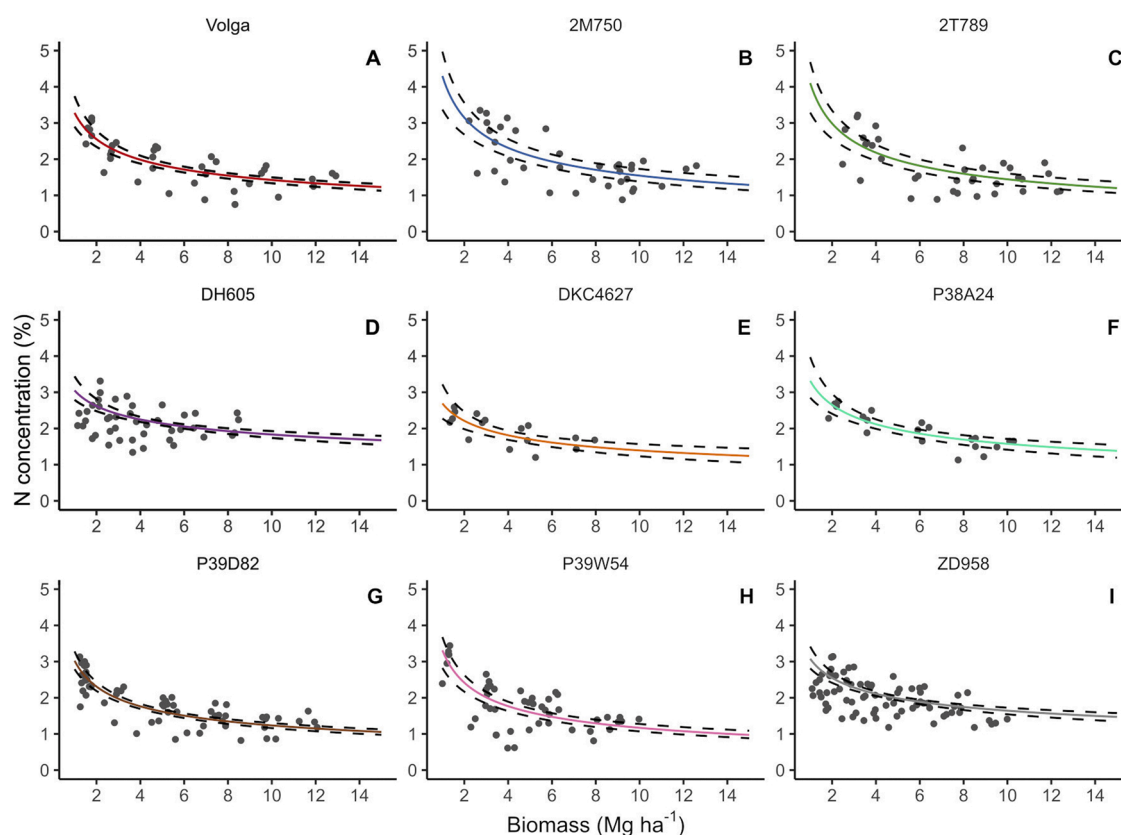
### 3. Results

Plant %N was diluted over time as plant aged (with increase of plant biomass), with hybrid-specific critical N curves fitted following the plant %N-biomass mathematical expression ([Fig. 1](#)). In a similar way across hybrids, the level of uncertainty (i.e. width of the 95 % credibility interval for the fitted curves) decreased with larger plant biomass levels. At greater plant biomass (13 Mg ha<sup>-1</sup>), the width of the credibility interval varied from 0.15 % to 0.38 % of plant %N ([Fig. 1, E,G](#)). In contrast, the level of uncertainty ranged from 0.49 % to 1.60 % of N (for P39D82 and 2MT50 hybrids, respectively) at lower biomass values, close to 1 Mg ha<sup>-1</sup>.

