

# Grain Nitrogen Source Changes over Time in Maize: A Review

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## ABSTRACT

Understanding the sources of grain N uptake (Grain N) in maize (*Zea mays* L.) and especially the trade-off between reproductive-stage shoot N remobilization (Remobilized N) and reproductive-stage whole-plant N uptake (Reproductive N) is needed to help guide future improvements in yield and N use efficiency (NUE). Therefore, a literature review was performed to investigate the knowledge gap concerning changes over time in Grain N sources and on N partitioning to the grain and stover plant fractions at maturity. The synthesis–analysis was based on 100 reports, which were divided into two time intervals: (i) research conducted from 1940 to 1990—“Old Era”—and (ii) research conducted from 1991 to 2011—“New Era.” The most remarkable results were (i) Grain N concentration was the main parameter that has changed over time, (ii) Reproductive N contributed proportionally more to Grain N for the New Era while Reproductive N and Remobilized N contributed equally to Grain N for the Old Era, (iii) Remobilized N was primarily associated with vegetative-stage whole-plant N uptake (Vegetative N), which was constant across eras, although the proportion of the Remobilized N itself seems to be driven by the ear demand, (iv) complex plant regulation processes (source:sink) appeared to influence Reproductive N, and (v) stover N concentration gains mirrored the grain N concentration as the plant N uptake increased at maturity in both eras. This new appreciation for the changes over time may assist directed selection for yield and NUE improvements.

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**Abbreviations:** %N, N concentration; %Ng, grain N concentration; %Ns, stover N concentration; Grain N, grain N uptake; HI, harvest index; NHI, N harvest index; NIE, N internal efficiency; NUE, N use efficiency; Plant N, whole-plant N uptake; Remobilized N, reproductive-stage shoot N remobilization; Reproductive N, reproductive-stage whole-plant N uptake; Vegetative N, vegetative-stage whole-plant N uptake.

THE IMPROVEMENT in maize grain yield through the last decades, largely due to increased tolerance to crowding intensity, has indirectly been accompanied by a decline in grain N concentration (%Ng) (Duvick, 1997; Ciampitti and Vyn, 2012). Therefore, gains over time in N use efficiency (NUE) (yield to applied N ratio) (Moll et al., 1982) were, from a physiological perspective, primarily explained by increasing yield at equivalent whole-plant N uptake (Plant N) levels that were, therefore, reflected in modern-era hybrid gains in N internal efficiency (NIE) (yield to Plant N ratio) (Ciampitti and Vyn, 2012). However, this documented history opens an important question for future progress in maize NUE: Is continued lowering of %Ng a sustainable approach to pursue further gains in NUE? The answer to this question can diverge depending on alternative destinations for grain maize as a final product (food, feed, fiber, and fuel). Coque and Gallais (2007) suggested three different approaches in expressing the grain protein yield (hereafter called “sources of grain N”), and these are used here relative to grain N at maturity: (i) yield multiplied by %Ng, (ii) Plant N multiplied by N harvest index (NHI) all at maturity, and lastly (iii) the sum of two reproductive-stage parameters,

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namely reproductive-stage shoot N remobilization (Remobilized N) plus the reproductive-stage whole-plant N uptake (Reproductive N). For inbreds, variation in Reproductive N was larger than that in Remobilized N (Coque and Gallais, 2007). In addition, a trade-off was documented between both the Remobilized N and vegetative-stage whole-plant N uptake (Vegetative N) versus the Reproductive N (Weiland and Ta, 1992; Triboi and Triboi-Blondel, 2002). Unfortunately, most scientific maize literature on Reproductive N and Remobilized N is focused on the inbred level (Di Fonzo et al., 1982; Lafitte and Edmeades, 1994; Bertin and Gallais, 2000). Thus, a scientific knowledge gap exists concerning understanding of Reproductive N and Remobilized N relationships at the hybrid level.

Partitioning of both dry plant mass and N in maize grain and stover at maturity were reported to be associated with different Plant N threshold levels (Massignam, 2003; Ciampitti, 2012). As Plant N increased, grain harvest index (HI) fitted a linear-plateau model, with N thresholds of 11 g N m<sup>-2</sup> (grain HI plateau at ~0.52) (Massignam, 2003) and 21 g N m<sup>-2</sup> (grain HI plateau at ~0.54) (Ciampitti, 2012) associated with different maximum Plant N (~20 vs. 35 g N m<sup>-2</sup>). Despite these preliminary indications, this association needs to be validated at a larger scale (e.g., multiple environments) and be investigated more thoroughly to provide insights into the main changes over time. Furthermore, the examination of the proportionality in the N allocation between grain and stover fractions should be pursued. Ciampitti (2012) reported equivalent slopes of the gain in %Ng and shoot N concentration (%N) as Plant N increased, but that conclusion was constrained to the genotypes, plant densities, N rates, and site-years explored. Therefore, a deeper understanding of the N partitioning process should be pursued at a broader scale.

This multifaceted study of grain N uptake (Grain N) attempted to answer the following overriding question: Are the parameters (Remobilized N, Vegetative N, Reproductive N, %Ng, and stover N concentration [%Ns]) related to Grain N changing (in quantity, direction [associated with negative or positive slope values], and/or strength [related to the *r*<sup>2</sup> values]) over time in response to continued maize improvement processes? In addition, the trade-off between Reproductive N and Remobilized N was also examined to address the following research questions: Is the time factor changing the direction and strength of the Reproductive N and Remobilized N? Are there any clues as to how source and sink effects over the Remobilized N, Vegetative N, and Reproductive N might be decorrelated? Lastly, the study of both biomass and N partitioning between grain and nongrain fractions was evaluated to answer the following questions: Is the N partitioning (grain vs. stover) changing over time? Is there any isometry between grain and stover mass and N partitioning?

A theoretical framework linking all components (biomass, Plant N, and yield parameters) is presented in Fig. 1. Grain N uptake is the main destination of the Plant N (since NHI averages close to 64%) (Ciampitti and Vyn, 2012) and is associated with yield in the NUE component named as NIE. Therefore, a proper assessment of the main changes in Grain N (and its sources) over time under each approach is relevant and important.

## MATERIALS AND METHODS

### Data Inclusion and Description

Information from the investigation performed on the physiological changes over time of maize yield dependency on plant N by Ciampitti and Vyn (2012) was used in this manuscript. A brief description of the data inclusion criteria, calculation, and descriptive analysis is presented here (full details can be reviewed at Ciampitti and Vyn, 2012). A total of 100 data sources published in international refereed journals as well as selected unpublished sources (~10 M.S. and Ph.D. dissertations) were used. Research was included only when specific criteria were met (e.g., minimum data inclusion criteria was for yield, plant density, and Plant N parameters at maturity, only treatment means were collected, and data was standardized for yield expressed at 155 g kg<sup>-1</sup> moisture and Plant N on a dry basis; information was mostly from hybrids). A total of approximately 3000 treatment means, from research performed in different parts of the globe, were collected and numerous data sets had more than the minimum data set for several parameters (e.g., Plant N and biomass at silk emergence and at maturity, yield components, grain HI, NHI, %Ng, %Ns, among others). The entire database was arbitrarily divided into two eras, named as “Old Era,” comprising research trials conducted from 1940 to 1990 (inclusive), and the “New Era,” associated with experiments performed from 1991 till 2011. Further details related to the latter justification and parameters evaluated can be reviewed at Ciampitti and Vyn (2012).

