



From past to future: Trends in maize N dilution curve

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Abstract

Background and Aims Maize (*Zea mays* L.) grain yield has increased over recent decades due to genetic improvement and advances in crop management, accompanied by greater nitrogen (N) fertilizer use. The N dilution curve provides a physiological framework to diagnose crop N status by describing allometric relationship between aboveground biomass and plant N concentration. However, it remains unclear

whether long-term breeding progress and recent architectural have altered this relationship. This study aimed to (i) evaluate historical changes in the maize N dilution curve and (ii) compare N dilution patterns between short- and tall-stature hybrids.

Methods Three published datasets including hybrids released between 1930 and 2020 were synthesized and combined with two experiments conducted in the United States and Argentina under non-limiting N conditions. Nitrogen dilution curves were fitted using Bayesian modeling, whole-plant and organ-level (leaf and stem) N dilution dynamics were evaluated.

Results The N dilution curve differed between the oldest hybrids (1930–1949) and modern hybrids (1990–2020). Posterior model parameters median across eras were 2.69 for a and 0.22 for b, with no shifts detected among hybrids released from 1950 to 2020. Across field experiments, short- and tall-stature hybrids exhibited similar N dilution patterns at the whole-plant, leaf, and stem levels.

Conclusion These findings demonstrate that neither long-term genetic improvement nor reduced plant height alters the core physiological relationships governing N dilution in maize. Overall, the results reinforce the robustness of the N dilution framework and support its use as a physiological reference for understanding crop N status.

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Abbreviations

N	Nitrogen
C	Carbon
%N	Nitrogen concentration
W	Aboveground plant biomass
YOR	Year of release

Introduction

Maize (*Zea mays* L.) grain yield increases over time are well documented and represent one of the greatest achievements in modern crop production (Duvick and Cassman 1999; DeBruin et al. 2017; Mueller et al. 2019). These gains have resulted from a combination of factors, including genetic improvement (Duvick 2005) and advances in crop management practices (Ciampitti and Vyn 2012; Assefa et al. 2018). Over the same period, the use of synthetic nitrogen (N) fertilizers has also risen substantially (Erisman et al. 2008; Zhang et al. 2015), driven by the need to meet the high N demand of maize (Ciampitti and Lemaire 2022), along with increases in plant density (Duvick and Cassman 1999) and shifts in soil management practices. However, many farmers apply N above the optimal rate (Houser 2022), thereby, generating a N surplus (N inputs greater than N outputs) that increase environmental impact risks. Therefore, development of better diagnostic tools to help farmers and agronomists to improve targeting the right N rate are still needed. An approach based on the eco-physiological basis of N nutrition, such as the N dilution curve proposed by Lemaire and Gastal (2009) nearly 30 years ago, could adequately serve progress towards a more universal science-based N recommendation.

The relationship between plant biomass and N concentration follows a characteristic dilution pattern, where increases in biomass are accompanied by a reduction in tissue N concentration (Lemaire et al. 1984). This relationship, known as the crop N dilution curve, describes the allometric scaling between aboveground biomass and plant N concentration and is typically represented by a power function ($\%N = a W^{-b}$), where a indicates the N concentration at 1 Mg ha⁻¹ of biomass and b is the dilution coefficient (Greenwood et al. 1990; Plénet and Lemaire 1999). This pattern is primarily driven by an increasing proportion of structural tissues with lower N concentration (e.g., stems) as plants develop, along with

reduced N concentration in shaded leaves as canopy closure progresses (Lemaire and Gastal 2009; Gastal et al. 2026). Given that breeding and management have substantially altered plant architecture (e.g., plant height, leaf angle, and density), it is reasonable to expect that these changes could influence N dilution dynamics through shifts in biomass (and N) partitioning and canopy structure. However, there is limited research assessing whether the advances in genetic and management achieved over time have altered this relationship, and the extent and direction of such potential changes remain unclear.