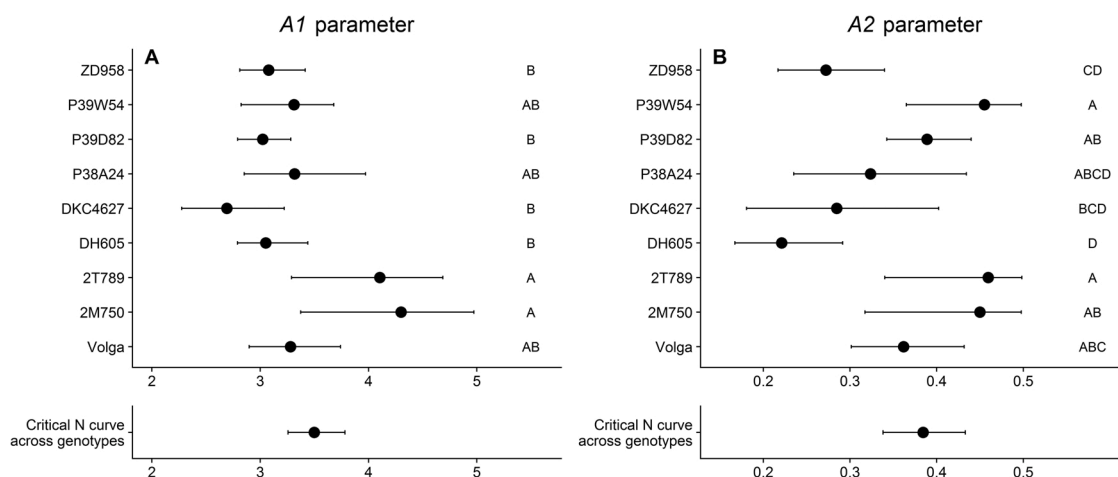
To compare the critical N curves across hybrids, the estimated  $A_1$  and  $A_2$  coefficients with their respective 95 % credibility intervals are

presented in [Fig. 2](#). For the parameter  $A_1$ , a statistical difference was only found between 2T789 and 2MT50 with respect to DKC4627, DH605, P39D82, and ZD958 ([Fig. 2A](#)). In particular, 2T789 and 2MT50 genotypes ([Ciampitti et al., 2013](#)) showed considerably higher posterior medians of  $A_1$  with respect to others hybrids (greater than 4), yet with larger credibility intervals. There was no statistical difference for the  $A_1$  parameter across the remaining seven hybrids, implying a relatively minor GxExM (e.g., hybrid x density x row spacing) variation for this coefficient for the tested modern maize hybrids. Posterior distributions of  $A_1$  and  $A_2$  coefficients are presented in the Supplementary Fig. 1.

Compared to  $A_1$ , results obtained for  $A_2$  showed a larger degree of variation across genotypes, with North American materials (P39W54, 2T789, 2MT50, and P39D82) presenting greater values relative to Asian hybrids (DH605 and ZD958) ([Fig. 2B](#)). It should be noted the reported differences between genotypes reflect not only the hybrid differences but the variation of management (e.g., plant density and row spacing) and environment (GxExM) scenarios as well (the hybrids are not cultivated in the same environment and the same management practices). Overall, uncertainty levels of  $A_2$  are larger than the ones reported for the  $A_1$  coefficients. The relative level uncertainty (i.e., the width of 95 % credible interval divided by the median and expressed in percentage)



**Fig. 1.** Relationship between plant N concentration (%N) and plant biomass for each hybrid included in the dataset from early vegetative growth to silking stage. Solid lines represent the critical N curves and their 95 % credibility intervals (dashed lines). Each circle represents individual observations.