### Data Calculations and Parameters Analyzed

The grain HI and NHI were related to the Grain N behavior and to the dry mass and N partitioning. When the grain HI and NHI were not directly reported, the grain HI and NHI were estimated and calculated as follows:

$$\text{Grain HI} = (\text{Yield}/\text{Plant Biomass}) \times 100, \text{ and}$$

$$\text{NHI} = \text{Grain N}/\text{Plant N},$$

in which the whole-plant biomass (Plant Biomass [on a dry weight basis]) and Plant N includes all stover fractions (i.e., that in leaves plus stem plus shank plus cobs plus husk organs) plus the grain fraction for biomass (yield, 155 g kg<sup>-1</sup> moisture) and N (Grain N), respectively.

The Remobilized N was determined using the “balance approach” as follows:

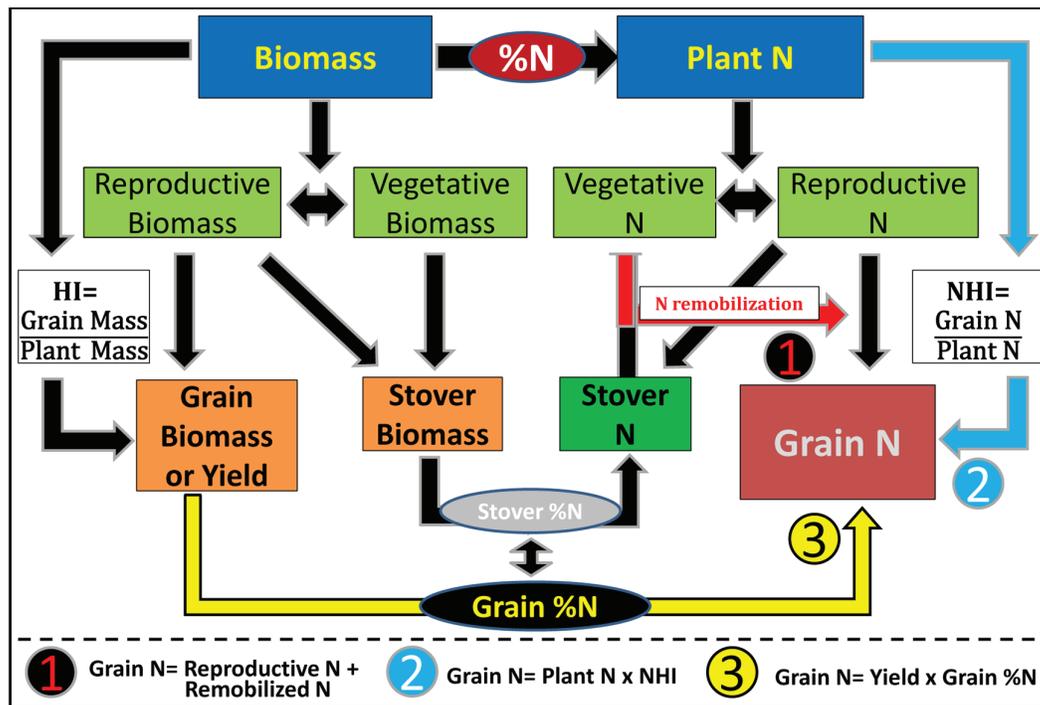


Figure 1. Theoretical framework linking whole-plant biomass (Biomass), whole-plant N uptake (Plant N), and grain yield (Yield) components evaluated in this research for maize. Vegetative-stage whole-plant N uptake (Vegetative N) or Biomass includes leaf and stem plant components. Reproductive-stage whole-plant N uptake (Reproductive N) or Biomass includes shoot (leaf plus stem organs) and ear (cob, husk, and grain fractions) plant components. Stover N or Biomass includes shoot plus cob and husk fractions (all whole-plant fractions except grain). The red arrow reflects the N remobilization process from the shoot to the grain during the grain filling period. Pathways of the different grain N sources (1,2, and 3) are highlighted within the flow diagram. %N, N concentration; Plant N, whole-plant N uptake; HI, grain harvest index; NHI, N harvest index; Grain N, grain N uptake; Remobilized N, reproductive-stage shoot N remobilization; Shoot N, shoot N uptake.

Remobilized N = Vegetative N – Stover N,  
 in which the vegetative N (cumulative whole-plant N at silk emergence) includes all leaves plus stem plus ear N and the stover N at maturity (i.e., leaves plus stem plus shank plus cob plus husk N fractions). This approach is less accurate than the <sup>15</sup>N method (requiring one measurement at maturity while the balance approach requires determinations at silking and at maturity). The balance approach can be flawed due to sampling error (comparable stover biomass measured at two diverse phenological stages and when the data is combined for the calculations) (Kichey et al., 2007), but it is nevertheless legitimate and used extensively.

The reproductive N was estimated as follows:

$$\text{Reproductive N} = \text{Plant N} - \text{Vegetative N},$$

in which the Plant N (cumulative whole-plant N at maturity) includes stover plus grain N.

### Descriptive Statistical Analysis

For both eras, mean, standard deviation, minimum, and maximum were determined for all parameters (Table 1). Furthermore, histograms were developed, using the “hist” function from the R program (R Development Core Team, 2009), to graphically show the distribution for the Remobilized N, Vegetative N, Reproductive N, Grain N, %Ng, and %Ns

(Supplemental Fig. S1). To quantify the variance in the Grain N (Fig. 2), the proportion of the variance (quantified by the *r*<sup>2</sup> values) was accounted for by linear regressions between Grain N and each source parameter (similar procedure as used by Duvick and Cassman [1999] and Sadras [2006]).

From all the evaluated relationships, the number of observations varied because not all parameters were measured or reported in all the studies used in the main dataset. For the associations between Remobilized N and Vegetative N, and between Reproductive N and Remobilized N (Fig. 3A and 3B), the bubble graph technique was used (R program [R Development Core Team, 2009]) to show these relationships as a function of a third parameter whereby different sizes refer to the yield or Plant N dimension (e.g., larger sizes, superior yields).

Allometric analyses were performed to statistically quantify the changes between eras. Therefore, reduced major axis was used to evaluate the slopes and intercepts. The SMATR package (version 3; Warton et al., 2012) from the R program (R Development Core Team, 2009) was used to test for common slopes and intercepts (Table 2). The parameters were log<sub>10</sub> transformed before the analysis, and the normality and residuals distributions were tested for each individual association evaluated.

