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Heterosis for Nitrogen Use Efficiency of Maize Hybrids Enhanced over Decades in China

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Abstract: The nitrogen use efficiency (NUE) of modern maize hybrids has been greatly improved, but for decades, little was known on whether the NUE heterosis of maize hybrids had increased. A two-year field study was conducted on eight maize hybrids and their parental inbred lines from the 1970s to 2000s under two N levels (0, 150 kg N ha^{-1}). These were the most popular hybrids in China's main maize-growing areas at the time. The results showed that the yield of the maize hybrids increased significantly at an average rate of 37.5% every 10 years. The evolution of NUE heterosis was mainly related to the increased kernel number per ear. The absolute NUE heterosis (AH_{NUE}) and the mid-parent NUE heterosis (MPH_{NUE}) of maize hybrids increased by 151.4% and 76.4% in the past four decades, or an average rate of 2.11 kg kg^{-1} and 19.1% every 10 years. Based on the coefficient of determination, the contribution of the mid-parent nitrogen internal efficiency heterosis (MPH_{NIE}) to MPH_{NUE} (43–57%) was significantly higher than that of the mid-parent nitrogen recovery efficiency heterosis (MPH_{NRE}) (19–32%), indicating that the evolution of maize NUE heterosis was mainly derived from its NIE heterosis evolution. The increase of NIE heterosis in the past 40 years was closely related to the increased heterosis of kernel number per ear, the pre-silking N accumulation and the post-silking N remobilization. Therefore, the enhancement of maize NUE heterosis can be attributed to (i) heterosis improvement in post-silking N remobilization, which results mainly from greater heterosis in pre-silking N accumulation; (ii) heterosis improvement in carbon and nitrogen sink capacity, which exhibit as heterosis enhancement in grain yield and grain nitrogen concentration. To further improve yield and NUE, the pre-silking N and carbon accumulation and post-silking N remobilization should both continue to increase in maize breeding. Our results will provide new insights into NUE and help breeders select genotypes with both higher yields and higher NUE for the future.

Keywords: maize; heterosis; yield; nitrogen use efficiency; decades

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Citation: Li, R.; Gao, J.; Li, Y.; Yu, S.; Wang, Z. Heterosis for Nitrogen Use Efficiency of Maize Hybrids Enhanced over Decades in China. *Agriculture* **2022**, *12*, 764. https://doi.org/10.3390/agriculture12060764

Academic Editor: Andreas S. Pacholski

Received: 20 April 2022
Accepted: 25 May 2022
Published: 27 May 2022

1. Introduction

Maize is the largest grain cereal crops in China, accounting for more than one-third of Chinese cereal production, and plays an important role in ensuring food security [1–3]. According to data from the National Bureau of Statistics, as of 2019, area coverage and yield of maize in China were 4.1×10^7 hectares and 2.6×10^8 t, respectively [3]. Because of over-application of N, annual bulk deposition of N in maize production increased by approximately 8 kg N per ha from the 1980s (13.2 kg N per ha) to the 2000s (21.1 kg N per ha) [4]. The main challenges in maize production were to limit the excessive use of nitrogen without reducing grain yield [5]. Exploring the potential to increase maize nitrogen use efficiency (NUE) is an important means to solve this problem. Maize NUE can be divided into nitrogen recovery efficiency (NRE) and nitrogen internal efficiency (NIE) (NUE = NRE × NIE) [6]. NRE reflects the ability of the plant to take up nitrogen from the soil, and NIE refers to the ability of the plant to utilize the absorbed nitrogen to produce grain yield [6–9].

In the past few decades, global maize grain yields have increased significantly, and about 50% of the increase was due to genetic improvement such as germoplasms [10], tropical maize [11], etc., and 50% to management practices including N application [12–17], irrigation, increasing plant population, chemical control of diseases and pests [18], while NUE has lagged far behind this increase due to excessive nitrogen application. The NUE itself and overall N stress tolerance of modern maize hybrids seem to have been improved [7]. However, when modern hybrids were grown under low nitrogen supplies, the loss of grain yield was still large [8]. Previous studies showed that the evaluation of maize NUE components promoted the understanding of individual physiological mechanisms and biochemical processes (such as nitrogen absorption, assimilation and remobilization) [6,19]. In order to achieve high nitrogen utilization, some authors observed that NRE components were more important in a high nitrogen supply environment, whereas the NIE components were more essential in low N availability environments [20,21]. The results showed that new genotypes with higher NUE, higher total biomass (BM) accumulation and higher N uptake at the post-silking stage. The high N use efficiency of the new genotypes were mainly related to the decrease of grain % N and increase of NIE [6].

