INFLUENCE OF NITROGEN APPLICATION RATES ON NITROGEN ACQUISITION AND UTILIZATION EFFICIENCY OF MAIZE VARIETIES UNDER DRIP IRRIGATION SYSTEMS

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Abstract. Maize yield is significantly influenced by nitrogen fertilization, yet excessive application poses risks to human health and the environment. A sustainable approach to maintaining high maize production involves reducing nitrogen application and selecting genotypes excelling in nitrogen uptake, utilization, and remobilization. This study aimed to assess the impact of various maize genotypes and nitrogen levels on nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE), and nitrogen utilization efficiency (NUtE) under drip irrigation. The research also sought to categorize these genotypes based on their NUE. The trials were conducted over two growing seasons, 2022 and 2023, at Anningqu Town, Urumqi City, Xinjiang Province, China. The study involved a combination of 17 maize genotypes and two nitrogen application rates, with lower (LN) and higher (HN) levels at 150 and 300 kg N ha^{-1}, respectively. Results indicated a decrease in total dry matter and total nitrogen uptake by 7.14% and 12.54% in 2022 and 2023, respectively. Conversely, the LN treatment enhanced NUtE, NUpE, and consequently NUE. Genotype, nitrogen application, and their interaction significantly influenced all traits ($p < 0.05$). NUE was strongly correlated with grain yield, total dry matter, and N uptake, and NUpE, but not with NUtE. The genotype Zhengdan958 demonstrated superior NUE under both LN and HN conditions. These findings indicate that certain maize genotypes can adapt to reduced nitrogen inputs, primarily through improved NUpE rather than NUtE.
Keywords: *dry matter yield, harvest index, N harvest index, nitrogen concentration, genotypic variation*

Introduction

Nitrogen (N) is one of the most important and limiting nutrients for crop production (Iqbal et al., 2020). It is the main component of several macromolecules, which is necessary for normal growth and productivity (Castro-Rodríguez et al., 2017). N fertilization has significantly improved crop production and thus reduces the pressure of global population growth (Iqbal et al., 2020). However, low N availability is a major constraint in crop production and can reduce yields by up to 50% (Jones et al., 2013; Rennenberg et al., 2015). In order to maintain better crop growth and yield, the demand for nitrogen fertilizer has increased (Rennenberg et al., 2015; Xu et al., 2012) and will continue to increase in the future (Iqbal et al., 2015). On the other side, plants can only utilize half of the applied fertilizer (Schroeder et al., 2013), and poses significant threats

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to human health and the environment (Miao et al., 2011; Cameron et al., 2013). Therefore, it is an urgent need to enhance NUE in plants to reduce N fertilizer application into soils.

NUpE is the ability of crops to absorb nitrogen from soil and fertilizer, and NUtE is the ability of crops to convert absorbed nitrogen into yield. To boost crop nitrogen use efficiency (NUE), it is crucial to enhance both the plant's ability to absorb nitrogen from the soil (NUpE) and the efficiency of nitrogen utilization during periods of rapid plant growth and biomass accumulation (NUtE) (Oliveira et al., 2020). However, for some cultivars, a large amount of absorbed N is retained in organs that no longer play a role in later development, such as senescent leaves, and cannot be transported to organs that need it to maintain their growth levels, resulting in inefficient N utilization (Guo et al., 2021). NUE is the yield capacity of a Crop genotype at low available N concentrations. To improve the NUE, it is necessary to improve the component traits such as NUpE (absorption/uptake), translocation (transport/partitioning/remobilization) and NUtE (utilization) (Peng et al., 2022). To improve NUE in crops, it is important to explore genetic variation in all relevant traits.

NUE is a complex trait affected by both genetic and environmental factors (Islam et al., 2021; Mauceri et al., 2020). It is composed of two main components, NUpE and NUtE, which are influenced by biochemistry, phenology, and architecture, as well as the external environment such as N availability (Hawkesford et al., 2019). Studies have shown that total dry matter, nitrogen absorption and root morphology of plant have a certain effect of improving NUE (Abenavoli, et al., 2016; Luo et al., 2019).

Maize is the third most important food crop after wheat and rice. Due to its increasing use in biofuel production, its demand is also increasing. At present, thousands of varieties have been bred. Nitrogen efficient maize genotypes are beneficial to improve nitrogen use efficiency and reduce nitrogen residue in low nitrogen soil and nitrogen sufficient soil (Simunji, et al., 2018). In production input, high nitrogen fertilization is a great challenge, which not only increases the cost of farmers, but also brings environmental and human problems. There are significant genotypic differences in NUE in some crops, including maize, rice and wheat (Simunji, et al., 2018; Hawkesford et al., 2019; Nehe, et al., 2018). The relationship between different maize genotypes affecting the related physiological traits of NUpE and NUtE has not been well evaluated. These studies will increase our understanding of the effects of genotype and nitrogen supply on NUE variability in maize under field conditions. It is of great significance for rational application of nitrogen fertilizer and maize breeding in drip irrigation agricultural system in arid area. Fertilizer along with water-drip irrigation is the process of Fertilizer entering to the field with water, a new technology of combination with irrigation and Fertilizer, product of the combination with a precise and accurate irrigation and fertilizer (Fan, et al., 2020).

The purpose of this study was to evaluate the contribution of NUpE and NUtE to nitrogen efficiency related traits of maize genotypes, and to classify genotypes according to their nitrogen efficiency (NUE) under low and high nitrogen supply in drip irrigation.