**Table 1. Summary statistics of the synthesis for the Old (1940–1990) and New (1991–2011) Eras relative to maize grain yield (155 g kg<sup>-1</sup> moisture content), whole-plant N uptake (Plant N), whole-plant biomass (Biomass) at different phenological stages (silking and maturity, all on dry basis) and variables related to the partitioning components of yield and N and to the N use efficiency.**

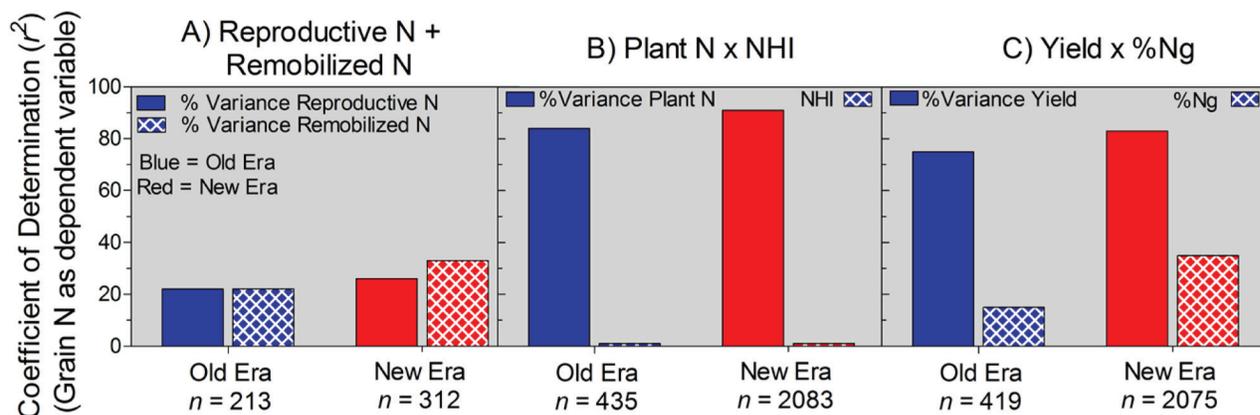
Variable	Old Era				New Era			
	<i>n</i>	Mean	SD	Min.–Max.	<i>n</i>	Mean	SD	Min.–Max.
For Grain N calculation (Grain N = Reproductive N + Remobilized N) <sup>†</sup>								
Grain N, g m <sup>-2</sup>	213	9.8	3.5	1.0–21.7	312	10.8	3.8	2.5–20.7
Reproductive N, g m <sup>-2</sup>	213	5.1	3.7	–3.5–16.7	312	6.0	3.6	–1.5–17.2
Vegetative N, g m <sup>-2</sup>	213	11.3	4.9	1.2–24.1	312	10.9	4.0	4.0–22.4
Remobilized N, g m <sup>-2</sup>	213	4.9	3.7	–3.2–14.7	312	4.3	3.3	–5.0–12.5
For Grain N calculation (Grain N = Plant N × NHI) <sup>‡</sup>								
Plant N, g m <sup>-2</sup>	435	15.7	5.9	3.3–38.7	2083	17.3	7.6	0.2–42.7
NHI, dimensionless	435	64.7	10.0	22.3–83.9	2083	63.4	8.6	9.6–86.6
Grain N, g m <sup>-2</sup>	435	10.1	4.0	1.0–21.7	2083	10.9	4.9	0.2–29.7
For Grain N calculation (Grain N = Yield × %Ng) <sup>§</sup>								
Grain N, g m <sup>-2</sup>	419	9.8	3.8	1.0–21.7	2075	11.0	4.9	0.1–29.6
%Ng, mg N g <sup>-1</sup>	419	13.6	2.8	6.0–22.9	2075	12.1	2.5	3.0–26.8
Yield, Mg ha <sup>-1</sup>	419	7.3	2.6	0.6–19.3	2075	8.9	3.3	0.3–16.9
For dry mass and N partitioning relationship <sup>¶</sup>								
%Ng, mg N g <sup>-1</sup>	523	13.3	2.5	7.9–22.9	1424	11.9	2.6	1.5–26.8
%Ns, mg N g <sup>-1</sup>	523	7.5	2.6	2.3–24.4	1424	6.9	2.2	1.0–21.1
Plant N, g m <sup>-2</sup>	523	14.5	5.1	3.3–38.7	1424	18.4	8.3	0.2–42.7
Biomass, g m <sup>-2</sup>	523	126	42	10.4–357	1424	176	64	2.8–313
Yield, Mg ha <sup>-1</sup>	523	6.8	2.3	0.6–19.3	1424	9.5	3.4	0.1–16.9
NHI, dimensionless	523	62.1	9.4	28.2–86.7	1424	63.4	9.1	9.6–83.9
Grain HI, dimensionless	523	47.6	6.9	12.0–63.0	1424	49.9	7.0	7.0–67.0

<sup>†</sup>Grain N, grain N uptake (at maturity); Reproductive N, reproductive-stage whole-plant N uptake; Remobilized N, reproductive-stage shoot N remobilization; Vegetative N, vegetative-stage whole-plant N uptake.

<sup>‡</sup>NHI, N harvest index.

<sup>§</sup>Yield, grain yield at maturity; %Ng, grain N concentration.

<sup>¶</sup>%Ns, stover N concentration; HI, harvest index.



**Figure 2. Coefficient of determination ( $r^2$ ) of grain N uptake (Grain N) (dependent variable) versus each parameter: the reproductive-stage whole-plant N uptake (Reproductive N) and the reproductive-stage shoot N remobilization (Remobilized N) (A), whole-plant N uptake (Plant N) and the N harvest index (NHI) at maturity (B), and grain yield (Yield) and grain N concentration (%Ng) at maturity (C) components for the Old Era (blue color: observations from 1940 to 1990) versus New Era (red color: research performed between 1991 and 2011). The proportion of the variance in Grain N explained by linear regression was documented by the  $r^2$  of the association between Grain N and each parameter measured (as documented by Duvick and Cassman [1999] and Sadras [2006]).**

## RESULTS AND DISCUSSION

### Study of the Grain Nitrogen Components

The Grain N parameter can be viewed as the final outcome of the interplay between (i) Remobilized N and Reproductive N, (ii) Plant N (Vegetative N plus Reproductive N) affected by the N partitioning efficiency (NHI), and iii) grain yield and %Ng.