Recent evidence indicates that the parameters of the critical N dilution curve, a and b , which define the curve describing the minimum N concentration required to maximize crop growth at a given biomass, are largely consistent across modern maize hybrids (from 1992 to 2016), with only minor variations under contrasting genotype $\times$ management conditions (Ciampitti et al. 2021). These findings support the robustness of the original curve proposed by Plénet and Lemaire (1999), suggesting that breeding progress and management changes have not substantially altered the overall N dilution pattern (Fernandez and Ciampitti 2026). However, slight shifts in the b coefficient, reflecting how N concentration declines with biomass, may be linked to differences in canopy architecture among hybrids (Ciampitti et al. 2021). For instance, compared with older hybrids, modern hybrids have a more erectophile leaf habit, enabling greater light penetration to lower canopy leaf layers (Cagnola et al. 2021). This has led to the hypothesis that structural traits, such as the more compact canopy of modern shorter hybrids, could subtly influence N dilution dynamics through changes in C-N allometry or in leaf-to-stem C and N ratios.

A recent study examining the breeding effect in commercial hybrids released from 1983 to 2017 in the US Corn Belt reported greater N dilution in newer hybrids when each of them were grown at their typical plant density across their decade of release, but no differences when all hybrids were grown under current, higher plant densities (Saenz et al. 2025). Likewise, another recent study reported no differences in N dilution patterns of US maize hybrids released from 1936 to 2011 despite the higher total N uptake and lower N stem content of modern hybrids (Fernandez and Ciampitti 2026). Regarding the new generation of short-stature hybrids, recent studies observed similar

total biomass and N uptake compared to traditional tall hybrids, but higher leaf biomass, leaf N concentration, and N partitioning to reproductive structures (Kosola et al. 2023; Ordóñez et al. 2024). However, the specific impact of the *BRACHYTIC2* gene mutation (Multani et al. 2003), the non-transgenic technology responsible for reduced stem elongation, on N biomass partitioning, and the N dilution curve remains unexplored. Overall, while N dilution patterns appear relatively stable in modern germplasm, it remains unclear how long-term breeding progress across multiple decades and current changes in plant architectures may have influenced these relationships. Understanding these historical shifts in N partitioning and plant architecture are important, as the N dilution concept supports diagnostic tools for assessing crop N status and guiding fertilizer management.

Following this rationale, the aims of this study were to (i) study historical changes (from 1930 to 2020) in maize N dilution curve and (ii) evaluate whether short-stature maize hybrids including iso-hybrids (with *BRACHYTIC2* gene mutation), exhibit similar or distinct N dilution patterns compared to conventional tall-stature hybrids. By linking historical hybrid development with novel plant architectures, this study provides insight into the stability and applicability of N dilution relationships for both past and future maize production systems.

Materials and Methods

Historical hybrid dataset

Data included in this study were obtained from three published studies on the literature covering historical maize hybrids: Emmett et al., (2018), Woli et al. (2016), and Mueller et al. (2019) hereafter referred to as study 1, 2, and 3 respectively (Table 1). Studies

were screened and selected based on the following criteria: (i) evaluation of historical maize hybrids under field conditions; (ii) inclusion of at least three hybrids released in different decades; (iii) reporting of grain yield, plant N concentration or N uptake, and plant dry biomass at a minimum of two maize growth stages, spanning from the six ligulated leaf stage to kernel milk (V6 to R3; Ritchie and Hanway 1982); and (iv) inclusion of at least one N fertilization rate considered sufficient to ensure non-limiting N conditions, as validated using the critical N dilution curve (Ciampitti et al. 2021) (Supplementary material Figure S1).

To analyze the effects of release period, hybrids were grouped into five chronological categories based on their year of release (YOR): A) 1930–1949, B) 1950–1969, C) 1970–1989, D) 1990–2000, and E) 2001–2020. This grouping allowed for comparison among genotypes across distinct breeding periods while capturing the progression of genetic improvements over time.

Field experiments

In addition to the historical hybrid dataset, two field experiments were conducted with short- and tall- stature maize hybrids under non-limiting N conditions (Table 1). One experiment was conducted at the Purdue Agronomy Center for Research and Education (ACRE) in West Lafayette, United States of America (40° 28'N, 86° 59'W) (study 4). The experiment followed a maize-soybean (*Glycine max* L.) rotation under rainfed conditions with four replications. N rates ranged from 0 to 269 kg N ha⁻¹ (0, 67, 134, 201, and 269 kg N ha⁻¹). The 201 kg N ha⁻¹ was selected for the analysis to ensure non-limiting N conditions, following the same methodology as in the historical studies. Two hybrids were compared, a short-stature hybrid PR111-20SSC and standard stature hybrid