Heterosis has led to a noticeable steady increase in crop yields over the past 90 years [13], and this mysterious phenomenon has been studied subsequently [22]. From the 1930s to the 1990s, the grain yield per hectare of maize in the United States of America increased by about 120%, while the grain nitrogen concentration dropped by about 35% [23]. In this progress, relative heterosis for maize grain yield has not increased and, in some cases, is slightly reduced. The genetic component of yield improvement has been mainly attributed to increased stress tolerance or drought [24]. Genotype groups (such as hybrids and inbred lines) differ not only under optimal growth conditions [25], but also their grain yield and physiological determinants in response to nitrogen [26,27]. Our previous study showed that maize hybrids displayed a significant heterosis for NUE, and about 52% was due to the heterosis in NIE. Heterosis for NIE, and thereby, for NUE in maize was ascribed to (i) an earlier establishment of pre-anthesis source for N accumulation, which phenotypically exhibited as a faster leaf appearance rate with higher maximum LAI and photosynthetic nitrogen use efficiency; (ii) a larger amount of N being remobilized from the vegetative tissues, especially from leaves, during the grain filling. Phenotypically, there were notably a rapid reduction in post-anthesis specific weights of leaves and stalk, but with maintained functionally stay-green ear leaves; and (iii) a higher productive efficiency per unit grain N, which were characterized by a reduced grain N concentration and enhanced sink strength. However, whether the heterosis for NUE of maize has increased or not was examined.

Modern maize breeding is usually carried out under adequate nitrogen input and fertile soil, and breeders are more concerned about nitrogen uptake, maintaining green and grain yield [28,29]. The differences in plant traits between nitrogen use efficiency (NUE) and its heterosis in maize hybrids are largely ignored. It would be interesting to study whether different varieties have any effect on the response of nitrogen application over the decades [30]. The objectives of our present study were to evaluate (i) the change of maize hybrid NUE heterosis in the past four decades and (ii) the physiological basis of NUE heterosis evolution characteristics.

2. Materials and Methods

2.1. Plant Materials and Experimental Location

A two-year field study was conducted under irrigated conditions on a farm near Tumed Right Banner County (40°32′22.23″ N, 110°30′37.58″ E, 996 m a.s.l.), Inner Mongolia Autonomous Region, China. The effective accumulated temperature and sun radiation during the spring maize growth periods during were 2986 °C d and 3699 MJ m^{-2}, respectively, in 2015 and 3119 °C d and 3660 MJ m^{-2}, respectively, in 2016. The effective cumulative temperature refers to the sum of the daily average temperature above 10 °C. The growth seasons rainfall was 358.5 mm in 2015 and 423.1 mm in 2016. The field has a sandy loam texture, with the following characteristics in the upper 30 cm soil layer: 16.5 g kg^{-1} organic matter, 19.8 mg kg^{-1} alkaline nitrogen (N), 16.5 mg kg^{-1} available phosphorous (P), 90.4 mg kg^{-1} available potassium (K) and pH 7.9 in 2015; and, respectively, 24.5 g kg^{-1}, 21.2 mg kg^{-1}, 26.7 mg kg^{-1}, 120.4 mg kg^{-1} and 7.5 in 2016. Maize was planted in late April to early May and harvested about late September to early October. To ensure adequate plant growth, 75 kg ha^{-1} P_2O_5, 30 kg ha^{-1} N, (diammonium phosphate, containing 46% P_2O_5 and 18% N) and 45 kg ha^{-1} K_2O (potassium sulfate, containing 50% K_2O) were applied annually as starting fertilizers. These were incorporated into 0–15 cm of soil by rotary tillage before planting.

2.2. Experimental Design

Eight maize hybrids and their parental inbred lines were used in this study (Table 1). These hybrids were the most popular varieties from the 1970s to 2010s in China's main maize producing areas. The experiment was treated with two levels of N, 0 (0 N) and 150 kg ha^{-1} (150 N), arranged in a randomized complete block design. Each plot consisted of ten rows of maize, with a row spacing of 0.6 m and row length of 5 m with three replications. Seeds dressing were used by 60% Imidacloprid (Gaoqiao, Bayer, Germany) before sowing. Seeds were seeded mechanized using a maize precision seeder (MASCHIO MTR, Qingdao, China) at an average depth of 5.0 cm. At the V3 stage, seeds were thinned to 75,000 plants ha^{-1}. At the V6 stage, 150 kg N ha^{-1} was applied in the 150 N plots in both years. In total, 50 mm of irrigation water at V10 and R1 stages was supplied through food irrigation in both years. Other cultivation practices, such as removal of weeds and chemical control of diseases and pests, were performed using conventional approaches.

Table 1. Details of the maize hybrids used in different decades.

Hybrids	Decade	Parental Combination	Breeding/Provide Institution	Year of Hybrid Release
Jidan 101	1970s	Ji 63 × M 14	Jilin Academy of Agricultural Sciences	1974
Zhongdan 2		Mo 17 × Zi 330	Chinese Academy of Agricultural Sciences	1973
Danyu 13	1980s	Mo 17 × E 28	Dandong Academy of Agricultural Sciences	1979
Nongda 60		Shen 5003 × Zong 31	China Agricultural University	1985
Yedan 13	1990s	Ye 478 × Dan 340	Laizhou Academy of Agricultural Sciences	1989
Nongda 108		X 178 × HangC	China Agricultural University	1991
Xianyu 335	2000s	PH6WC × PH4CV	Seed-Pioneer Tieling Co., Ltd.	2000
Zhengdan 958		Zheng 58 × Chang 7–2	Henan Academy of Agricultural Sciences	1996

2.3. Sampling and Measurements

2.3.1. Plant Sampling

At silking (R1) and maturity stages (R6), three representative plants with uniform growth were cut at the stem base in each plot each year. The shoot was separated into leaves, stalks (leaf sheaths, tassel, bracts and either ear-shoots at R1 or cob at R6) and grain. All samples were heat-treated at 105 °C for 30 min, and dried at 70 °C to a constant weight to obtain the dry matter (DM). The samples were then ground into fine powder for further analysis [31].