Materials and methods

Site description

The research was undertaken during the 2022 and 2023 growing seasons at the "National Gray Desert Soil Fertility and Fertilizer Benefit Monitoring Station" in

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Anningqu Town, Urumqi City, Xinjiang Province, Northwest China (43°56′28″N, 87° 28′ 35″E). This area, characterized by a temperate arid to semi-arid climate, is situated at an elevation of 600 meters. It experiences an average annual temperature of 7.6℃, with an effective accumulated temperature of 1734℃. The region receives an annual rainfall of 310 mm, has a frost-free period of 156 days, and enjoys approximately 2594 h of sunshine annually. In 2022, the soil at a depth of 0-20 cm exhibited the following characteristics: a pH of 8.10, an electrical conductivity (EC) of 0.04 dS/m in a 1:5 soil to water solution, an organic matter content of 16.90 g/kg, ammonium nitrogen levels at 0.88 mg/kg, nitrate nitrogen at 34.84 mg/kg, Olsen P of 14.03 mg/kg, and available potassium levels of 401.05 mg/kg.

Experimental design

The experiment was set up in a split-zone design. N rates were classified to main plots and genotypes to subplots randomized into three blocks. In the experiment, treatments consisted of a factorial combination of 17 maize genotypes (*Table 1*) and two N fertilization rates (150 and 300 kg N ha-1 for LN and HN, respectively). Each plot was mulched with a sheet of transparent polyethylene film (1.2 m wide × 3 m long). The plastic film was held in place by burying the edges with soil. Two drip irrigation lines were installed under the plastic film. There was a 0.6-m-wide bare strip between each plot. Each plot had three rows of maize plants. The maize plants were sown at 20 cm intervals within each row. Row spacing configuration of 55 m + 55 cm + 60 cm. The plant population was 8.7×10^4 plants/ha. Maize was sowed on April 25, 2022 and May 6, 2023, respectively.

Nitrogen fertilizers use urea, all of which was used as a top dressing. 50% of phosphorus (superphosphate) and potassium fertilizer (potassium sulfate) were used as basal fertilizers and applied before planting, while the remaining 50% phosphorus (potassium dihydrogen phosphate) and potassium fertilizer (potassium sulfate) were used as top dressing fertilizers and applied through the drip irrigation system. All topdressing fertilizers were applied in five equal amounts 45, 52, 59, 69, and 82 days after planting. The fertilizer solution was stored in a 20 L iron container and pumped into the irrigation system. All plots were fertilized with 120 kg P_2O_5/ha and 90 kg K_2O/ha. The plots were irrigated nine times (every 7 to 10 days) between June and August. These irrigation practices were similar to those used by local farmers. Other cultivation management measures refer to local field.

Table 1. *The origin of maize genotypes*

Genotypes	Origin	Genotypes	Origin
Heyu187	Jilin, China	Xinyu54	Xinjiang, China
KWS2564	Xinjiang, China	Xinyu69	Xinjiang, China
Huamei1	Xinjiang, China	Xinyu80	Xinjiang, China
M751	Xinjiang, China	Xinyu102	Xinjiang, China
Xinsiyu13	Xinjiang, China	Xinyu108	Xinjiang, China
Xinyu9	Xinjiang, China	Xinyu110	Xinjiang, China
Xinyu24	Xinjiang, China	Zhengdan958	Henan, China
Xinyu29	Xinjiang, China	Xianyu335	Liaoning, China
Xinyu47	Xinjiang, China		

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Sampling and measurement methods

Upon reaching maturity, all the plants were harvested at the same time. The maize plants were harvested by cutting at ground level and separated into two components: stovers and grains. The samples were initially dried at 105°C for 30 min to remove surface moisture, followed by further drying at 75°C until they reached a constant weight. The dry weight of each sample was then documented. Subsequently, the dried samples were ground to a fine consistency, ensuring they could pass through a 1 mm sieve. This preparation was followed by a digestion process using sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). The nitrogen content within the plant tissues was determine to use the Kjeldahl method, a widely recognized analytical technique for quantifying nitrogen levels (Singh et al., 2020).

The measured traits, abbreviations, calculations and units are shown in *Table 2*. N uptake in stovers and grains was calculated as the product of dry matter yield and N concentration. N harvest index was calculated as the ratio between N uptake in grain and total N uptake. Harvest index was determined as the ratio between grain dry matter yield and total dry matter yield (grain plus stover biomass). NUE (grain dry matter yield (kg ha^{-1})/N supply (kg N ha^{-1})) was calculated as the product of NUtE and NUpE (*Table 2*), following the approach of previous studies assessing N and/or other nutrients (Presterl et al., 2002; Hawkesford et al., 2019; Ranjan et al., 2023). NUtE was calculated as the ratio between grain dry matter yield and total N uptake (grain dry yield (kg ha^{-1})/N uptake (kg N ha^{-1})), while the NUpE was calculated as the ratio between total N uptake and N supply (N uptake (kg N ha^{-1})/N supply (kg N ha^{-1})) (Adotey et al., 2024).