Table 1 Characteristics of the studies included in the dataset

Study	Location	Year	Number of hybrids	Sampling stages	N rate (kg N ha ⁻¹)
1	Aurora, NY, US	2015	12	V6, R1, R3	170
2	Ames, IA, US	2008	10	V6, V10, V14, VT, R1	168
3	West Lafayette, IN, US	2016–2017	7	V13, R1, R2	176
4	West Lafayette, IN, US	2025	2	V6, V9, V12, V15, R1	201
5	Buenos Aires, Argentina	2023	4	V7, V12, R1	250

DKC62-70 (Bayer Crop Science). Hybrids were sown on 9 May 2025 at a target density of 84,000 plants ha^{-1} . Plots were six rows wide (0.76 m spacing) and 12 m long. All plots, except the control, received 44 kg N ha^{-1} as starter fertilizer, and sidedress applications were made at the four ligulated leaf stage (V4; Ritchie and Hanway 1982) using sub-surface coulter-injected urea ammonium nitrate (28% UAN) between the maize rows.

A second field experiment was conducted in the experimental field of the Department of Plant Production of the Faculty of Agronomy, University of Buenos Aires, Buenos Aires, Argentina (34° 35'S, 58° 29'W) (study 5). Two pairs of maize isohybrids were evaluated and sown on December 5, 2023 (Table 1). Each pair includes a traditional stature hybrid (tall; T) and its respective reduced stature isohybrid (short; S) due to the mutation of the *BRACHYTIC2* gene (T1 and S1; T2 and S2 respectively). Hybrids were cultivated at a target density of 80,000 plants ha^{-1} and fertilized with 250 kg N ha^{-1} in a randomized complete block design with 3 replicates. Each plot involved 4 rows 6 m long distanced at 0.52 m. The experiment was conducted with assisted irrigation, free of diseases, pests, and weeds. All plots received 250 kg N ha^{-1} at the three ligulated leaf (Ritchie and Hanway 1982) as urea ammonium nitrate (28% UAN). Fertilizer was applied along the rows and incorporated into the soil with a hoe. Data from study 5 was averaged due to non-significant differences between pairs. Weather and soil information for both studies are presented in the supplementary material (Figure S2 and Table S1).

Plant measurements and N determination

Aboveground biomass was sampled at different stages during vegetative and early reproductive stages (Table 1) by collecting four whole plants per plot at ground level. In Studies 4 and 5, harvested plants were partitioned into leaves and stems before subsequent analyses. Samples were oven-dried at 60 °C in a forced-air dryer until constant weight to determine dry matter. Plant N concentration was subsequently analyzed using the micro-Kjeldahl method (Nelson and Sommers 1973). Crop phenology was taken bi-weekly in 5 tagged plants within each plot.

Statistical analysis

The N dilution curve was obtained for each YOR group. Bayesian modeling was used to characterize the relationship between plant N concentration (%N) and aboveground plant biomass (W , Mg ha^{-1}) employing a power function $\%N = a \times W^{-b}$, where a and b are parameters and W represents aboveground biomass. Data from studies 1, 2, and 3 (Table 1) were included in the analysis. A detailed description of statistical analyses is provided in the Supplementary Material.

Briefly, posterior distributions were obtained using a Markov chain Monte Carlo algorithm (MCMC). Four parallel chains were run with 50,000 iterations each, and a burn-in period of 10,000 and a thin rate of 2. The convergence of the models and mixing of the chains were assessed via trace plots and Gelman-Rubin diagnostic (Gelman and Rubin 1992). Priors were defined as weakly informative distributions centered on biologically realistic parameter values reported in previous maize N dilution studies (Ciampitti et al. 2021), ensuring consistency with established physiological ranges while allowing the data to drive posterior inference. The models were fit using the *brms* package (Bürkner 2017) in R software (R Core Team 2025). Parameter estimates a and b were considered different across YOR groups if the 95% credible intervals for the difference between groups did not include zero.