At physiological maturity, two rows of plants were harvested for determining the yield. The grains were dried and weighed. Grain yield was reported on a standard moisture content of 14%. The yield components, including the number of ears ha^{-1}, kernels per ear and 1000-kernel weight, were recorded.

2.3.2. Nitrogen and Heterosis Indices

Nitrogen concentration was measured with an Auto Analyzer III (SEAL, Hamburg, Germany). N content of each component was calculated as the product of N concentration by its DM (kg ha^{-1}). Based on these measurements, the NUE indices were calculated according to the following equations [5,31,32].

GY_{fert} is the grain yield of a treatment receiving 150 kg N ha^{-1} fertilizer, and GY_{unfert} is the grain yield of 0 N treatment.

$$NUE = (GY_{fert} - GY_{unfert})/\text{N fertilizer applied} \tag{1}$$

$$NRE = \left(Nupt_{fert} - Nupt_{unfert}\right)/\text{N fertilizer applied} \tag{2}$$

$$NIE = (GY_{fert} - GY_{unfert})/\left(Nupt_{fert} - Nupt_{unfert}\right) \tag{3}$$

$$\text{Plant N} = \text{plant N content at maturity} \tag{4}$$

$$\text{Grain N} = \text{grain N content at maturity} \tag{5}$$

$$Veg_N = \text{total plant N content at silking} \tag{6}$$

$$Rep_N = \text{N content at maturity} - \text{N content at silking} \tag{7}$$

$$\text{Rem N} = \text{vegetative N content at silking} - \text{vegetative N at maturity} \tag{8}$$

$$NHI = \text{grain nitrogen accumulation at maturity}/\text{nitrogen accumulation at maturity} \tag{9}$$

$$HI = \text{grain weight at maturity}/\text{biomass weight at maturity} \tag{10}$$

Absolutely heterosis (AH) was calculated with the following formula:

$$AH = F_1 - MP \tag{11}$$

Mid-parent heterosis (MPH) is the heterosis of a hybrid relative to the average value of the parent:

$$MPH = [(F_1 - MP)/MP] \times 100 \tag{12}$$

where F_1 is the mean of single-cross hybrids and MP is the mid-parental value. The statistical significance of the heterosis value of each trait was determined, as well as its comparison between N treatments by *t*-test [33].

2.4. Statistical Analysis

The experimental data were statistically analyzed by ANOVA using the SPSS 21.0 software (SPSS Institute Inc, Chicago, IL, USA). Analysis of variance test was done based on RCBD design. The effects and interaction of the two main factors: nitrogen and genotype, on the different parameters were analyzed through a two-way analysis of variance (ANOVA). Correlations between variables were also examined with Pearson correlation coefficients and considered statistically significant at $p < 0.05$. The figures were constructed using the SigmaPlot 12.5 software (Systat Sofware Inc., San Jose, CA, USA).

3. Results

3.1. Heterotic Performance of Yield and Yield Components of Hybrids

The yield of hybrids in each decade were significantly higher under 150 N than under 0 N, with an average increasing a rate of 0.7 t·ha^{-1} per 10 years. At 150 N, the number of grains per ear of hybrids in each decade showed a significant increase (Table 2, Figure 1), from a stable increment of 16–18% in the 1970s, 1980s and 1990s to 39.6% in the 2000s (Table 2). Nitrogen application did not affect the 1000-kernel weight (TKW) of the 1970s and 1980s hybrids, but significantly increased TKW by 7.3% of the 1990s hybrids and by 6.6% of the 2000s hybrids. It showed that during the 20 years from 1970s to 1980s, the yield gain (YG) of hybrids were mainly due to the gain in grain number per ear (KnG), while the gains in the recent 20 years from 1990s to 2000s were attributed by the increase of the grain number per ear (GN) and TKW.

Table 2. Effect of N applications on yield and yield components of hybrids.

Index	**Grain Yield ($t{\cdot}ha^{-1}$)**		**Yield Gain ($t{\cdot}ha^{-1}$)**	**Grain Number per Ear**		**Grain Number Gain**	**1000-Kernel Weight (g)**		**1000-Kernel Weight Gain (g)**
	150 N	**0 N**		**150 N**	**0 N**		**150 N**	**0 N**	
	Era of Hybrids								
1970s	11.5 d	10.0 b	1.5 d	559.7 d	499.1 b	60.6 b	341.2 b	331.7 b	9.5 b
1980s	11.9 c	10.3 b	1.6 c	587.3 c	514.4 a	72.9 c	333.3 c	328.7 b	4.6 c
1990s	12.6 b	10.1 b	2.5 b	594.5 b	482.6 c	111.9 b	343.1 b	319.5 c	23.6 a
2000s	14.3 a	10.8 a	3.5 a	603.2 a	426.5 d	176.7 a	369.9 a	347.0 a	22.9 a
	Source of variance								
Era (E)	**	ns	**	**	ns	**	ns	ns	ns
N rate (N)	** ns		-	** **		-	**		-
E × N	ns ns		-	ns ns		-	ns ns		-

Note: ** indicate significant differences at 0.01 probability. Different letters in the same nitrogen treatment indicate significant differences at 0.05 probability.