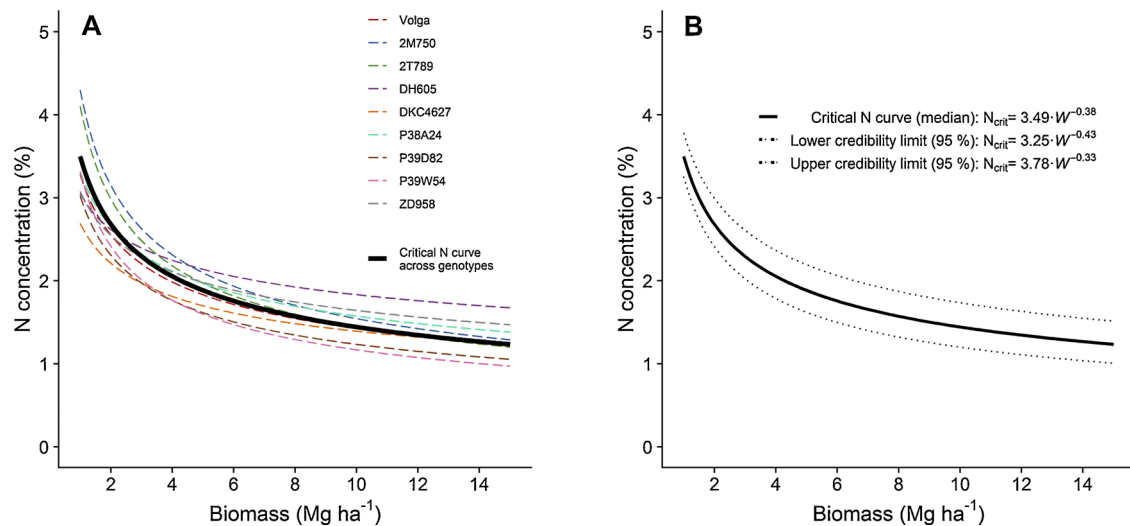
**Fig. 2.** Hybrid-specific and global estimated values (posterior medians) and 95 % credibility intervals (horizontal lines) for parameters  $A_1$  (panel A) and  $A_2$  (panel B). Different letters represent significant differences across hybrids based on the 95 % credibility intervals of the pairwise comparison between hybrids.

was roughly 11.4 % for  $A_1$  and 22.3 % for  $A_2$ , averaged across all nine hybrids.

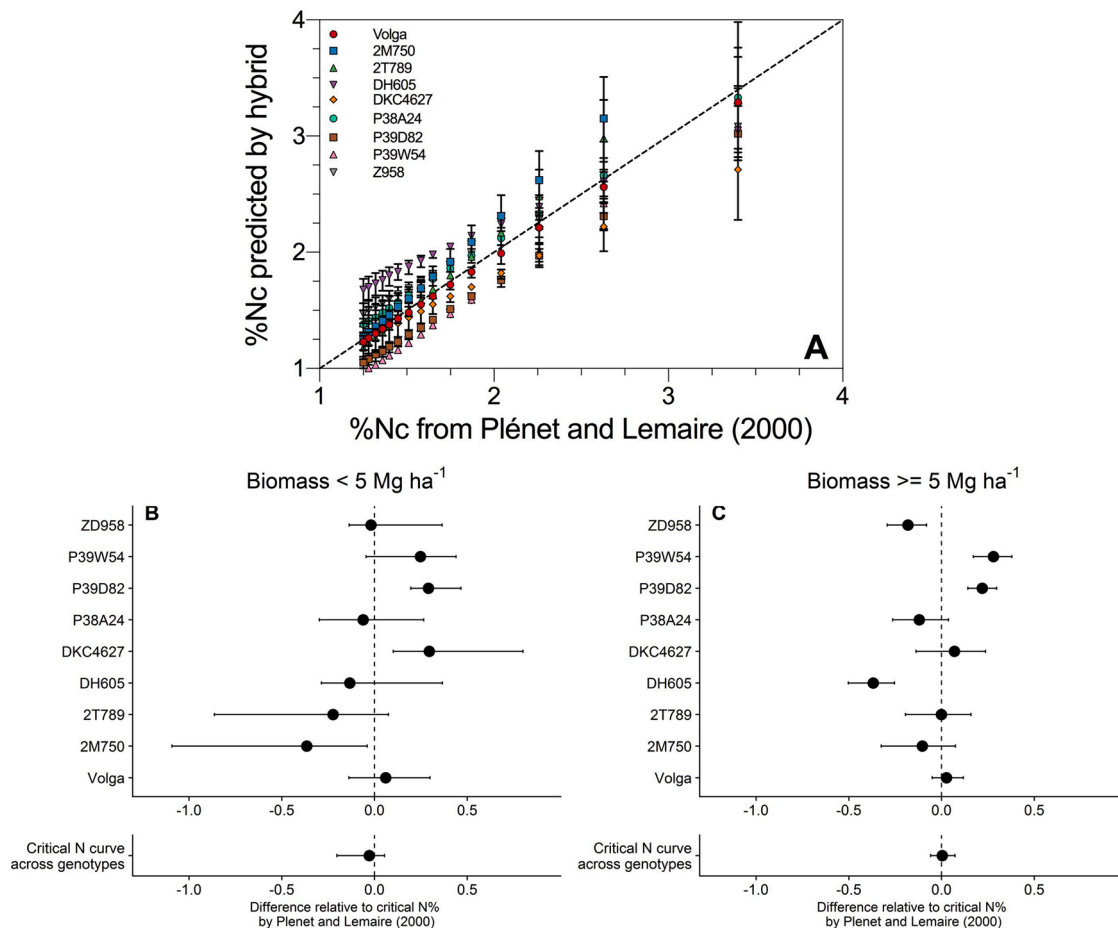
Synthesizing the entire database from the 20 unique GxExM combinations, an overall critical N curve was fitted across all nine tested hybrids (Fig. 3A). Posterior medians obtained were equal to 3.49 [95 % CI = (3.25, 3.78)] for the estimated  $A_1$  coefficient and to 0.38 [95 % CI = (0.33, 0.43)] for the  $A_2$  coefficient (Fig. 2A and 2B, respectively). Relative to the individual curves for each hybrid, the width of the credibility intervals for the overall critical N curve was reduced to 0.52 %N (at around 1 Mg ha<sup>-1</sup> of biomass) and to 0.14 %N (at 13 Mg ha<sup>-1</sup>), depicting a larger uncertainty with lower relative to greater plant