The same modeling approach was used to study the N dilution curve from short- and tall-stature hybrids, as well as the specific relationships of leaf (%N_{leaf}) and stem (%N_{stem}) N concentrations with aboveground biomass (W , Mg ha^{-1}) (Studies 4 and 5 from Table 1). In these hierarchical models, hybrid stature was included as a random effect and BLUPs were estimated for short- vs. tall-stature hybrid comparisons. Analyses were conducted separately for each experiment due to differences in genetic structure: study 5 evaluated two pair of maize isohybrids with or without mutation of the *BRACHYTIC2* gene, while study 4 included a short- and a tall- stature hybrids with different genetic background. More details about models and priors are provided in the supplementary material.

Results

Historical changes in maize N dilution

The N dilution curve differed between the oldest (1930–1949) and more modern hybrids (1990–2020), driven by shifts in both parameters a and b (Fig. 1, Table 2). The oldest hybrids exhibited a lower initial N content (a parameter) and a slower N dilution rate (b parameter) compared to the modern counterparts. However, no shift in the N dilution curve was observed among hybrids released between 1950 and 2020 (Fig. 1, Table 2). In summary, posterior distribution median for parameter a was 2.69 ($q_{0.025} = 2.37$; $q_{0.975} = 2.98$), while parameter b presented a median of 0.22 ($q_{0.025} = 0.15$; $q_{0.975} = 0.27$) across all eras

(Table 2). Regarding the YOR groups created in this study, both biomass and N concentration ranges were very similar. Across groups, biomass ranged from 1.3 to 18 Mg ha^{-1} , while N concentration presented values from 3.80% to 0.88%.

Short- vs. tall-stature hybrid N dilution curve

The N dilution curve did not differ between short- and tall-stature hybrids in both studies, with no differences observed in parameters a or b (Fig. 2A and D, Table 3). In study 4, plant N concentration ranged from 4.48% to 1.56% (short), and 4.82% to 1.49% (tall), while aboveground biomass varied from 0.46 to 11.9 Mg ha^{-1} (short) and 0.28 to 9.40 Mg ha^{-1}