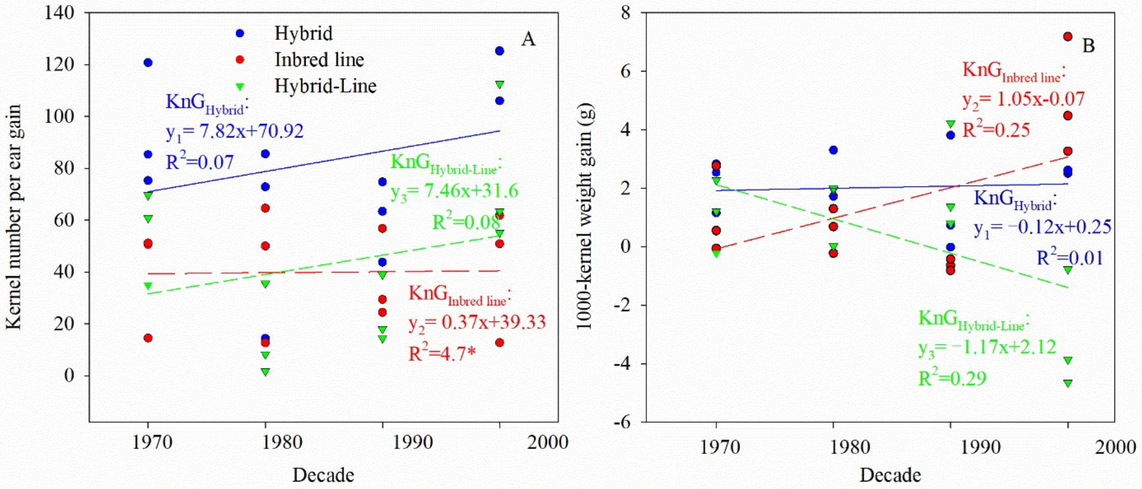

Figure 1. Changes of KnG and KwG of maize hybrids and inbred lines in different decades. Note: (**A**), Kernel number per ear gain; (**B**), 1000-kernel weight gain. * indicate significant differences at 0.05 probability.

At 150 N, GN of maize hybrids were greater than that of parental inbred lines in each decade. However, TKW of hybrids and parental inbred lines gradually converged, and the heterosis of TKW of hybrids gradually decreased (Figure 1).

3.2. The Heterosis of NUE and Its Related Parameters Indexes

The effect of interaction of hybrid era × N rate on heterosis for grain N, leaf Rem N and Rep_N are significant (r = 6.20–44.3, $p < 0.05$), and heterosis responses mainly depend on era of hybrids (Table 3). Maize hybrids had obvious heterosis for NUE over inbred lines in each decade (Figure 2). From the 1970s to the 2000s, the AH_{NUE} of maize hybrids increased significantly, at a rate of 2.11 kg kg^{-1} every 10 years. The AH_{NUE} (13.99 kg kg^{-1}) of maize hybrids from 2000s was 151.4% higher than that from 1970s (5.56 kg kg^{-1}). The MPH_{NUE} of maize hybrids in 2000s, 1990s, 1980s and 1970s were 312.88% (247.10–367.53%), 186.24% (176.96–199.03%), 223.76% (187.27–313.13%) and 177.39% (139.32–207.54%), respectively. The 40-year MPH_{NUE} increased by 76.4%, and at a rate of 19.1% every 10 years ($R^2 = 0.38$; $p < 0.05$).

Table 3. Mean squares from the ANOVA of heterosis for NUE, GY and related traits of hybrids (F value).

Heterotic Index	Source of Variation		
	Era of Hybrids (E)	**N Rate (N)**	**(E × N)**
NUE	5.93 *	-	-
NIE	13.06 *	-	-
NRE	9.12 *	-	-
Plant N	61.45 **	22.29 **	1.75
Grain N	31.71 **	23.79 **	7.07 *
Rem N	25.87 **	32.96 *	3.07
Rem Leaf N	8.23 *	2.11	6.20 *
Rem Stalk N	9.56 *	0.56	3.77
Veg_N	23.71 **	17.32 *	1.36
Rep_N	59.87 **	32.96 **	44.3 *
NHI	4.12	0.99	0.84
BMR_{silk}	11.46 *	30.25 **	1.61
BMR_{pm}	10.17 *	31.09 **	0.58
HI	0.36	0.01	3.57

Note: * and ** indicate significant differences at 0.05 and 0.01 probability, respectively. NUE, nitrogen use efficiency; NIE, nitrogen internal efficiency; NRE, nitrogen recovery efficiency; Plant N: plant N content at maturity per unit area; Grain N: grain N content at maturity per unit area; Rem N: reproductive-stage shoot N remobilization; Rem Leaf N: reproductive-stage leaf N remobilization; Rem Stalk N: reproductive-stage stalk N remobilization; Veg_N: vegetative-stage whole plant N uptake; Rep_N: reproductive stage whole plant N uptake; PNUE: Photosynthetic nitrogen use efficiency; BM_{silk}: biomass at silking per unit area; BM_{pm}: biomass at maturity per unit area; HI: harvest index.