Table 2. *Traits, abbreviations, calculation and units of traits measured in 17 genotypes of maizes under150(LN) and 300 kg N ha^{-1} (HN) in 2022 and 2023*

Trait	Abbreviation	Calculation	Unit
Grain dry matter yield	GDY		kg ha^{-1}
Stover dry matter yield	SDY		kg ha^{-1}
Total dry matter yield	TDY		kg ha^{-1}
Harvest index	HI	GDY/TDY	kg kg^{-1}
Grain N concentration	GNC		%
Stover N concentration	SNC		%
Grain N uptake	GNU	GNC × GDY	kg ha^{-1}
Stover N uptake	SNU	SNC × SDY	kg ha^{-1}
Total N uptake	TNU	GNU + SNU	kg ha^{-1}
N harvest index	NHI	GNU/TNU	kg kg^{-1}
N utilization efficiency	NUtE	GDY/TNU	kg grain dry matter yield kg^{-1} N uptake
N uptake efficiency	NUpE	TNU/N supply	kg total N uptake kg^{-1} N supply

Data analyses

The data analysis was conducted using SPSS statistical software version 22.0 (SPSS Inc., 1996), employing a two-factor split-zone ANOVA test to assess the significance at the 0.05 level. This test considered maize genotypes and nitrogen application rates as independent variables. To identify significant differences among specific treatments, a Duncan multiple range test was applied at a P-value threshold of less than 0.05.

Additionally, correlation and regression analyses were performed using the same version of SPSS software, while principal component analysis (PCA) was conducted with Origin software version 21.0.

Results

Analysis of variance and genotypic variation in traits

The mean, coefficient of variation and ANOVA of the measured traits are shown in *Table 3*. All traits were influenced by genotype, nitrogen application and their interaction ($N < 0.05$) (*Table 3*). The variation coefficients of each trait reflected the variability of maize genotypes at HN and LN levels (*Table 3*). On average, the total dry matter (grains, stovers and total), in 2022 and 2023 decreased by 7.14 and 12.54, respectively; total nitrogen uptakes (in grains, stovers and total) decreased by 20.16 and 20.13%, respectively. As expected, HN reduces NUtE, NUpE, and therefore NUE (*Table 3*).

Table 3. *Mean coefficient of variation (CV%) and analysis of variance (nitrogen (N), genotype (G) and N×G interaction effects) for traits measured in 17 maize genotypes under 150 (LN) and 300 kg N ha^{-1} (HN) in 2022 and 2023*

Year	Trait	LN	CV (%)	HN	CV (%)	Analysis of variance (P value)		
		Mean		Mean		N	G	N×G
2022	GDY	15503.31	14.24	17126.87	14.29	< 0.001	< 0.001	0.002
	SDY	15923.85	14.43	16717.71	11.60	< 0.001	< 0.001	< 0.001
	TDY	31427.15	14.21	33844.58	12.78	< 0.001	< 0.001	< 0.001
	HI	0.49	1.96	0.51	2.46	< 0.001	< 0.001	< 0.001
	GNC	1.08	14.00	1.18	19.60	< 0.001	< 0.001	< 0.001
	SNC	0.45	36.94	0.59	24.83	< 0.001	< 0.001	< 0.001
	GNU	168.05	20.15	201.63	25.07	< 0.001	< 0.001	< 0.001
	SNU	71.02	38.06	97.82	28.36	< 0.001	< 0.001	< 0.001
	TNU	239.07	21.93	299.45	22.14	< 0.001	< 0.001	< 0.001
	NHI	0.71	9.17	0.67	8.90	< 0.001	< 0.001	< 0.001
	NUtE	66.60	17.18	58.69	17.07	< 0.001	< 0.001	< 0.001
	NUpE	1.59	21.93	1.00	22.14	< 0.001	< 0.001	< 0.001
	NUE	103.36	14.24	57.09	14.29	< 0.001	< 0.001	< 0.001
2023	GDY	11306.22	18.66	13368.67	20.38	< 0.001	< 0.001	< 0.001
	SDY	12468.46	19.09	13814.69	21.11	< 0.001	< 0.001	< 0.001
	TDY	23774.69	18.79	27183.36	20.61	< 0.001	< 0.001	< 0.001
	HI	0.48	2.14	0.49	2.50	< 0.001	< 0.001	0.058
	GNC	1.22	19.28	1.29	16.14	< 0.001	< 0.001	< 0.001
	SNC	0.51	21.78	0.57	36.46	< 0.001	< 0.001	< 0.001
	GNU	137.05	25.92	171.26	23.29	< 0.001	< 0.001	< 0.001
	SNU	62.36	21.23	78.40	38.35	< 0.001	< 0.001	< 0.001
	TNU	199.41	20.67	249.66	24.06	< 0.001	< 0.001	< 0.001
	NHI	0.68	7.92	0.69	9.91	0.036	< 0.001	< 0.001
	NUtE	57.47	15.00	54.64	15.87	< 0.001	< 0.001	< 0.001
	NUpE	1.33	20.67	0.83	24.06	< 0.001	< 0.001	< 0.001
	NUE	75.37	18.66	44.56	20.38	< 0.001	< 0.001	< 0.001

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Dry matter yield and total N uptake

On average, during the 2022 and 2023, LN decreased ($N < 0.05$) grain dry matter yield by, 9.48% and 15.43% (*Table 3*). However, the grain dry matter yield reduction of different genotypes in different years was different. The yield reduction in 2022 was between 5.06% and 13.32%, and the yield reduction in 2023 was between 11.20% and 25.97%. The reduction in grain dry matter yield under LN conditions was positively correlated with ($P < 0.05$; $R^2 = 0.8177$) the reduction in total dry matter yield (*Fig. 1a*). LN decreased the total N uptake by 20.16% and 20.13%. Total N uptake in the LN treatment was positively correlated ($P < 0.05$; $R^2 = 0.5412$) with total N uptake in HN treatment, but the slope was lower than 1; therefore, the total nitrogen uptake in the LN treatment was significantly lower than the total nitrogen uptake in the HN treatment (*Fig. 1b*). Under both fertilization conditions, total nitrogen uptake was positively correlated with total dry matter yield, with no difference in regression slope (*Fig. 1c*). More interestingly, different total nitrogen uptake was observed at the same total dry matter yield (*Fig. 1c*), indicating an important variation in NUpE. Grain dry matter yield was positively correlated with total N uptake under the LN treatment ($N < 0.05$; $R^2 = 0.5328$) and HN treatment ($N < 0.05$; $R^2 = 0.5711$) (*Fig. 1d*). Moreover, at the same level of total nitrogen uptake (under LN and HN conditions), grain dry matter yields varied considerably among genotypes (*Fig. 1d*).