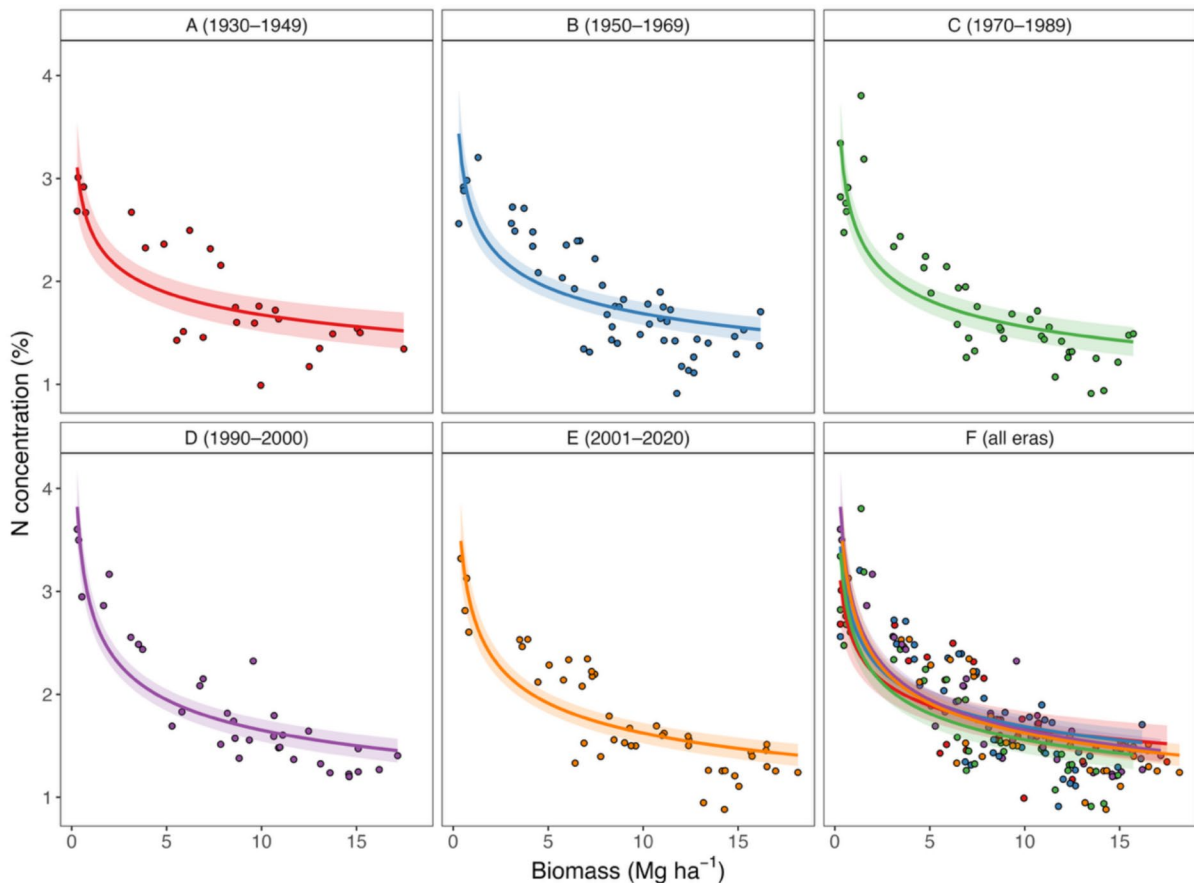


Fig. 1 Relationship between plant N concentration (%) and aboveground biomass (Mg ha^{-1}) for each hybrid era group (A to E). Curves represent the dilution model for each group, while shaded areas indicate the 95% credible interval of each

curve. Points represent the observed data from studies 1, 2, and 3 within each group. Colors refer to each hybrid era group, and F represents combined data from all groups

Table 2 N dilution curve parameters (*a* and *b*) for each hybrid era, including the 2.5th, 50th, and 97.5th percentiles of their posterior distributions, and pairwise comparisons among hybrid eras. Different letters indicate significant differences for each parameter based on the 95% credible intervals of the differences between parameter estimates. Uppercase letters indicate differences for *a* parameter, while lowercase letters indicate differences for the *b* parameter

Hybrid era	Parameter	q 2.5%	q 50%	q 97.5%	Pairwise comparison
A (1930–1949)	<i>a</i>	2.28	2.51	2.74	A
	<i>b</i>	0.13	0.18	0.23	a
B (1950–1969)	<i>a</i>	2.47	2.69	2.89	AB
	<i>b</i>	0.16	0.21	0.24	ab
C (1970–1989)	<i>a</i>	2.39	2.58	2.76	AB
	<i>b</i>	0.18	0.22	0.26	ab
D (1990–2000)	<i>a</i>	2.68	2.86	3.03	B
	<i>b</i>	0.21	0.24	0.27	b
E (2001–2020)	<i>a</i>	2.59	2.81	3.02	B
	<i>b</i>	0.21	0.24	0.28	b

(tall) (Fig. 2A). In study 5, plant N concentration ranged from 3.51% to 1.20% (short), and 3.28% to 0.90% (tall), with biomass ranging from 0.43 to 12.43 Mg ha⁻¹ (short) and from 0.47 to 13.20 Mg ha⁻¹ (tall) (Fig. 2D). Analysis of the BLUPs for the N dilution curve parameters (*a* and *b*) indicated that the 95% credible intervals for the differences between hybrids included zero in both experiments (Table 3).

No difference between the short- and tall-stature hybrids was observed when exploring leaf N concentration (%Nleaf) and stem N concentration (%Nstem) relationship with aboveground biomass in both studies (Fig. 2 B–C and E–F). Additionally, analysis of the BLUPs for the curve parameters (*a* and *b*) indicated that the 95% credible intervals for hybrids-specific deviations from the global curve included zero (Table 3). This confirms that deviations from the overall mean were not statistically distinguishable from zero, consistent with the observed similarity in the N dilution relationship at crop level. In study 4, the short-stature hybrid leaf N concentration presented a median of 3.0% with values ranging from 4.7% to 2.3%, comparable to the tall hybrid with median of 2.87%, varying from 4.75% to 2.5%. Regarding stem N concentration, the short-stature hybrid values ranged from 3.98% to 0.86%, with median of 1.68%, and tall hybrid presented values from 4.97%