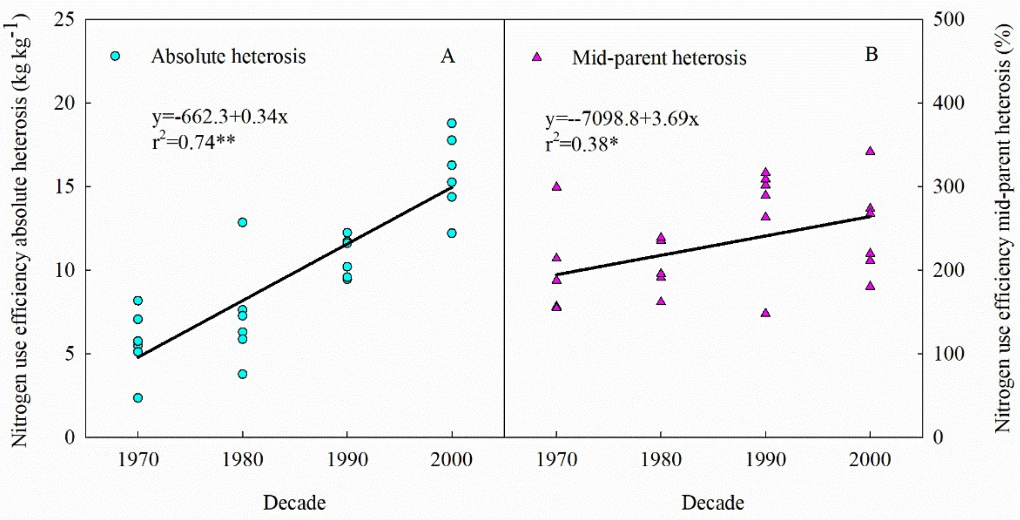

Figure 2. Changes in absolute heterosis and mid-parent heterosis of nitrogen use efficiency of maize hybrids in different decades. Note: (**A**), nitrogen use efficiency absolute heterosis; (**B**), nitrogen use efficiency mid-parent heterosis (%). * and ** indicate significant differences at 0.05 and 0.01 probability, respectively.

3.3. Physiological Basis Underlying the Heterosis for NUE

The NIE heterosis of maize hybrids from 2000s were 275.4%, 607.3% and 230.0% higher than that from 1970s, 1980s and 1990s in AH_{NIE}, and 203.3%, 824.2% and 352.9% higher in MPH_{NIE} (Tables 4 and 5). In contrast, the NRE heterosis of maize hybrids manifests a significant decrease over time (Table 5). The MPH of Grain N, Veg_N, Rep_N, BM_{silk} and BM_{pm} in 2000s were higher than those in 1970s, 1980s and 1990s (Table 5).

Table 4. The mean absolute heterosis value of NUE and related parameters of hybrids.

Index	1970		1980		1990		2000	
	150 N	0 N	150 N	0 N	150 N	0 N	150 N	0 N
NUE	8.7 ab	-	7.3 b	-	8.4 ab	-	14.0 a	-
NIE	10.2 a	-	5.4 a	-	11.5 a	-	38.1 a	-
NRE	0.2 a	-	0.1 a	-	0.1 a	-	−0.1 a	-
Plant N	68.0 ab	45.1 b	43.3 bc	25.5 b	30.5 c	20.5 b	96.4 a	114.3 a
Grain N	45.5 b	30.8	30.2 b	14.0	34.2 b	8.7	82.1 a	84.6 a
Rem N	33.7	16.2	75.1	12.9	51.9	11.1	77.4	16.8
Rem Leaf N	21.9	10.5	48.8	8.4	33.7	7.2	47.3	16.7
Rem Stalk N	11.8	5.7	26.3	4.5	18.2	3.9	25.5	9.0
Veg_N	56.2	30.5	88.1	24.5	48.3	22.9	91.7	46.5
Rep_N	11.8	14.6 ab	−44.8	1.1 b	−17.8	−2.4b	4.7	67.8 a
BM_silk	3.1	2.5 ab	3.0	1.4 b	3.5	1.7 ab	3.4	2.6 a
BM_pm	7.9 a	5.7	6.9 ab	4.5	4.4 b	4.0	7.8 ab	8.8

Note: Different letters in the same nitrogen treatment indicate significant differences at 0.05 probability. NUE, nitrogen use efficiency; NIE, nitrogen internal efficiency; NRE, nitrogen recovery efficiency; Plant N: plant N content at maturity per unit area; Grain N: grain N content at maturity per unit area; Rem N: reproductive-stage shoot N remobilization; Rem Leaf N: reproductive-stage leaf N remobilization; Rem Stalk N: reproductive-stage stalk N remobilization; Veg_N: vegetative-stage whole plant N uptake; Rep_N: reproductive stage whole plant N uptake; BM_silk: biomass at silking per unit area; BM_pm: biomass at maturity per unit area.