biomass (Fig. 3B). This is because the summary model used the whole set of data points for the estimation of the posterior distribution of the parameters.

Benchmarking the predicted plant %N for the critical curve, %N<sub>c</sub> (median, lower and upper credibility intervals) relative to those reported from the original work (Plénet and Lemaire, 2000), a consistent deviation was visible for maize hybrids DH605, ZD958, P39D82 and P39W54. Maize hybrids DH605 and ZD958 were consistently overestimated (above the 1:1 line) while P39D82 and P39W54 were underestimated (below 1:1) for %N<sub>c</sub> parameter all relative to the original critical N curve equation from Plénet and Lemaire (2000) (Fig. 4A).



**Fig. 3.** Relationship between plant N concentration (%N) and plant biomass from early vegetative growth to silking stage including all maize hybrids and the average critical N curve (panel A) and the 95 % credibility intervals for both upper and lower levels of the average critical curve across all hybrids (panel B). The solid line represents the global critical N curve for maize for all five papers included in the dataset. Dashed lines correspond to a specific maize hybrid presented in each study (Fig. 1).



**Fig. 4.** Predicted plant N concentration (%N) critical values, %Nc, for each maize hybrid (median and credibility intervals) and %Nc (1:1 line) estimated with the equation  $\%N = 3.40(W) - 0.37$  from Plénet and Lemaire (2000) (panel A), and posterior medians and 95 % credibility intervals for the %Nc per hybrid and for the average across genotypes for observations below (panel B) and above the level of  $5 \text{ Mg ha}^{-1}$  of plant biomass (panel C).