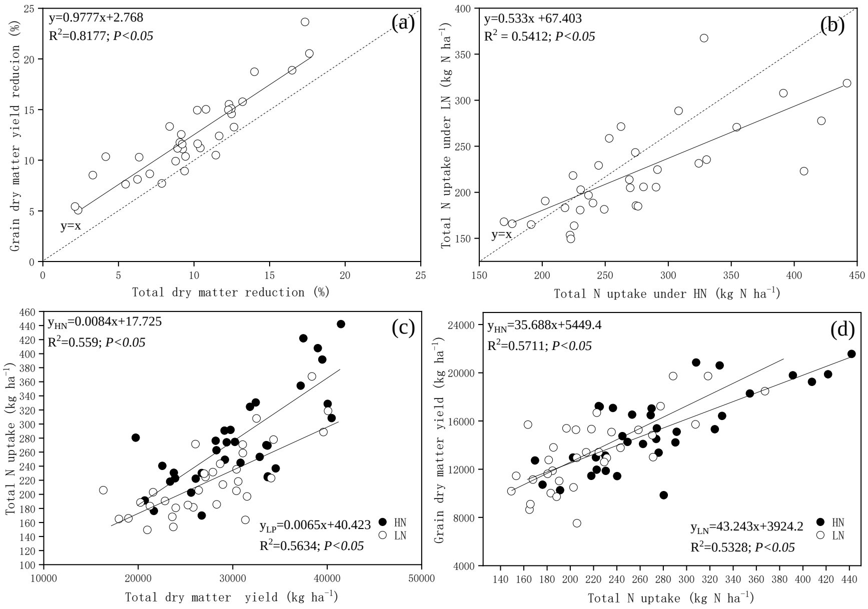

***Figure 1.** Relationships between Grain dry matter yield and total dry matter yield reductions (a), between total N uptake under LN and HN (b), between total N uptake and total dry matter yield (c) and between grain dry matter yield and total N uptake (d) in 17 maize genotypes under 150 (LN) (open symbols) and 300 kg N ha^{-1} (HN) (closed symbols)*

NUtE, NUpE and their importance to NUE

In this experiment, the variation of NUtE in 2022 and 2023 had different responses to N levels, genotypes and their interactions (*Table 4*). Under HN conditions in 2022, NUtE ranged from 47.13 to 76.86 kg grain dry matter yield kg^{-1} total N uptake, the genotypes M751 and KWS2564 exhibited the highest NUtE (76.86~76.27 kg grain dry

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matter yield kg^{-1} total N uptake), Heyu187 was the lowest. These values are higher in LN than in HN (47.94 ~ 96.06), the highest was still KWS2564, and Xinyu24 was the lowest. Under HN conditions in 2023, NUtE ranged from 35.11 to 75.22 kg grain dry matter yield kg^{-1} total N uptake, the KWS2564 exhibited the highest NUtE, while Xinyu9 had the lowest NUtE. In the case of LN, the highest value of Xinyu 47 was 74.56, and the lowest value of Xinyu 9 was only 36.52. On average, HN decreased NUtE by 11.88% in 2022 and 4.92% in 2023; However, the sensitivity of NUtE to LN varied among maize Genotypes (*Table 4*).

Table 4. *N utilization efficiency (NUtE, kg grain dry matter yield kg^{-1} total N uptake), N uptake efficiency (NUpE, kg total N uptake kg^{-1} N supply) and N use efficiency (NUE, kg grain dry matter yield kg^{-1} N supply) in 17 maize genotypes under 150 (LN) and 300 kg P ha^{-1} (HN) in 2022 and 2023*