Table 5. The mean mid-parent heterosis value of NUE and related parameters of maize hybrids in different decades.

Index	1970		1980		1990		2000	
	150 N (%)	0 N (%)	150 N (%)	0 N (%)	150 N (%)	0 N (%)	150 N (%)	0 N (%)
NUE	177.4	-	223.8	-	186.2	-	312.9	-
NIE	76.6 b	-	25.1 b	-	51.3 b	-	232.2 a	-
NRE	64.6 a	-	32.9 ab	-	27.3 ab	-	−13.9 b	-
Plant N	27.4 ab	20.2 b	23.7 b	21.6 b	15.5 c	17.7 b	32.9 a	52.3 a
Grain N	24.4 b	23.7 b	32.9 b	27.3 b	24.7 b	15.0 b	50.2 a	64.7 a
Rem N	79.9	37.2	122.8	56.9	94.0	28.1	193.6	56.4
Rem Leaf N	79.9	37.2	122.8	56.9	94.0	28.1	172.6	78.2
Rem Stalk N	79.9	37.2	122.8	56.9	94.0	28.1	172.6	78.2
Veg_N	33.0	23.8 ab	45.6	29.8 a	27.4	15.2 b	53.3	32.4 a
Rep_N	20.2	13.0 bc	−18.2	1.7c	−4.8	87.5 ab	5.9	101.9 a
BM_silk	38.8	33.5 ab	35.7	16.4 c	40.6	20.1 bc	45.1	39.1 a
BM_pm	52.1 a	38.0 b	40.6 ab	36.4 b	24.0 b	27.1 b	45.2 a	64.6 a

Note: Different letters in the same nitrogen treatment indicate significant differences at 0.05 probability. NUE, nitrogen use efficiency; NIE, nitrogen internal efficiency; NRE, nitrogen recovery efficiency; Plant N: plant N content at maturity per unit area; Grain N: grain N content at maturity per unit area; Rem N: reproductive-stage shoot N remobilization; Rem Leaf N: reproductive-stage leaf N remobilization; Rem Stalk N: reproductive-stage stalk N remobilization; Veg_N: vegetative-stage whole plant N uptake; Rep_N: reproductive stage whole plant N uptake; BM_silk: biomass at silking per unit area; BM_pm: biomass at maturity per unit area.

From the 1970s to 2000s, the coefficient of determination of MPH_NIE on MPH_NUE (43–57%) was significantly higher than that of MPH_NRE on MPH_NUE (19–32%), which was 26% higher on average, indicating that the evolution of heterosis for NUE of maize hybrids was mainly derived from the evolution of NIE heterosis (Figure 3).

The comprehensive analysis of all inbred lines and hybrids showed that NIE of era hybrids were closely related to biomass accumulation at silking (BM_silk) and plant N uptake in vegetative stage (Veg_N). Hybrids had greater BM_silk and higher Veg_N than inbred lines in each decade, and the gap between hybrids and inbred lines gradually increased (Figure 4). The relationships of NIE with BM_silk and Veg_N all exhibited plateau with linear increase. NIE stayed at around 9.66 $\text{kg}\cdot\text{kg}^{-1}$ and 9.49 $\text{kg}\cdot\text{ha}^{-1}$ when BM_silk and Veg_N were below 8.73 t ha^{-1} and 38.63 kg ha^{-1}, respectively, when BM_silk was above 8.73 t ha^{-1}, NIE increased linearly. (Figure 4). Compared with inbred lines, Rem N of maize hybrids in each era had stable heterosis. The contribution of nitrogen remobilization (NCR) to grain nitrogen yield of maize hybrids also had a significant heterosis compared with inbred lines, and this differences gradually expand over time (Figure 5A). Grain N yield of hybrids also showed similar patterns with the gradual progression (Figure 5B).

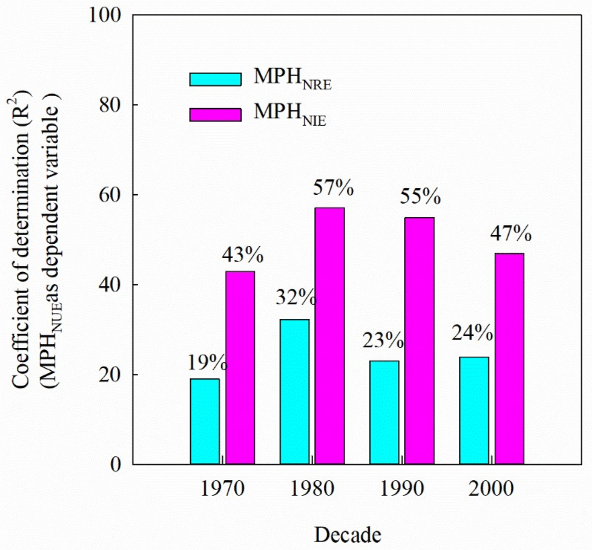

Figure 3. Coefficient of determination (R^2) of MPH_{NUE} (dependent variable) versus MPH_{NIE} and MPH_{NRE} of maize hybrids in different decades.