Estimating the posterior medians based on plant biomass below  $5 \text{ Mg ha}^{-1}$  (similar to  $>\%Nc$  above 2%) and above  $5 \text{ Mg ha}^{-1}$  ( $<\%Nc$  below 2%) as a difference for the value of each hybrid relative to the predicted

by the standard and original equation by Plénet and Lemaire (2000), larger differences in %Nc were reported as plant growth increases, with high biomass levels. With lower biomass levels (below  $5 \text{ Mg ha}^{-1}$ ) only



two hybrids differ relative to the average and standard values previously reported in maize (P39D82 and DKC4627), presenting slightly larger values of %Nc for early growth (Fig. 4B). On the other side, for higher biomass levels (above 5 Mg ha<sup>-1</sup>) a significant deviation was documented for with lower values for %Nc for DH605 and ZD958 maize hybrids and with higher %Nc values for P39D82 and P39W54 all relative to the standard reference of %Nc values originally established by Plénet and Lemaire (2000) (Fig. 4C). In summary, the prediction of %Nc under low biomass levels (below 5 Mg ha<sup>-1</sup>; with high %N > 2%) when the response of W to total N supply (soil + fertilizer) is low (large increase in %N with low increase in W) is more uncertain than the scenario presented with high biomass levels (above 5 Mg ha<sup>-1</sup>; with high %N < 2%) when the increase in W is larger (to total N supply) with low increase in plant %N.

As previously stated differences between hybrids cannot be isolated only due to the hybrid effect but to conditions nested with this factor in each location such as management and environmental factors. Therefore, the results presented in this review provide an evidence of minor differences in the N dilution curve as result of different GxExM combinations. Besides the hybrid effect, one of the most contrasting factors among the evaluated studies is related to the variations documented in the overall M component across studies. The most relevant to be highlighted are targeted plant density (ranging from 60,000 to more than 90,000 plants ha<sup>-1</sup>), row spacing (from 60 to 80 cm), and duration of vegetative growth (planting until silking, ranging from 68 to 96 days) (Table 1). Lastly, variations in the critical N curve and its coefficients could be linked to corresponding changes in these main factors, GxM scenarios, modifying the response of the crop to light, canopy architecture, overall plant growth rate, plant N demand, and the consequent relationship of N dilution within the canopy as the plant aged over time.

#### 4. Discussion

In the present study, we have successfully implemented the Bayesian approach recently proposed by Makowski et al. (2020) with a large maize dataset including % N and biomass measurements collected for nine recent hybrids in four countries. Results suggest that the critical N curve for maize and its parameter values ( $A_1$  and  $A_2$ ) show small variations across the tested modern maize hybrids and are similar to those initially reported by Plénet and Lemaire (2000). Likewise, although not included in this review due to the constraint of the ability to individualize hybrid contribution, the data from Herrmann and Taube (2004) also confirmed with an independent dataset from Germany that the N dilution curves (when averaged across hybrids) did not differ relative to the one obtained by Plénet and Lemaire (2000), with comparable  $A_1$  and  $A_2$  coefficients. More precisely, the coefficient  $A_1$  referring to the plant N concentration for a biomass level of 1 Mg ha<sup>-1</sup> marginally differ among genotypes, highlighting that the initial process of N dilution when plants are earlier in the growth process (grown in isolated plants) is similar for all the maize GxExM scenarios explored in this review. The second coefficient in the critical N curve,  $A_2$ , refers to the specific pattern of N dilution, changes in plant %N as the plant aged, factor slightly influenced by the maize hybrids-environment-management tested in this review analysis. As initially reported by Makowski et al. (2020), the level of uncertainty of the critical N curve depended on the plant biomass levels, with larger uncertainty documented for lower relative to greater biomass values. Although this study is limited in the number of evaluated investigations due to their completeness of the datasets, to the extent of our knowledge, this is the first review testing the universality of the plant %N-biomass relationship in maize across varying GxExM combinations. Future studies should focus on dissecting the potential contributions of individual and interacting effects caused by G, E, M on the N dilution patterns for maize crop.