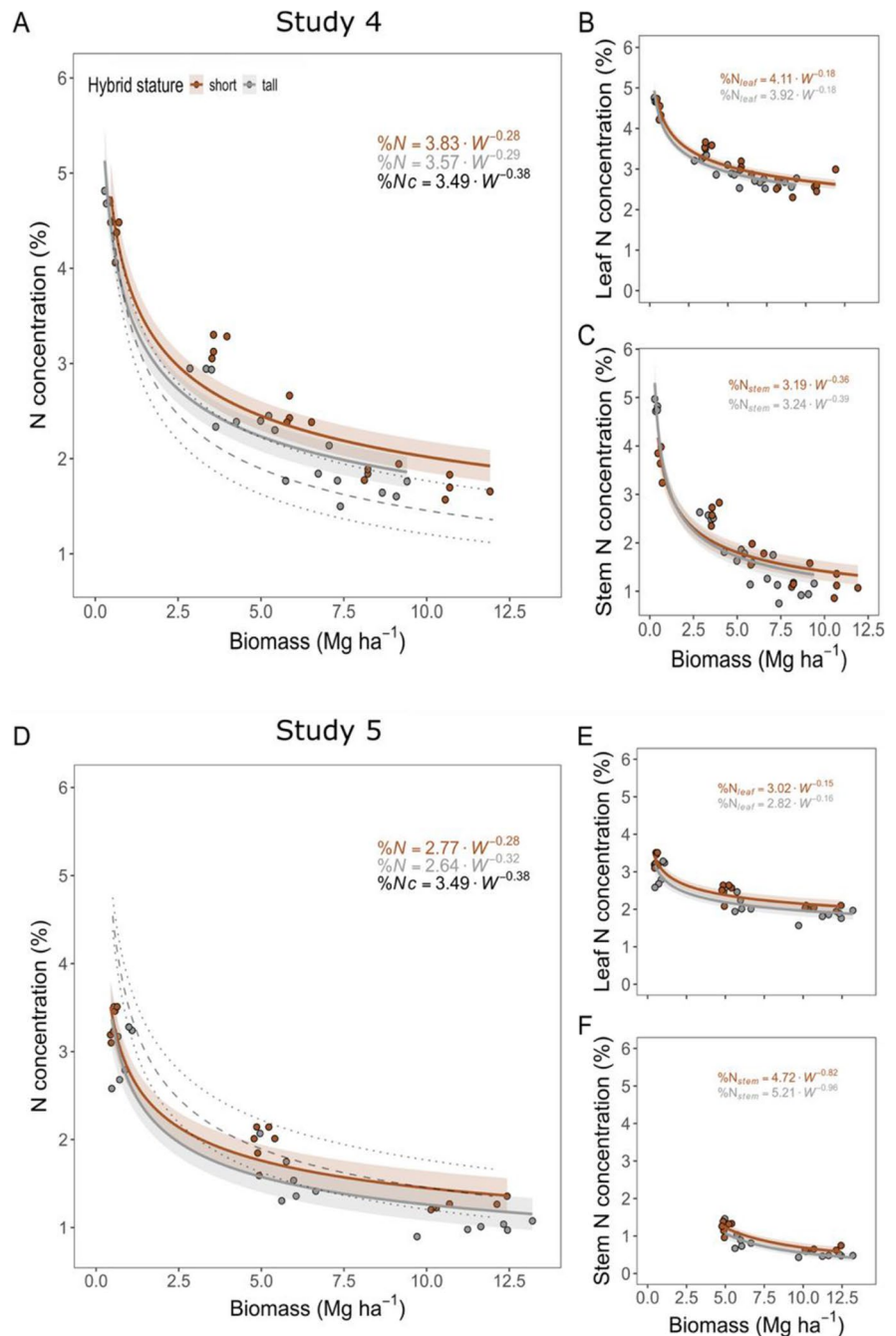
to 0.75%, with median of 1.79% (Fig. 2B and C). On the other hand, in study 5, short-stature hybrids leaf N concentration median was 2.54% with values ranging from 3.51% to 1.94%, while for tall hybrids the median was 2.12%, with values ranging from 3.28% to 1.57%. Regarding stem N concentration, the short-stature hybrids values ranged from 1.39% to 0.59%, with median of 0.85%, and for the tall hybrids values ranged from 1.46% to 0.43%, with median of 0.59% (Fig. 2E and F).