Year	Genotype	NUtE		NUpE		NUE	
		LN	HN	LN	HN	LN	HN
2022	Heyu187	62.07efA	47.13iB	1.85cdA	1.41bB	114.82cA	66.24cB
	KWS2564	96.06aA	76.27aB	1.09mA	0.75iB	104.66dA	57.28eB
	Huamei1	74.56bcA	63.13dB	1.37ijA	0.90fB	101.81dA	56.79efB
	M751	70.63cdA	76.86aA	1.45hiA	0.75iB	102.13dA	57.44eB
	Xinsiyu13	74.88bcA	47.16iB	1.49ghA	1.36bB	111.25cA	64.13dB
	Xinyu9	47.94hA	53.67fgB	1.81deA	0.88fgB	86.68fA	46.96iB
	Xinyu24	78.19bA	72.17bB	1.31jkA	0.79hiB	102.56dA	56.92efB
	Xinyu29	70.37cdA	57.26eB	1.21lA	0.83ghB	85.11fA	47.55iB
	Xinyu47	64.07eA	49.66hiB	1.57fgA	1.10cB	100.56dA	54.71gB
	Xinyu54	74.46bcA	56.10efB	1.24klA	0.91fB	92.04eA	51.30hB
	Xinyu69	50.25hA	62.76dB	2.45aA	1.10cB	123.13bA	68.67bB
	Xinyu80	63.05efA	48.96hiB	1.37ijA	0.97eB	86.32fA	47.39iB
	Xinyu102	59.74efgA	51.84ghB	1.50ghA	0.97eB	89.44efA	50.34hB
	Xinyu108	59.02fgA	65.32cdB	1.72eA	0.84gB	101.75dA	55.07fgB
	Xinyu110	56.59gA	53.00fgA	1.62fA	0.91fB	91.80eA	48.35iB
	Zhengdan958	68.42dA	67.69cA	1.92cA	1.03dB	131.47aA	69.50bB
	Xianyu335	61.94efA	48.80hiB	2.12bA	1.47aB	131.50aA	71.88aB
2023	Heyu187	55.93efA	47.22gB	1.54cA	1.08cB	86.25dA	51.04dB
	KWS2564	66.35bcB	75.22aA	1.12gA	0.57iB	74.28ghA	42.39eB
	Huamei1	54.68fgA	52.50eA	1.22fA	0.73gB	66.75jkA	38.16fB
	M751	57.97eA	64.08bB	1.27fA	0.67hB	73.46hA	43.22eB
	Xinsiyu13	54.96fgA	60.21cdB	1.53cA	0.82eB	83.95dA	49.13dB
	Xinyu9	36.52iA	35.11hA	1.37deA	0.93dB	50.10nA	32.81hB
	Xinyu24	64.25cdA	48.42fgB	1.23fA	0.92dB	79.13eA	44.56eB
	Xinyu29	52.43ghA	53.67eA	1.10gA	0.64hB	57.62mA	34.20ghB
	Xinyu47	74.56aA	58.40cdB	1.02hA	0.74gB	76.31fgA	43.24eB
	Xinyu54	51.71hA	51.47efA	1.35eA	0.77fgB	69.93iA	39.54fB
	Xinyu69	62.70dA	61.26bcA	1.42dA	0.90dB	89.32cA	54.95cB
	Xinyu80	54.92fgB	60.76cA	1.10gA	0.59iB	60.62lA	35.70gB
	Xinyu102	51.63hA	47.57gB	1.26fA	0.80efB	64.78kA	38.08fB
	Xinyu108	64.42cdA	57.10dB	1.20fA	0.77fgB	77.41efA	43.79eB
	Xinyu110	68.13bA	53.78eB	1.00hA	0.74gB	67.81ijA	39.87fB
	Zhengdan958	54.80fgA	51.56efB	1.80bA	1.18bB	98.86bA	60.93bB
	Xianyu335	51.09hA	50.54efgA	2.05aA	1.30aB	104.80aA	65.94aB

Different lowercase letters in the same column and different capital letters for the same item in the same row indicate significant differences at the 0.05 level

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In this study, the responses of NUpE (0.57~2.45 kg total N uptake kg^{-1} N supply) to N levels, genotypes and their interactions in 2022 and 2023 were widely variable (*Table 4*). Under HN conditions, the NUpE in 2022 ranges from 1.47~0.75 kg total N uptake kg^{-1} N supply, and 1.30~0.57 kg total N uptake kg^{-1} N supply in 2023. As expected, the NUpE increases greatly with LN treatment. More interestingly, there were important differences in NUpE between maize genotypes under LN conditions. The maximum difference in NUpE in 2022 is between 2.45~1.09 kg total N uptake kg^{-1} N supply. The difference in 2023 is relatively small, but it is also between 2.05~1.00 kg total N uptake kg^{-1} N supply. In the experiments, the genotypes Xinyu69 and Xianyu335 showed the highest NUpE (2.05~2.45 kg total N uptake kg^{-1} N supply) under LN treatment in 2022 and 2023, respectively, whereas KWS2564 and Xinyu110 had the lowest NUpE (1.00~1.09 kg total N uptake kg^{-1} N supply). The genotypes Xianyu335 showed the highest NUpE (1.30~1.47 kg total N uptake kg^{-1} N supply) under HN from 2022 to 2023, whereas KWS2564 was lower in both years (0.57~0.75 kg total N uptake kg^{-1} N supply) (*Table 4*).

NUE varied between 32.81 to 131.50 kg grain dry matter yield kg^{-1} N supply (*Table 4*). Under HN conditions, the nitrogen use efficiency decreased by 44.77% in 2022 and 40.88% in 2023. The average NUE in 2022 is higher than that in 2023. Under the HN condition, the NUE in 2022 and 2023 were 57.09 and 44.56 kg grain dry matter yield kg^{-1} N supply, respectively, while under the LN condition, the NUE were 103.35 and 79.50 kg grain dry matter yield kg^{-1} N supply, respectively. Interestingly, due to the influence of maize genotypes, the difference of NUE under the same treatment in the same year was significant. In this experiment, the NUE of Xianyu 335 was significantly higher than that of other varieties under different treatments in different years, and that of Xinyu 9 was significantly lower than that of other varieties (*Table 4*). In this experiment, NUE was significantly correlated with ($P < 0.01$; $R^2 = 0.53$~0.57) NUpE (*Fig. 2a*) and not to NUtE (*Fig. 2b*).