From the differential N dilution patterns, Caloin and Yu (1984) reported changes in the plant chemical composition attributing to changes in the proportion of structural and metabolic plant tissues. Following

this rationale, the N dilution during crop mass can be understood as the outcome of two processes: (i) increasing proportion of plant structural tissues with low plant %N relative to metabolic tissues with high %N (Caloin and Yu, 1984); and (ii) general decline in leaf N concentration as W increases, in addition to an increase in the amount of structural tissues accompanied by increments in leaf area (Niklas, 1994). In general, maize plants grow without competition until early in the growth cycle (e.g., five-leaf stage; Girardin, 1992; Maddonni et al., 2001), presenting a very low N dilution mainly composed of metabolic tissues under well-illuminated environments. Therefore, the coefficient  $A_1$ , referring to the %Nc in early stage, can be considered as relatively constant across GxExM combinations. The latter situation was reflected in our review paper with minor differences in this coefficient across genotypes, usually considering N dilution when plants grow in a more isolated canopy. Likewise, Ziadi et al. (2009) reported that the %Nc of the uppermost leaf (fully illuminated) remained about 3.4 %N during the growth period. Similarly, Drouet and Bonhomme (2004) studying the light attenuation and plant density effect reported values around 3.4 %N for the tip part of the maize leaves fully exposed to light (less sensitive to density variations). This value is comparable to the one reported here for the coefficient  $A_1$ , indicating that when the canopy reaches 1 Mg ha<sup>-1</sup> with the plant structure mainly composed by leaves and all fully illuminated growing in sparse canopy this value remains constant (or present minor variations). In a study investigating the effect of plant density on plant growth and N uptake, the highest density (104,000 plants ha<sup>-1</sup>) displayed a growth rate of 40 % greater than the lowest level (54,000 plants ha<sup>-1</sup>) even early in the season, by five-leaf growth stage (V5) (Ciampitti et al., 2013). This scenario reflects that a low plant density canopy will reach 1 Mg ha<sup>-1</sup> later than at a higher density stand, with larger plants having more structural tissue (e.g., sheath and midrib) relative to the smaller plants at high density.

Lemaire and Gastal (1997) showed that for crop species the critical N curve can be dissected in two phases: i) emergence to crop biomass of 1.5 Mg ha<sup>-1</sup> (roughly LAI = 1) for maize, when N dilution is low for plants growing on isolated and fully illuminated canopies, and ii) between this point and flowering for maize with a rapid N dilution due to competition for light between plants of the community. The limit established by Plénet and Lemaire (2000) of 1.5 Mg ha<sup>-1</sup> to the onset of the critical N curve is relatively unprecise and depends on factors related to heterogeneity in plant arrangement (Seginer, 2004) leading potentially to a theoretical family of N dilution curves according to canopy arrangement (e.g., plant density dilution). Thus, as reflected in the theoretical curve a minor shift could be expected for the parameter  $A_1$  at varying plant densities earlier in the maize growing season. However, as plant biomass and LAI developed this initial shift will be then compensated at different plant densities with plant %N converging at greater plant biomass values, which leads to a correlative effect of  $A_1$  on the value of  $A_2$ . Still, one weakness for this dataset is that many of the studies did not report the final plant density achieved at mid-growing season (herein reported only the targeted plant density per study; Table 1), limiting the possibility of more formally testing the contribution of this factor in the uncertainty met with this dataset for the critical N dilution curve and their parameters.

From a GxM standpoint, changes in maize plant canopy architecture (also related to the variation in growth cycle; Table 1, and in potential total number of leaves) can be related to variations in the light extinction coefficient (Maddonni et al., 2006; Li et al., 2018), which are mainly dependent on factors such as genotype, plant density and row spacing (Maddonni et al., 2001). Changes in the vertical light distribution and attenuation are related to non-uniform leaf N partitioning within the plant canopy (Charles-Edwards et al., 1987; Field, 1983; Hirose and Werger, 1987; Drouet et al., 1999; 2004). Therefore, variations on the plant canopy architecture due to changes in GxM scenarios can plausible affect N dilution patterns as reflected on the  $A_2$  coefficients for the critical N curve in this review analysis. Exploring the most contrasting maize hybrid differences for the  $A_2$  coefficient between the Chinese