Discussion

This study provides new insights into the stability of maize N dilution dynamics by combining a retrospective analysis of historical hybrids with a forward-looking perspective on short-statured genotypes. Our findings suggest that the N dilution curve remains robust from 1950 onwards across diverse maize hybrids, which confirms the N dilution curves reported in earlier studies (Plénet & Lemaire, 1999; Ciampitti et al. 2021) and reinforce the continued relevance of this established approach. Furthermore, understanding and characterizing the physiological basis for N allocation and dilution in short-statured maize, a growing architectural trend designed to improve crop resilience and resource use efficiency, is fundamental for guiding N research and integrating these new hybrids into future cropping systems.

In retrospect, the robustness of the N dilution curve over the past seven decades aligns with recent findings indicating minor variation in curve parameters for modern genotypes, including the short-stature type (Ciampitti et al. 2021; Fernandez and Ciampitti 2026). Specifically, the consistency of the coefficient *b* suggests that although breeding has dramatically increased biomass and yield (Duvick 2005; Lee and Tollenaar 2007; Messina et al. 2022; Mueller et al. 2019; Ordóñez et al. 2025), it has not fundamentally altered the rate of N dilution along different values of shoot biomass. These results further support the original curve proposed by Plénet and Lemaire (1999) and more globally tested by Ciampitti et al. (2021), reinforcing the stability of this concept despite substantial genetic shifts. One possible hypothesis for the observed differences in older maize hybrids is linked to historical differences in plant density. Lower plant densities, which

Fig. 2 Relationship between plant N concentration (%), leaf N concentration (%), stem N concentration (%) and aboveground biomass (Mg ha^{-1}) A, B, C respectively for study 4 and D, E, F for study 5, short- (brown) and tall- (gray) stature hybrids. Curves represent the dilution model for each hybrid, while shaded areas indicate the 95% credible interval of each curve. Black dashed and dotted lines refer to Ciampitti et al. (2021) maize critical N dilution curve and its 95% credible interval



were more common in earlier production systems, may have resulted in larger individual plants at early growth stages. This could, in turn, lead to lower values of the allometric coefficient a , as plants reach an initial biomass of around 1 Mg ha^{-1} with greater individual size, potentially influencing N dilution patterns (Seginer 2004).

Shifting to a future perspective, our study highlights a similar N dilution pattern in the novel short-stature and conventional tall-hybrids. The consistency of the parameters a and b implies that the foundational N status assessment tools derived from this concept remain applicable to short-stature maize, a vital prerequisite for successfully employing

Table 3 Whole plant, leaf and stem N dilution curve parameters (*a* and *b*) BLUPs differences between hybrids by study, including the 2.5th, 50th, and 97.5th percentiles of their posterior distributions

Experiment	Plant Fraction	Parameter	BLUPs differences		
			<i>q</i> 2.5%	<i>q</i> 50%	<i>q</i> 97.5%
Study 4	Whole plant	<i>a</i>	−0.01	0.28	0.57
		<i>b</i>	−0.06	−0.01	0.04
	Leaf	<i>a</i>	0	0.19	0.39
		<i>b</i>	−0.03	0	0.03
	Stem	<i>a</i>	−0.38	−0.03	0.25
		<i>b</i>	−0.12	−0.04	0.03
Study 5	Whole plant	<i>a</i>	−0.12	0.12	0.41
		<i>b</i>	−0.13	−0.04	0.03
	Leaf	<i>a</i>	−0.02	0.21	0.43
		<i>b</i>	−0.06	−0.01	0.04
	Stem	<i>a</i>	−4.25	−0.18	2.12
		<i>b</i>	−0.29	−0.12	0.16

in-season N diagnostic tools in these new hybrids and for advancing sustainable N management practices. Mechanistically, this stability can be understood through three interconnected processes. First, N partitioning between leaves and stems followed comparable dilution trajectories across stature types, indicating that modifications to canopy architecture did not alter the fundamental coordination between organ-level N allocation and whole-plant growth. Second, leaf N concentration, closely linked to photosynthetic capacity and canopy-level carbon assimilation (Sinclair and Horie 1989), declined similarly across stature types, suggesting that the functional relationship between light interception, biomass accumulation, and N investment in photosynthetic tissues was maintained despite differences in canopy structure. This point is particularly relevant because short-stature hybrids differ in leaf angle and canopy architecture, which could in principle alter light distribution and the resulting C:N balance at the canopy level. However, the similar leaf N dilution trajectory (even when the short type presents more N concentration at same biomass) suggests these architectural differences did not translate into divergent N demand per unit biomass. Third, stem N concentration, primarily associated with structural support and transient N storage (Ciampitti & Vyn 2012; Mueller et al. 2019), exhibited comparable dilution behavior, indicating