For maize, the N remobilization process occurs preferentially from stem and older leaves (from bottom layers) sustaining the upper leaf %N layers (especially those around the ear leaf). In addition, the leaf senescence process is affected not only by N but also by water supply, light quality, and crowding stress, among other factors (Eik and Hanway, 1965; Pearson and Jacobs, 1987; Muchow and

**Table 2. Summary of reduced major axis regression analyses of log10-transformed data for all the relationships evaluated in this research.**

Log y vs. log x <sup>†</sup>	Factor <sup>‡</sup>	r <sup>2</sup>	n	Statistic for slopes (95% CI <sup>§</sup> )	Elevation (95% CI)	Statistic for intercepts (Wald Statistic)
Grain N vs. Reproductive N	Old and New Eras	0.28	525	0.54 (0.51 to 0.57)	0.61 (0.59 to 0.64)	All unique intercepts for different eras
Grain N vs. Remobilized N	Old and New Eras	0.28	525	0.49 (0.47 to 0.52)	0.69 (0.67 to 0.71)	All unique intercepts for different eras
Grain N vs. Plant N	Old and New Eras	0.93	2518	1.07 (1.06 to 1.08)	-0.36 (-0.37 to -0.34)	All unique intercepts for different eras
Grain N vs. NHI	Old and New Eras	0.14	2518	3.6 (3.5 to 3.7)	-4.6 (-4.8 to -4.4)	All unique intercepts for different eras
Grain N vs. Yield	Old and New Eras	0.85	2504	1.20 (1.19 to 1.22)	-0.10 (-0.11 to -0.09)	All unique intercepts for different eras
Grain N vs. %Ng	Old Era	0.17	419	2.1 (1.9 to 2.2)	-1.4 (-1.5 to -1.2)	Old vs. New (-2.0 vs. -1.8)
	New Era	0.40	2075	2.7 (2.6 to 2.8)	-1.9 (-2.0 to -1.8)	
Remobilized N vs. Vegetative N	Old and New Eras	0.66	525	1.35 (1.30 to 1.40)	-0.64 (-0.70 to -0.58)	All unique intercepts for different eras
Reproductive N vs. Remobilized N	Old and New Eras	0.14	525	-0.95 (-1.0 to -0.9)	1.90 (1.85 to 1.96)	All unique intercepts for different eras
1) Grain and 2) stover HI vs. Plant N	Old and New Eras	0.19	1947	1) 0.29 (0.28 to 0.30)	1.04 (1.03 to 1.06)	All unique intercepts for (grain and stover) different eras
		0.12	1947	2) -0.23 (-0.24 to -0.22)	2.21 (2.20 to 2.22)	
1) %Ng and 2) %Ns vs. Plant N	Old and New Eras	0.34	1947	1) 0.41 (0.40 to 0.42)	0.59 (0.58 to 0.61)	Grain vs. stover (0.49 vs. 0.24)
		0.34	1947	2) 0.59 (0.58 to 0.61)	0.12 (0.10 to 0.14)	

<sup>†</sup>Grain N, grain N uptake (at maturity); Reproductive N, reproductive-stage whole-plant N uptake; Remobilized N, reproductive-stage shoot N remobilization; Plant N, whole-plant N uptake (at maturity); NHI, N harvest index; Yield, grain yield at maturity; %Ng, grain N concentration; Vegetative N, vegetative-stage whole-plant N uptake; HI, harvest index; %Ns, stover N concentration.

<sup>‡</sup>Different levels separated for each factor within a specific relationship are related to significant differences among slopes ( $P < 0.05$ ), based on the likelihood ratio statistic test (slopes were not equal). For each factor, levels that did not present significant differences among slopes ( $P > 0.05$ ) were jointly analyzed, but intercepts were individually evaluated through the Wald statistic test (assuming common slope among levels).

<sup>§</sup>CI, confidence interval.

Carberry, 1989; Borrás et al., 2003). Greater Grain N was tightly linked to superior yields in response to different genetic backgrounds (Swank et al., 1982).

For the first Grain N approach highlighted in Fig. 1, both Remobilized N and Reproductive N ( $n = 525$ , ~35 studies; Table 1) contributed equally to the Grain N (~50%) for the Old Era while for the New Era, the Reproductive N accounted for approximately 56% of the Grain N. In the early 1950s, Hay et al. (1953) already documented similar contributions of the Remobilized N and Reproductive N to Grain N in varying genotypes. Notwithstanding the statistical dependence between Remobilized N and Reproductive N, the latter relationship has a physiological foundation (also reported by Coque and Gallais, 2007). Previous research already documented Remobilized N and Reproductive N variations among hybrids (Beauchamp et al., 1976; Below et al., 1981; Ta and Weiland, 1992; Rajcan and Tollenaar, 1999) and inbreds (Bertin and Gallais, 2000; Coque and Gallais, 2008). For the Old Era, both Remobilized N and Reproductive N accounted for approximately 22% of the Grain N variation (Fig. 2A;  $n = 213$ ). For the New Era, both variables accounted for a higher fraction of the Grain N variation (~26 for Reproductive N and 33% for Remobilized N, respectively;  $n = 312$ ; Fig. 2A).

Overall, the log-log analysis showed that the slopes (and also intercepts) for the associations between Grain N versus Reproductive N and Remobilized N were equivalent and did not change across eras (Table 2). The documented improvement in the New Era, therefore,

was mainly associated with reduced variation in both Reproductive N and Remobilized N in studies involving newer genotypes. Across eras, both Reproductive N and Remobilized N mean values were 50% lower at low grain yield levels (~3 vs. 6 g N m<sup>-2</sup> for reproductive N and ~2.5 vs. 5 g N m<sup>-2</sup> for Remobilized N for <6 Mg ha<sup>-1</sup> and >6 Mg ha<sup>-1</sup>, respectively). Thus, higher yield increments positively impacted both Reproductive N and Remobilized N parameters (bubble sizes; Supplemental Fig. S2A and S2B) in an approximately similar manner.

Regardless of eras (and genotypes), from the same database, Ciampitti and Vyn (2012) demonstrated that as the N supply improved (higher fertilizer N rate applied) the yields increased. Therefore, N supply is one of the factors regulating the Grain N parameter. The N status influence can be understood from the association between Plant N at silk emergence and yields at a macro scale (Ciampitti and Vyn, 2012) as well as at a micro scale for yield components (Ciampitti et al., 2012). The latter was also documented for wheat (*Triticum aestivum* L.) by Martre et al. (2006).

For the second Grain N approach, the association between the Plant N and NHI was investigated as an alternate approach to quantifying the Grain N sources ( $n = 2518$ , ~80 studies; Table 1; Fig. 2B). Since higher yields required more N supply, maintaining %Ng can be achieved via two possible scenarios: one by increasing Plant N or second by achieving greater remobilized N efficiency (NHI) (from stover to grain). The latter, NHI, is firmly linked to the grain HI as demonstrated by Ciampitti and Vyn (2012). In the current analysis, the Plant N and NHI variables

were unrelated ( $r^2 = 0.001$ ;  $P > 0.05$ ). Although Plant N explained most of the Grain N variation (as expected), the latter was higher for the New (91%) versus Old Era (84%). In addition, the allometric analysis (log-log scale) demonstrated that the models (slopes and intercepts) for the Grain N and Plant N relationship did not change across eras (Table 2). Nonetheless, the Grain N and Plant N quantities increased approximately 9 to 10% from Old to New Era (Table 1). In contrast to the relative importance of the Plant N, the NHI accounted for almost none on the Grain N variation (~1% for both eras). The minor NHI influence on the Grain N can be explained by the relative inflexibility in NHI at different Grain N levels (i.e., NHI plateaued at 65% when grain N ranged from 10 to 30 g N m<sup>-2</sup>; Supplemental Fig. S3B). As demonstrated for the Plant N and Grain N relationship, the Grain N and NHI association presented comparable allometric models (log-log analysis) across eras (Table 2). Overall, NHI declined approximately 2% from the Old to the New Era. To some extent, the latter can be accounted for by the addition of low yielding points (<1.5 Mg ha<sup>-1</sup>), which presented low NHI values (<30 units). The low NHI values were also associated to low grain HI levels, as summarized by Ciampitti and Vyn (2012). There seems to be little scope for improvement in maximum NHI in maize (Sinclair and Vadez, 2002), but minimum NHI should be further improved (as well as minimum grain HI) to increase the N and C partitioning efficiency at low yield levels.