Correlations between traits under HN and LN conditions

Figures 3 and *4* show the correlation of traits under HN and LN conditions, respectively. In general, the correlation among the traits was consistent at the two N levels. Grain dry matter yield was positively correlated ($N < 0.01$; r = 0.70~0.99) with total dry matter yield, grain N uptake and total N uptake. By contrast, at both N levels, Stover dry matter yield was positively correlated ($N < 0.01$; r = 0.99) with total dry matter yield. Total dry matter yield was significantly correlated with total N uptake and grain N uptake ($N < 0.01$; r = 0.69~0.77). Grain N uptake was highly correlated ($N < 0.01$; r = 0.92) with total N uptake. NUtE was significantly negatively correlated ($N < 0.01$; r = -0.81~-0.85) with grain N concentration (*Figs. 3* and *4*). NUpE was positively correlated with grain dry matter yield, total dry matter yield, grain N uptake and total N uptake ($N < 0.01$; r = 0.73~1) under both N conditions (*Figs. 3* and *4*).

Principal components analyses under LN and HN conditions

At the low nitrogen (*Fig. 5a*) levels, the first principal component (PC1) explained 52.4% of the variance, primarily influenced by factors related to productivity (total, stover, and grain dry matter yields), nitrogen uptake (both in grain and overall), nitrogen use efficiency NUpE (*Fig. 5a*), and NUE. The second principal component (PC2)

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represented 30.3% of the variance, with a focus on NUpE, the nitrogen harvest index, and to a lesser extent, the general harvest index. The acute angle between the load vectors suggested a strong positive correlation between NUE and the aforementioned productivity measures, nitrogen uptake, and NUpE. The harvest index showed a positive correlation with the nitrogen harvest index, with moderate linkages to NUpE. NUpE inversely correlated with stover nitrogen uptake and concentrations in both stover and grain.

Under high nitrogen (*Fig. 5b*) conditions, similar patterns were observed, with PC1 and PC2 accounting for 55.0% and 26.7% of the variance, respectively. PC1 was again dominated by productivity indicators, nitrogen uptake, NUpE, and NUE, while PC2 was influenced by NUpE and the nitrogen harvest index. NUE was significantly correlated with productivity, nitrogen uptake, and NUpE, whereas the nitrogen harvest index and NUpE exhibited negative correlations with stover and grain nitrogen concentrations.

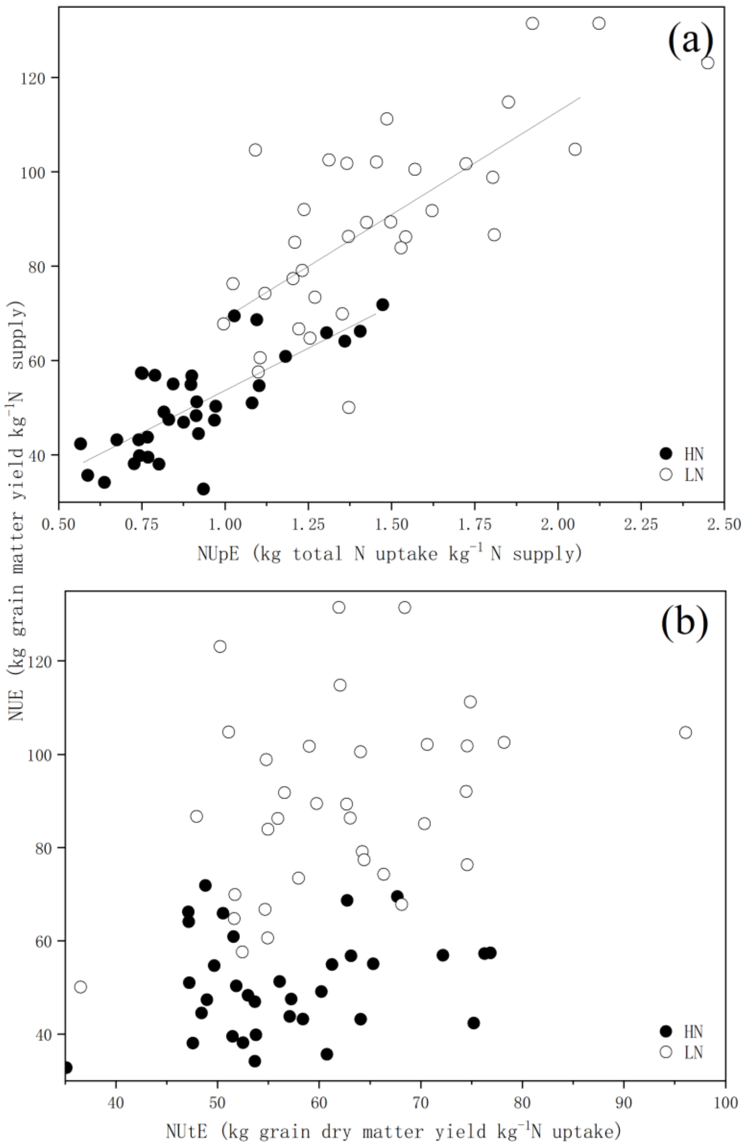

Figure 2. *Relationship between NUE and NUpE (a) and between NUE and NUtE (b) in 17 maize genotypes under150 (LN) (open symbols) and 300 kg P ha−1 (HN) (closed symbols)*