that internal N remobilization and storage dynamics within vegetative tissues were preserved. Collectively, these organ-level responses suggest that the allometric relationship between N concentration and biomass, which is the foundation of the dilution curve concept, is governed by conserved plant physiological processes that so far are robust to the documented changes in plant stature. Thus far, reduced stature type does not appear to disrupt the coordination between growth, N allocation, and dilution at either the organ or whole-plant level (Briat et al. 2020). In addition, weather conditions and soil characteristics across studies did not show extreme variability, supporting the consistency of the dataset and reducing the likelihood that environmental heterogeneity drove the observed patterns.

Our findings are subject to three main limitations. First, the retrospective analysis relied on N dilution patterns under non-limiting N conditions rather than on the critical N dilution curve. This was a constraint imposed by the scarcity of comprehensive historical datasets spanning several decades that included different N rates and adequate sampling times and (at least three during the season; Fernandez et al. 2022). Additionally, the datasets available for the historical analysis were all conducted in the United States. Second, the analysis focused on hybrid era effects and did not account for other potential drivers of variation in N dilution across the historical datasets. Third, although there are multiple genetic and breeding strategies to reduce plant height in maize, this study only focused on a limited number of this variation. However, the non-transgenic nature of this trait facilitates its acceptance in sensitive regulatory markets (Turnbull et al., 2021), positioning it as a tool with great potential to reduce yield losses from stem lodging (Barten et al. 2022). Finally, while this study characterized the reference N dilution curve, reflecting N uptake dynamics under non-limiting conditions, future work should prioritize establishing the critical N dilution curve (defined as the minimum N concentration required to maximize biomass production) for the short-stature maize hybrids relative to the benchmark N dilution curve (Ciampitti et al., 2021). Defining this critical threshold is essential for validating existing N diagnostic tools (i.e., N nutrition index; Lemaire et al. 2008; Bosche et al. 2025) for this novel architecture. Additionally, further research is needed in diverse environments and with different

N management practices to detect subtle agronomically significant variations in the N dilution parameters (*a* and *b*) that could affect future N management guidelines.

Conclusion

This study shows that the maize N dilution curve seemed to remain stable over the last seven decades of genetic improvement despite significant changes in grain yield and plant architecture. Short-stature hybrids exhibited N dilution patterns similar to conventional tall-stature hybrids at the whole-plant and organ levels. This indicates that reductions in plant height may not alter the fundamental physiological relationships that govern N dilution. The conservation of leaf and stem N concentration dynamics further supports the robustness of N allocation and dilution processes across contrasting plant heights. Mechanistically, this stability reflects the conservation of core processes governing biomass accumulation and N partitioning, specifically, the coordinated relationship between carbon assimilation, structural tissue development, and N demand, across diverse genetic backgrounds and plant architectures. The comparable dilution trajectories, obtained from the tested genetic backgrounds, observed for leaf and stem N concentration suggest that neither long-term genetic improvement nor architectural modifications associated with reduced stature disrupt the allometric scaling between aboveground biomass and plant N concentration. These relationships are expected to remain structurally stable under moderate stress conditions, as they emerge from conserved growth-N coordination rather than condition-specific responses, but warrants further investigation under severe stress environments.

However, we acknowledge that the evaluation of short-stature hybrids is based on a limited number of genotypes, and further studies encompassing greater genetic diversity are required to confirm these patterns. Additionally, our analysis is based on N dilution relationships under non-limiting N conditions rather than on the critical N dilution curve, a constraint imposed by the limited availability of historical datasets with multiple N rates and adequate temporal sampling.

From a practical standpoint, the stability of N dilution across the tested maize hybrids supports the

continued applicability of existing N status diagnostic frameworks, including the use of the NNI as a relevant diagnostic tool. If this could be transfer to other short-stature hybrids, the application of the published framework could assist in reducing calibration burden on N demand and management nutrient decision tools; however, this scenario warrants further investigation as new short-stature hybrids are commercially available.

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Authors Contributions All authors contributed to the study conception and design. The first draft of the manuscript was written by Leonardo Bosche, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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