In the third Grain N approach, the quantification of yield and %Ng as sources of Grain N was also pursued ( $n = 2494$ , ~80 studies; Table 1). The yield and %Ng were unrelated ( $r^2 = 0.02$ ;  $P > 0.05$ ). An expected high autocorrelation was observed between yield and Grain N, reflected in the proportion of the Grain N variation explained by yield (>75%; Fig. 2C). In addition, yield accounted for a greater fraction of the Grain N variation for the New Era (75% in the Old vs. 83% in the New Era). The allometric model (slopes and intercepts) for Grain N and yield relationship did not show any difference across eras (Table 2). Yield was modified more than Grain N from Old to New Eras (~22% for yield vs. 12% for Grain N; Table 1). In addition, the increase in biomass and grain HI were large relative to the changes in the Plant N and NHI; thus, the increment resulted in the yield parameter being favored concomitantly with a reduction in the %Ng (~12% from Old to New Era; Table 1). Similar trends in %Ng were documented not only for maize but also for wheat and soybean [*Glycine max* (L.) Merr.] crops (Duvick and Cassman, 1999; Wilcox and Cavins, 1995; Cober and Voldeng, 2000; Triboi et al., 2006). For this research, the %Ng accounted for 15% of the Grain N variability for the Old Era but 35% for the New Era (Fig. 2C). The gain in proportion of variability explained by %Ng was the outcome of a more consistently positive Grain N trend (less variation) as %Ng increased (Supplemental Fig.

S4B). The allometric analysis demonstrated that the slopes and intercepts were significantly different for the New and Old Eras (Table 2) but also that at comparable Grain N levels, the %Ng was greater for the Old versus the New Eras (a dilution effect was observed in that New Era yields were superior to Old Era yields when compared at similar %Ng). The latter confirmed the uncoupled stoichiometric improvement in the ratio between C and N, with larger accumulation and effectiveness in partitioning (stover to grain) for C (biomass) than for N (Triboi and Triboi-Blondel, 2002).

### Trade-Off Mechanisms between Vegetative-Stage and Reproductive-Stage Whole-Plant N Uptake and Reproductive-Stage Shoot N Remobilization

A remaining and critical topic related to the N process is the understanding of the fundamental contribution of the Remobilized N and Reproductive N processes to the Grain N. Pan et al. (1986) clearly stated that even when superior Remobilized N is a highly desirable trait, a trade-off was reflected as a decline in Reproductive N. Furthermore, the same authors recognized that NUE improvement will result from a more balanced contribution of both N processes. The latter was also investigated by Gallais and Coque (2005). In our research, the negative association documented between the Reproductive N and Remobilized N can be physiologically explained from the positive linear relationship between the Remobilized N and the Vegetative N (Fig. 3A and 3B). The latter association can be visualized, for example, under low Reproductive N when Grain N is relying on the Vegetative N and on the effectiveness and onset timing of the Remobilized N. The previous trade-off was observed not only for maize but also for wheat (Kichey et al., 2007; Bogard et al., 2010).

The Remobilized N and Vegetative N relationship was strong ( $r^2 = 0.67$ ;  $n = 525$ ), as reported by Coque and Gallais (2007), and had similar slope (0.6) as also documented by Pan et al. (1984, 1986, 1995) for prolific and nonprolific materials and by Ciampitti (2012) for modern single-eared hybrids. The allometric analysis (log-log scale) did not reflect changes across eras for the slopes and intercepts of the Remobilized N and Vegetative N relationship (Table 2). To explain the previous association, three hypotheses were postulated by Coque and Gallais (2007), one related to soil processes and the other two related to physiological mechanisms. The first hypothesized that under low Vegetative N, more Reproductive N is taken up during the seed filling. The latter, even when plausible, seems too simplistic to be the main cause but cannot be answered with this review. Nonetheless, data reported from wheat demonstrated that even when N supply after anthesis was high, the Reproductive N was unaltered (Kichey et al., 2007; Bancal, 2009). Still, different research studies show contrasting outcomes as related to the

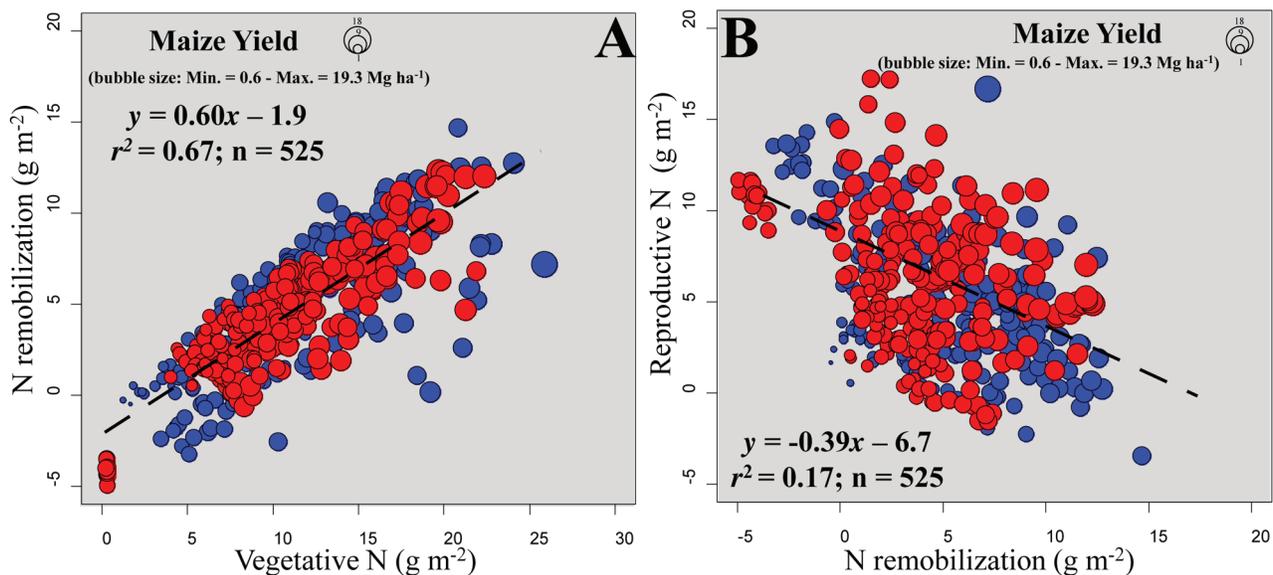


Figure 3. Reproductive-stage shoot N remobilization (Remobilized N), from silking till maturity, versus vegetative-stage whole-plant N uptake (Vegetative N) (A), and reproductive-stage whole-plant N uptake (Reproductive N) versus Remobilized N (B) for the Old (blue color: observations from 1940 to 1990;  $n = 213$ ) versus New Eras (red color: observations from 1991 and 2011;  $n = 312$ ). For both panels A and B, the different sizes of the bubbles correspond to grain yield (Yield) values ranging from 0.6 to 19.3 Mg ha<sup>-1</sup>. A unified slope was fitted to represent both Old and New Era relationships.