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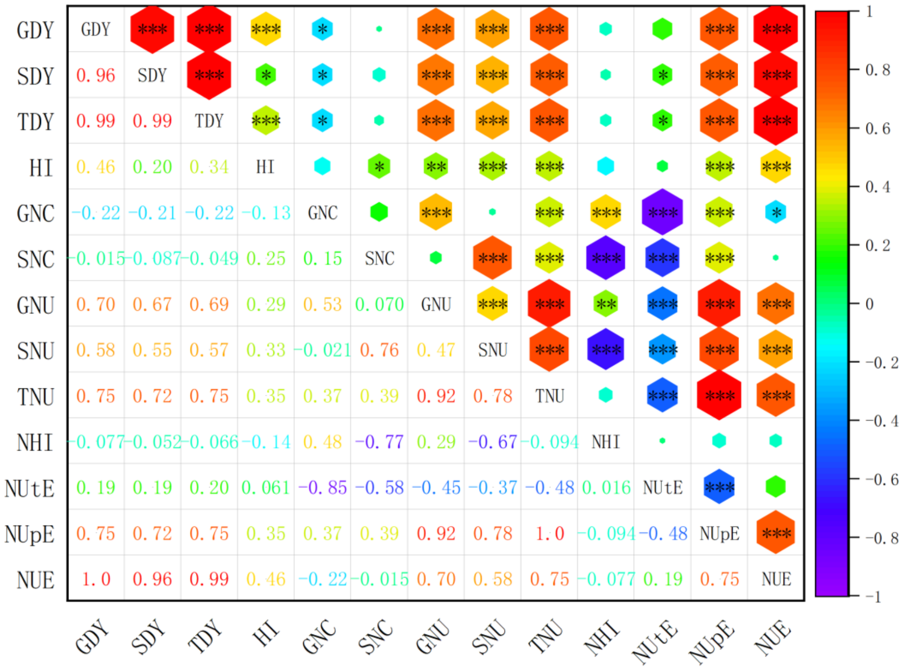

Figure 3. *Pearson correlations among traits measured in 17 maize genotypes under 300 kg N ha^{-1} (HN)*

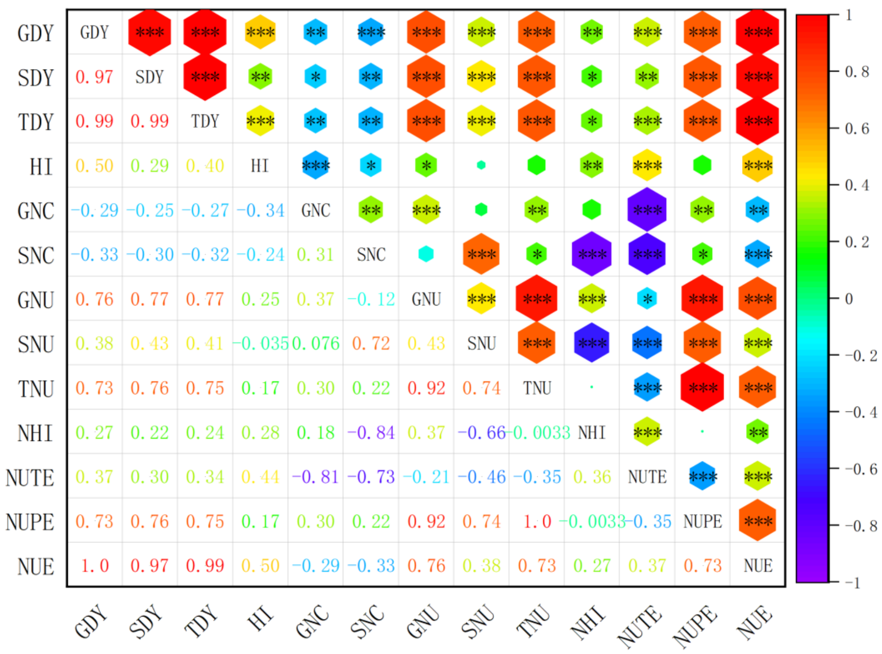

Figure 4. *Pearson correlations among traits measured in 17 maize genotypes under 150 kg N ha^{-1} (LN)*

Across both nitrogen conditions, genotypes in quadrant I, like Zhengdan958, were associated with high productivity, nitrogen uptake, harvest indices, and efficiencies. In contrast, genotypes in quadrants II and III, such as Xinyu29 and Xinyu80, displayed lower values for productivity, nitrogen uptake, NUpE, and NUE (*Fig. 5a*, *b*).