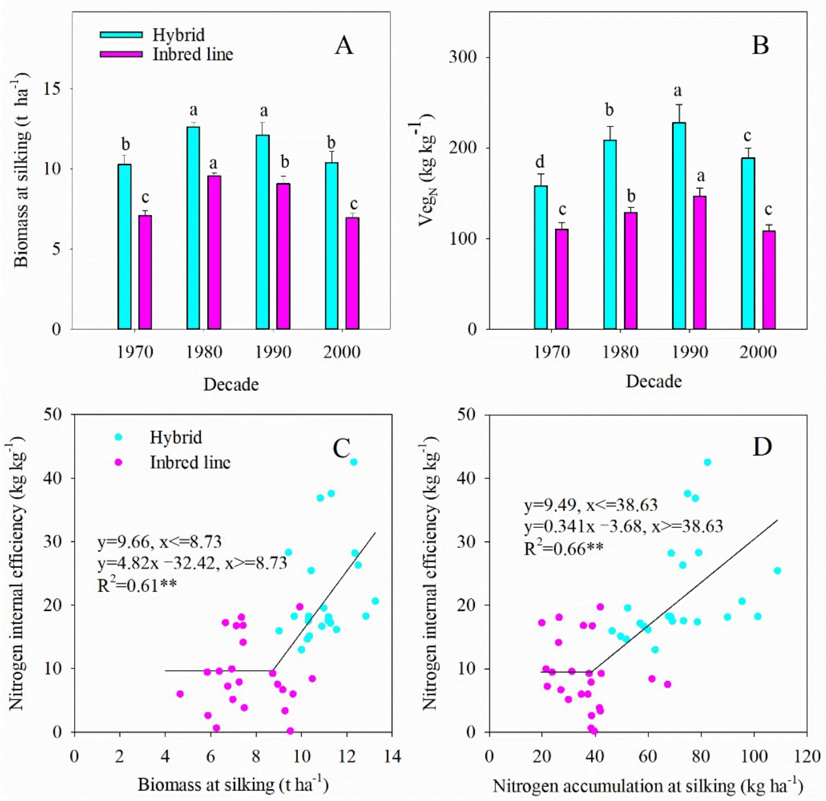

Figure 4. Relationship of BM_{silk}, Veg_N and NIE of maize hybrids and inbred lines in different decades. Note: (**A**), biomass at silking; (**B**), Veg_N; (**C**), Relationship of BM_{silk} and NIE; (**D**), Relationship of Veg_N and NIE. ** indicate significant differences at 0.01 probability. Different letters in the same nitrogen treatment indicate significant differences at 0.05 probability.

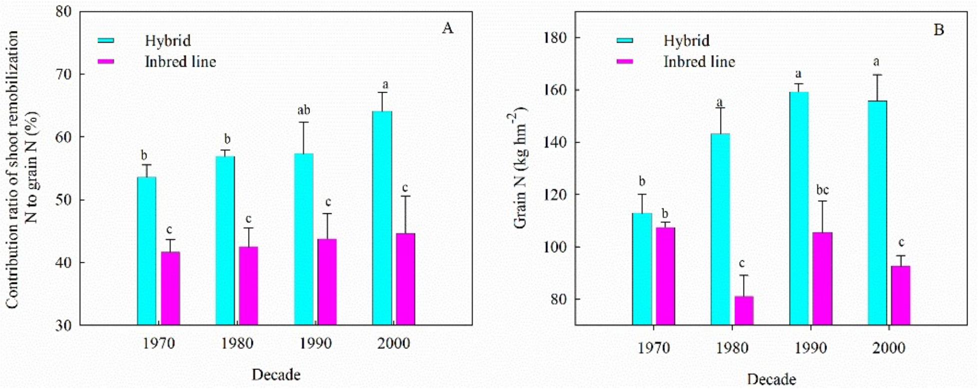

Figure 5. Contribution rate of nitrogen contribution ratio and grain nitrogen content of maize hybrids and inbred lines in different decades. Note: (**A**) Contribution rate of nitrogen contribution ratio; (**B**) grain nitrogen content. Different letters in the same nitrogen treatment indicate significant differences at 0.05 probability.

4. Discussion

Heterosis of hybrids have been commercially developed for a century, contributing about half of the yield increase during that period [22,34,35], but it is still unclear whether there is heterosis in maize nitrogen efficiency. The yield gap between hybrids and inbred lines under high nitrogen application was significantly higher than under low nitrogen application [26,33]. Hisse et al. [26] studied that the yield of hybrids (10–45%) was more responsive than inbred lines (5–11%), the same as our results (Table 3). The NUE heterosis of maize hybrids were higher than inbred lines in each decade (Figure 2, Table 3). The results of this study indicated that absolute heterosis and mid-parent heterosis for NUE increased linearly ($p < 0.05$), and the absolute heterosis for NUE improvements in the inbred lines from the 1970s to the 2000s (151.4%) was greater than mid-parent heterosis for the NUE in the lines from the 1970s to the 2000s (73.4%) (Figure 2). From the 1950s to 1990s, the NUE of maize hybrids in China increased at a rate of 0.15 kg·kg^{-1} year^{-1}, and decreased at a rate of 0.43 kg·kg^{-1} year^{-1} during the 1990s to 2000s. However, the NUE growth rate of inbred lines was 0.094 kg·kg^{-1} year^{-1} in the 20 years from the 1960s to 1980s, and decreased at the rate of 0.02 kg·kg^{-1} year^{-1} in the 1980s to 2000s [36]. The AH_{NUE} and MPH_{NUE} of maize hybrids from 1970s to 2000s were 7.3–14, and 177.4–312.9%, respectively (Tables 4 and 5).