effect of the N fertilization during seed filling on Grain N (Jung et al., 1972; Below et al., 1984; Binder et al., 2000). The other two approaches are related to physiological modifications. One approach is linked to the yield level explored, “mechanical influence” of sink limitation (Coque and Gallais, 2007). As the sink strength is reduced (e.g., fewer kernels per plant) the Remobilized N is proportionally more affected than the Reproductive N, thus uncoupling the ratio and trade-off strength (direction) between the latter two parameters. The second physiological hypothesis is linked to the positive relationship between N content and photosynthesis processes. Under any reproductive stress, the photosynthetic functionality is affected, enhancing the leaf senescence and Remobilized N (Triboi and Triboi-Blondel, 2002) but reducing the Reproductive N. The opposite (low Remobilized N—early in the grain filling period—and high Reproductive N) is expected under nonstress conditions.

To shed some light over these hypotheses, the dataset was divided by the yield level achieved at maturity into two groups (across eras), from approximately 1 to 8 Mg ha<sup>-1</sup> (low yield) and from 8.1 to 19.3 Mg ha<sup>-1</sup> (high yield) (different bubble sizes represent yield levels; Fig. 3A). Briefly, comparing the high yield (mean yield = 10.5 Mg ha<sup>-1</sup>;  $n = 313$ ) versus low yield (average yield = 6.0 Mg ha<sup>-1</sup>;  $n = 212$ ), improvements of 93% in the Remobilized N (from 2.9 vs. 5.6 g N m<sup>-2</sup>), 71% in the Vegetative N (from 7.6 vs. 13.0 g N m<sup>-2</sup>), and 45% in the Reproductive N (from 4.4 vs. 6.4 g N m<sup>-2</sup>) were recorded. Thus, superior yield promoted a greater change in the Remobilized N (and Vegetative N) than in the Reproductive N. For the Reproductive N and

Remobilized N relationship, greater yield (larger bubble sizes) represented an improvement in the “population mean” without modifying the trade-off direction (negative slope; Fig. 3B). In addition, the Reproductive N and Remobilized N ratios averaged approximately 2:1 units for the low yield group and approximately 1:1 unit for the high yield although the ratio variation was large at approximately 300 to 400% (calculated as [maximum – minimum/minimum] × 100; Sadras, 2007). Similar negative associations between Reproductive N and Remobilized N were documented by Pan et al. (1984) at plant level, with lower Reproductive N as the prolificacy trait (more ears per plant) was increasingly expressed. For single-eared modern hybrids, Ciampitti (2012) observed superior “late” Remobilized N (R3 to R6 period) or longer Reproductive N (later in grain development) as the per-plant yield increased. Uncoupling the potential causes of the trade-off, under superior yield (at-community area) or ear strength (at-plant scale) if the current N uptake is outweighed by the high N demand, the Remobilized N should be accelerated (Triboi and Triboi-Blondel, 2002).

Further proof of the role of sink strength in promoting N uptake arises out of strong relationships at community and at individual plant scales between the Vegetative N and yield (Ciampitti et al., 2012; Ciampitti and Vyn, 2012). Vegetative-stage whole-plant N uptake status influences on maize yield and its components were previously reported (Lemcoff and Loomis, 1986; Plénet and Cruz, 1997; Uhart and Andrade, 1995b). Similar trends were also documented for wheat by Bancal (2009), suggesting that the sink strength is regulated, to some extent, by the source activity (e.g.,

Vegetative N) at the anthesis stage (Sinclair and Jamieson, 2006). When the sink was artificially constrained in wheat (Mi et al., 2001; Bancal, 2009) and maize (Pan et al., 1995), a disruption in the Remobilized N and Vegetative N association resulted in lower N remobilization efficiency. Support for this phenomenon in maize was reported by Uhart and Andrade (1995a); they found that the Remobilized N was negatively associated to the source:sink ratio during grain filling period (lower Remobilized N was related to superior source-sink). Under source-limiting environments (e.g., shading during vegetative period) greater Remobilized N was documented (Reed et al., 1988). Therefore, for maize two scenarios can be clearly distinguished: (i) under “superior yields (nonstress),” Remobilized N is primarily driven by the vegetative N (i.e., more source driven) and (ii) under “constrained environments (biotic and/or abiotic stresses),” the proportionality between Remobilized N and Vegetative N is disrupted.

Based on the results above, the two physiological hypotheses proposed by Coque and Gallais (2007) can be reformulated into just one postulation: at a plant level, superior yield potential (larger size and number of kernels) increases the ear N demand (due to the close association between ear C and N) (Ciampitti and Vyn, 2011) and under a nonstress scenario, increases in Reproductive N are expected to occur as a consequence of longer photosynthetic activity (He et al., 2005), and “early” Remobilized N (V15–R3 period) would be minimized in favor of a “late” Remobilized N (R3–R6 period [“early” vs. “late” Remobilized N are negatively correlated; Ciampitti, 2012]). Therefore, Grain N can be portrayed as the final outcome of a series of complex interrelationships. Decades ago, Pan et al. (1984) already stressed the importance of a high Remobilized N but, because of the acknowledged downside effects on the Reproductive N, they suggested the use of prolific hybrids to enhance the Vegetative N (also summarized by Ciampitti and Vyn, 2012). In present times, the prolific genotypes can be further enhanced with biotechnology to increase the synchrony between primary and secondary ears, sustain high reproductive N (e.g., “stay-green” trait), and ideally be capable of rapid “late” Remobilized N after midgrain filling period. Achieving such goals would help mitigate the trade-off between Reproductive N and Remobilized N and be one of the potential avenues to further improve the achievable yield at the community level (and close the gaps with the potential yields).