(DH605) and Canadian (P39W54 and P39D82), the primary differences seem to be related to their canopy architecture (genotypic effect) and the management implemented. From the G component, DH605 is a compact-type maize hybrids with 20 leaves in total (Wei et al., 2019), but planted around mid-June as a summer crop with a plant density level ranging from 60,000 pl ha<sup>-1</sup> (Zhao et al., 2018), and from the other side the hybrids Pioneer 39W54 and 39D82 were all planted around mid-May but with a greater plant density level 93,300 pl ha<sup>-1</sup> (Ziadi et al., 2007). Differences related to plant density (management) and genetics (canopy architecture) can provide a plausible response to the changes in the N dilution pattern as biomass increases. In addition, changes in row spacing (and canopy arrangement) can contribute to the hypothesized plant density driven N dilution effect. Reanalyzing data from Lemaire et al. (1996) at equivalent levels of plant density, it is evident that the narrower row spacing (50 cm) under relatively low N supply (60 kg N ha<sup>-1</sup>) presented a lower NNI earlier in the season (50 days after emergence) relative to the wider row spacing (75 cm). If the narrower row spacing is interpreted in a similar approach as a high plant density stand, the reduced NNI can be hypothesized to be the outcome of a different critical N curve with lower critical N dilution and therefore with a NNI closer to the sufficiency range. It can be also postulated that this effect is less likely to be evident on species that can compensate in crop arrangement such as sorghum (*Sorghum bicolor* L.), soybeans (*Glycine max* L.), and wheat by tillering or branching. However, it is evident that a more detailed study investigating different hybrids, mainly linked to changes in canopy architecture, under varying management (e.g., duration of vegetative growth, contrasting plant density and row spacing) should be pursued to test the hypothesis related to crop N dilution driven by contrasting changes in canopy arrangement.

The uncertainty on the estimation of the %Nc is a step forward in the NNI-based N status diagnosis framework for obtaining more precise and reliable data to assess the crop N status. Although this review provides useful insights on the N dilution curve of maize and its uncertainty, it still left behind a few critical future research lines on the effect of more contrasting factors affecting canopy crop arrangement early in the season and linking the overall plant N status at flowering with ear N dilution (Zhao et al., 2020).

## 5. Conclusions

The critical %N–W fitted in this study with a recent Bayesian method provided a more precise estimation of %Nc for maize while allowing for an in-depth analysis of the uncertainty in the estimated parameter values. Although variations for the A<sub>2</sub> coefficient, N dilution pattern as biomass increases, were documented in this review, the most contrasting changes seem to be related to maize hybrids with different plant canopy architecture due to the interaction with varying GxM combinations. The universality of the critical N curve is still under question, but this review provides evidence that small variations in the critical N curve parameters might be expected for maize hybrids grown under contrasting GxM combinations. In addition, a hypothesis of changes in plant architecture, related to variations in GxM (e.g., plant density, row spacing, hybrid) affecting the N dilution curve was postulated as a potential interpretation of the results observed in this review but a more formal testing and separation of these GxM factors should be further evaluated as a part of future research lines on this topic. Lastly, refining the estimation of %Nc (mean and uncertainty) will lead to a more precise assessment of plant N status via improving the calculation of NNI at any point in the growing season until the end of the vegetative growth for maize crop.

## CRediT authorship contribution statement

**Ignacio A. Ciampitti:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition. **Javier Fernandez:** Data curation, Writing - review & editing, Formal analysis, Visualization. **Santiago**

**Tamagno:** Data curation, Writing - review & editing, Investigation, Visualization. **Ben Zhao:** Data curation, Writing - review & editing. **Gilles Lemaire:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - review & editing. **David Makowski:** Software, Formal analysis, Methodology, Writing - review & editing, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors gratefully acknowledge the financial support provided by Fulbright Program, the Argentine Ministry of Education and Kansas Corn Commission for sponsoring J.A. Fernandez's studies and Dr. I.A. Ciampitti's research program, and the CLAND institute of convergence (16–CONV-0003). This is contribution no. 21-125-J from the Kansas Agricultural Experiment Station.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eja.2020.126202>.

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