Maize NUE is affected by both NRE and NIE; Ciampitti and Vyn [8] believed that compared with old varieties, the increase in NUE of modern hybrids was mainly due to the significant decrease in grain nitrogen concentration and the increase in NIE. In this study, the AH_{NIE} and MPH_{NIE} of maize hybrids from 1970s to 2000s increased from 10.2 to 38.1, and 76.6% to 232.2%, which was consistent with the evolution trend of NUE heterosis (Tables 4 and 5). From 1970s to 2000s, the coefficients of determinations of MPH_{NIE} on MPH_{NUE} (43–57%) were significantly higher than that of MPH_{NRE} on MPH_{NUE} (19–32%), which was 26% higher on average, indicating that maize hybrids NUE heterosis were mainly derived from NIE heterosis (Figure 3), indicating that efficient internal nitrogen transport and redistribution during post-silking.

The accumulation of nitrogen in grain mainly depended on the nitrogen uptake after silking and N remobilization from vegetative organs [37]. Chen et al. [37] studied that 66–85% of the grain N content was contributed by pre-silking N remobilization. The lower NIE was mainly contributed by the lower N remobilization in the leaf. Ciampitti and Vyn [38,39] believed that the amount of post-silking nitrogen transport was mainly related to the pre-silking nitrogen accumulation in the plant. Pre-silking dry matter (BM_{silk}) and nitrogen accumulation (Veg_N) of hybrids were higher than inbred lines, which were significantly correlated with NIE heterosis of hybrids. NIE stayed at around 9.66 kg·kg^{-1} and 9.49 kg·ha^{-1} when BM_{silk} and Veg_N were below 8.73 t ha^{-1} and 38.63 kg ha^{-1}, respectively;

when BM_{silk} was above 8.73 t ha^{-1}, NIE increased linearly (Figure 4). Moreover, hybrids of different ages had a stable transport advantage in Rem N after silking, which should be one of the main factors determining the heterosis of NIE. A study showed that in a wide range of genotypes and environments, 27–39% of the nitrogen accumulated at pre-silking was transported, and 43–44% of nitrogen content was produced by transported N [37,39–41]. Wang et al. [6] found the contribution ratio of shoot remobilization N to the grain N of hybrids was 13.4% higher than inbred lines. The trade-off between RemN and PostN varied considerably among hybrid decades and N rates [37,42]. From 1970s to 2000s, the contribution of nitrogen remobilization of maize hybrids (from 54% to 69.7%) were higher than that of inbred lines (53–56%) (Figure 5). These results are consistent with previous maize studies that reported the response of F1 and inbred lines to nitrogen availability [26], indicating that nitrogen internal efficient transport and redistribution during post-silking. In other words, it is necessary to analyze the relationship between Rem N, Veg_N and Rep_N to improve maize hybrids NUE and its heterosis.

According to our results, plant N, grain N, Rem N, Veg_N, Rep_N and grain number per ear had significant quadratic relationships with era of hybrids (r = 13.6–61.45, $p < 0.01$), and NUE, NIE, NRE, Rem Stalk N, BM_{silk} and BM_{pm} (r = 5.93–13.06, $p < 0.05$) (Table 3). In total, the increase in hybrids maize yield were due to the increase in grain number per ear and 1000-kernel weight (Table 2). Yield formation mainly depends on the dry matter and nitrogen uptake at the post-silking stage [16]. However, higher NIE was the main reason for higher NUE, which was higher in pre-silking dry matter and pre-silking nitrogen accumulation. Therefore, hybrids maize needs to be improved to meet future food security.

5. Conclusions

Maize variety replacements over time resulted in increased yield and NUE. The evolution of heterosis for NUE mainly originated from the heterosis for NIE. The heterosis of pre-silking nitrogen accumulation and post-silking nitrogen transport in maize hybrids has increased response to nitrogen application. Across four decades, the AH_{NUE} and MPH_{NUE} increased by 2.11 kg kg^{-1} and 19.1% every 10 years. The higher pre-silking dry matter and nitrogen accumulation of maize hybrids should be the main reasons for evolution of NUE heterosis. To further improve yield and NUE, the pre-silking N and carbon accumulation and post-silking N remobilization should both continue to be increased for maize breeding. There is much room for further improvement in the yield and NUE advantages of maize hybrid in the future.

Author Contributions: Designed research, R.L. and Z.W.; performed research, R.L., J.G., Y.L. and S.Y.; analyzed data, R.L., S.Y. and Z.W.; wrote the paper, R.L. and Z.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported in part by the Science Foundation for Fostering Outstanding Young Scholars of IMAU (2017XYQ-1), the National Natural Science Foundation of China (31660359) and the National Key Research and Development Program of China (2016YFD0300103).

Data Availability Statement: The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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