### Partitioning of Dry Mass and Nitrogen at Varying Plant Nitrogen Levels at Maturity

Dry mass partitioning was analyzed by determination of changes in stover and grain dry mass relative to variations in Plant N at maturity (Fig. 4A). For the entire pertinent dataset ( $n = 1947$ , ~80 studies; Table 1), two different linear plateau associations were fitted for each grain and stover dry mass

fractions (both expressed as a ratio to biomass). An inflection point (after which the linear trend changed to a plateau model) was established at the Plant N of  $15 \text{ g N m}^{-2}$  (grain and stover plateau level at ~0.50), and grain HI declined as the Plant N decreased. Similar results were documented by Linden et al. (2000), Massignam et al. (2009), and Ciampitti (2012) but with different thresholds for Plant N and plateau levels for grain HI. The latter authors showed superior Plant N thresholds and grain HI plateau levels as yield improved. Although the latter grain HI and Plant N association is expressed across eras, the mean yield improved from Old to New Eras (from  $6.8$  to  $9.2 \text{ Mg ha}^{-1}$ ; Table 1) mostly because of superior whole-plant biomass with some additional (minor) improvement in the grain HI (~5%; Table 1). The trend documented in the dry mass components is, to a large extent (even more under low grain HI), associated with the yield levels and with a presumably larger impact (reduction) over the reproductive rather than vegetative mass from stress conditions (N deficiency, drought, and heat stresses, among others). In general, low grain HI ( $<0.3$ ;  $n = 30$ ) was associated with low yield levels ( $<3 \text{ Mg ha}^{-1}$ ) and sink limitations (primarily driven by restrictions in kernel number) presumably promoted by biotic or abiotic stresses. Additionally, as expressed by Triboi and Triboi-Blondel (2002), under an early kernel abortion (but under a posterior nonstress scenario) the grain HI and NHI can become very low (C and N influxes constrained by kernel number and size).

Overall, the latter paragraph provides support to the theory that superior yield levels (for a given genotype and environment under a nonlimiting sink scenario) are likely to show greater grain HI and, concomitantly, NHI (as documented by Ciampitti and Vyn, 2012). In addition to the era changes in biomass at maturity, a greater biomass at silking was documented for the New Era (Ciampitti and Vyn, 2012). In an earlier era study with a few hybrids, Tollenaar et al. (1992) demonstrated that the biomass accumulation during the bracketing silking period was an important change related to the maize yield improvement. Notwithstanding the changes documented in biomass, the models fitted for grain and stover HIs as compared to plant N (Fig. 4A) did not change between eras (Table 2). Thus, even when the improvement in maize biomass, grain HI, and yield is clearly demonstrated, the proportionality in the ratio (e.g., between grain HI and NHI; Ciampitti and Vyn, 2012) was equivalent for Old and New Era hybrids.

To fully understand the N partitioning within the plant, the study of the differential trends in the plant %N components was also evaluated for the stover and grain fractions (Fig. 4B). Gains in the %Ns and %Ng followed a similar stoichiometry ratio as the Plant N increased at maturity. For each plant component, the comparison across eras did not reveal changes in the allometric models (slopes and intercepts) and therefore, equivalent models can be used for grain and stover as compared to

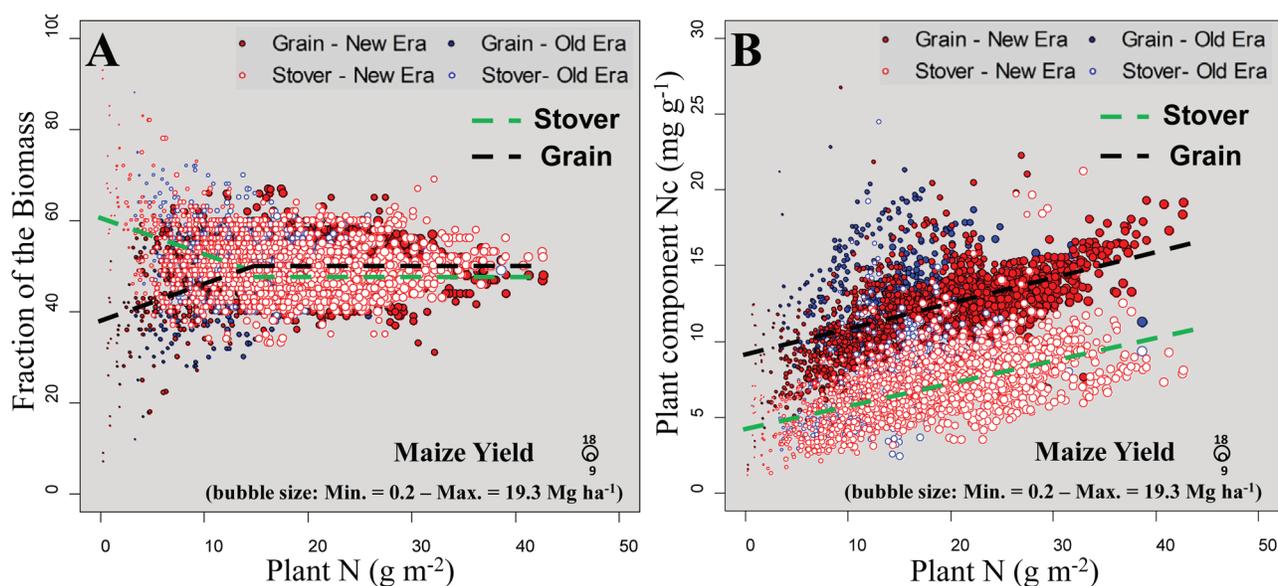


Figure 4. Fraction of the whole-plant biomass (Biomass) (A) and plant component N concentration (Nc) (B) for the grain N concentration (%Ng) and stover N concentration (%Ns) versus the whole-plant N uptake (Plant N) all determined at maturity for the Old Era (blue color: observations from 1940–1990;  $n = 523$ ) versus New Era (red color: research from 1991–2011;  $n = 1424$ ). For both panels A and B, different bubble sizes correspond to yield values ranging from 0.2 to 19.3 Mg ha<sup>-1</sup>. For panel A, the equation for the stover biomass fraction was  $y = -0.8x + 61$  with plateau at  $y = 49$ ,  $r^2 = 0.15$ , and  $n = 1947$ ; for the grain component the linear plateau was expressed as  $y = 0.8x + 39$  with plateau at  $y = 51$ ,  $r^2 = 0.15$ , and  $n = 1947$  for both plant N threshold level (inflection point in the linear-plateau association  $\sim 15$  g m<sup>-2</sup>). For panel B, for %Ng the equation was  $y = 0.17x + 9.2$ ,  $r^2 = 0.26$ , and  $n = 1947$  while for the %Ns the fitted equation was  $y = 0.15x + 4.5$ ,  $r^2 = 0.25$ , and  $n = 1947$ .

Plant N at maturity for the Old and New Eras (Table 2). Nonetheless, the %Ng and %Ns declined from Old to the New Era (Table 1) in similar proportions ( $\sim 11.7\%$  for %Ng and  $\sim 8.7\%$  for %Ns) associated with a concomitant increase in yield (from  $\sim 7$  to  $9$  Mg ha<sup>-1</sup> from Old to New Era). A similar decline in %Ng was previously recorded by Duvick and Cassman (1999), with an absolute %Ng drop around  $-1.5$  mg g<sup>-1</sup> from approximately 1930 to 1990 (as compared to our research:  $-1.4$  mg g<sup>-1</sup> from  $\sim 1940$  to 2011; Table 1). Trend-line slopes for %Ng ( $\sim 0.17$ ) and %Ns ( $\sim 0.15$ ) were very similar with different intercepts (9.2 and 4.5 for %Ng and %Ns, respectively). Similar results (for much smaller databases) were documented by Ciampitti (2012) and can be calculated from Wortmann et al. (2009, 2011) and Setiyono et al. (2010). It should be properly acknowledged that the latter studies are included in our database, but together they represent just approximately 25% of the entire dataset. Essentially equivalent slopes and intercepts for %Ng and %Ns ( $\%Ng = 0.18 - 0.17x + 7.3 - 8.7$  and  $\%Ns = 0.17x + 3.9 - 4.2$ ) response to N occurred previously in irrigated (Nebraska) and rainfed (Indiana; Ciampitti, 2012) maize studies. Furthermore, across eras, means for %Ng and %Ns in this study (12 and 7 mg g<sup>-1</sup>) were similar to the previously mentioned studies (12 and 8 mg g<sup>-1</sup>) across all the different plant N levels. However, despite the clear proportionality between %Ng and %Ns components, the variation documented ( $r^2 = 0.3$  to  $0.4$ ) reveals the limitation in using universal N partitioning

parameters between the grain and stover plant components. Nonetheless, the latter is a clear advancement in the physiological knowledge related to the N partitioning in diverse N status situations, which might be useful to incorporate into simulation models to improve modeling and to obtain more accurate predictions.

## CONCLUSIONS AND PROSPECTS

The review of available studies since 1940 highlighted the dominant changes over time in grain N sources and enabled answers to the questions posed earlier. The answer to the first research question (Are the parameters [Remobilized N, Vegetative N, Reproductive N, %Ng, and %Ns] related to Grain N changing [in quantity, direction {associated with negative or positive slope values}, and/or strength {related to the  $r^2$  values}] over time in response to continued maize improvement processes?) is that the %Ng was the main parameter that has changed over time (New Era had lower %Ng and accounted for more Grain N variation and a higher isometric ratio with Grain N). The Grain N was unrelated to NHI, a result that demonstrated the conservative NHI property.

For the second question (Is the time factor changing the direction and strength of the Reproductive N and Remobilized N? Are there any clues as to how source and sink effects over Remobilized N, Vegetative N, and Reproductive N might be decorrelated?) our answer is that the time did not affect the direction and strength of the

association, but an improvement in the proportion of Grain N variation accounted for by both Reproductive N and Remobilized N. In absolute terms, Reproductive N mean values were higher and Remobilized N means were lower for the New Era while both factors contributed equally to the Grain N for the Old Era. Source (Vegetative N) acted as a primary driving force for the Remobilized N process, but relative sink strength also seemed to have a regulating role in this complex interplay, presumably because higher ear N demand (not satisfied by the Reproductive N) enhances the N remobilization process (greater Remobilized N).

Finally, the answer for the third research question (Is the N partitioning [grain vs. stover] changing over time? Is there any isometry between grain and stover mass and N partitioning?) is that the grain N and nongrain N (stover N) partitioning at maturity did not change over time and across eras, and analogous gains in %N for the grain and stover N were documented.

In recent decades, improvements in maize NUE were attained largely as by-products of continuous yield progress and lower %Ng. Simultaneous improvements in both C and N dynamics are required, hopefully with more intentional physiology-based interventions, to overcome the future challenges in achieving greater Reproductive N and higher efficiency of Remobilized N in maize. Specific suggestions at the plant level include reducing kernel abortion (more kernels and larger kernel sizes increase ear demand for C and N), increasing Plant N, and expanding the duration of grain fill (sustaining functional photosynthesis by delaying senescence). Specific suggestions at the plant community scale are reducing the trade-off between Vegetative N and Remobilized N versus Reproductive N (i.e., uncouple source-sink effects), improving grain HI (also NHI) under low yield levels, increasing the capacity to accumulate vegetative-stage N (with emphasis on the vegetative N as the primary N source for ear N demand), and continuing the improvement in overall biomass (in both vegetative and reproductive phases). Future research should attempt to elucidate avenues related to improvements in N trade-off mechanisms, which can be potentially explored through the use of prolific materials (inherently larger Vegetative N and Remobilized N capacities) so long as ear synchrony and stalk strength are not compromised and leaves of maize hybrids with “functional stay-green trait” are sufficiently capable of sustaining high Reproductive N rates.

## Supplemental Information Available

Supplemental material is included with this manuscript.

Supplemental Figure S1. Mean, median, and 25 to 75% quartiles (IQR, 25%Q, and 75%Q; 50% confidence interval of all observations) of the reproductive-stage shoot N remobilization (Remobilized N) (A), reproductive-stage whole-plant N uptake (Reproductive N) (B), vegetative-stage whole-plant N uptake (Vegetative N) (C), grain

N concentration (%Ng) (D) and stover N concentration (%Ns) (E), and grain N uptake (Grain N) (F) at maturity in studies conducted from 1940 to 2011.

Supplemental Figure S2. Grain N uptake (Grain N) versus reproductive-stage whole-plant N uptake (Reproductive N) (A) and reproductive-stage shoot N remobilization (Remobilized N) (B) all determined from the time interval between silk emergence till maturity for the Old Era (blue color: observations from 1940–1990;  $n = 213$ ) versus New Era (red color: research from 1991–2011;  $n = 312$ ). For both panels A and B, different bubble sizes correspond to whole-plant N uptake (Plant N) values ranging from 3.3 to 38.7 g m<sup>-2</sup>. For panels A and B, both Old and New Eras shared the same slope and intercepts.

Supplemental Figure S3. Grain N uptake (Grain N) versus whole-plant N uptake (Plant N) (A) and N harvest index (NHI) (B) all determined at maturity for the Old Era (blue color: observations from 1940–1990;  $n = 435$ ) versus New Era (red color: research from 1991–2011;  $n = 2083$ ). For both panels A and B, different bubble sizes correspond to plant N values ranging from 0.7 to 42.7 g m<sup>-2</sup>. For panel A, both Old and New Eras shared the same slope (0.61) with different intercepts. For panel B, each era dataset shared the same NHI and plateau level and grain N threshold value, with slightly different intercepts for the linear section of the linear-plateau models.

Supplemental Figure S4. Grain N uptake (Grain N) versus maize grain yield (Yield) (A) and grain N concentration (%Ng) (B) all determined at maturity for the Old Era (blue color: observations from 1940–1990;  $n = 419$ ) versus New Era (red color: research from 1991–2011;  $n = 2075$ ). For both panels A and B, different bubble sizes correspond to whole-plant N uptake (Plant N) values ranging from 0.7 to 42.7 g m<sup>-2</sup>. For panel A, both Old and New Eras shared the same slope (1.36) with different intercepts. For panel B, each era dataset resulted in different equations (slopes and intercepts) for the association between Grain N and %Ng.

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