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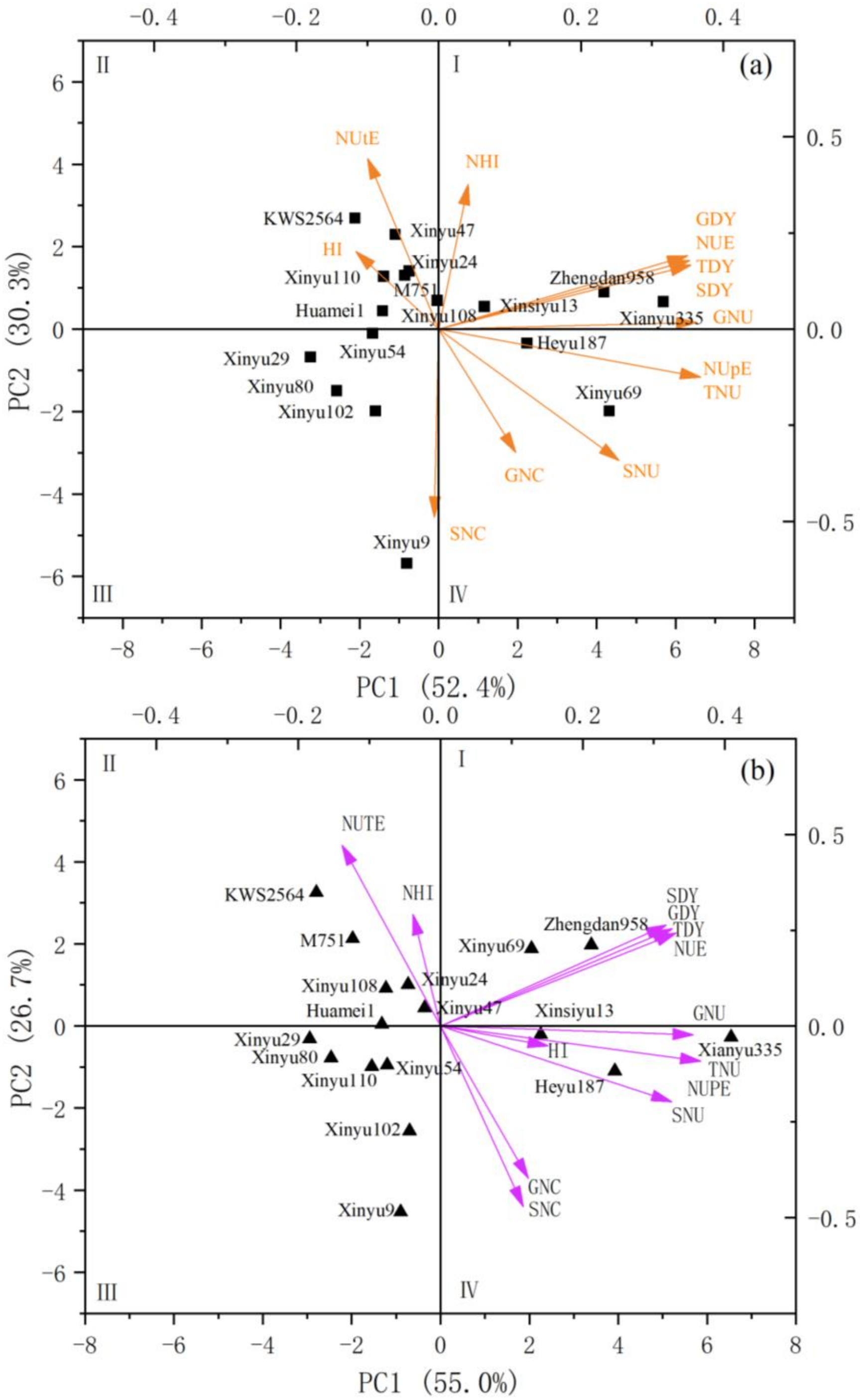

Figure 5. *Principal components analysis for 17 maize genotypes under 150 kg N ha^{-1} (a) and 300 kg N ha^{-1} (b)*

Discussion

Recent research has increasingly focused on enhancing NUE in maize by leveraging the potential of NUpE and NUtE. Developing and selecting varieties that are both nitrogen-efficient and tolerant to low nitrogen conditions is a promising strategy to address the issue of low NUE in crops. Our findings indicate that there are significant genotypic variations in NUE, NUpE, and NUtE across different nitrogen levels, a

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pattern observed in numerous other crops (Castro-Rodríguez et al., 2017; Oliveira et al., 2020; Mauceri et al., 2020).

Our study revealed that under drip irrigation, NUpE played a more critical role in determining grain dry matter yield and NUE in maize than NUtE. Consistent with this, genotypic variation in NUpE tends to exceed that of NUtE under both low and high nitrogen conditions (Vazquez-Carrasquer et al., 2021). However, it has also been demonstrated that NUtE is the primary factor accounting for the genetic variation in NUE under both low and high nitrogen scenarios (Nehe et al., 2018).

This study identified notable variability in NUpE among different maize genotypes, with NUpE showing a positive association with grain dry matter yield, total dry matter yield, and both grain and total nitrogen uptake. Enhancing total dry matter production could be a strategy to boost NUpE, especially in nitrogen-deficient conditions. The observed differences in total nitrogen uptake despite similar total dry matter production highlight the importance of genotypic diversity in NUpE.

Maize genotypes with higher NUpE, like Xinyu69 and Xianyu335, exhibit a reduced requirement for soil nitrogen to respond positively to fertilizer inputs, contrasting with those with lower NUpE, such as Xinyu110 and KWS2564. Cultivating these high NUpE genotypes can optimize nitrogen fertilizer use, mitigate environmental challenges like erosion and water pollution from runoff and leaching, and substantially enhance crop productivity in agricultural systems (Iqbal et al., 2015; Cameron et al., 2013). While our study did not assess root traits, understanding the role of root characteristics in NUpE is crucial for selecting nitrogen-efficient cultivars and improving NUE. Future research should, therefore, focus on NUpE and the underlying root traits to further optimize nitrogen use in crops.

NUtE tends to be elevated under conditions of nitrogen scarcity, which suggests that plants can enhance their nitrogen use when faced with limited availability (Hawkesford et al., 2019). This response has been documented across a range of crops, including an increase in NUtE when subjected to nitrogen deficiency (Oliveira Silva, 2020; Mauceri et al., 2020). To improve NUtE, strategies such as increasing the nitrogen harvest index and overall harvest index, or reducing plant nitrogen concentration, have been suggested (Sandaña et al., 2021).

Our findings indicate that a lower grain nitrogen concentration is associated with higher NUtE across different nitrogen levels. This correlation suggests that selecting for maize genotypes with reduced grain nitrogen concentration could be a strategy for developing varieties with improved NUtE. Furthermore, since grain nitrogen concentration is not linked to grain yield and is highly heritable, it is possible to select for lower nitrogen concentration without compromising yield.

While NUtE contribution to overall NUE may not be substantial, selecting for maize genotypes that minimize grain nitrogen concentration and extract less nitrogen from the soil can contribute to sustainable land use by reducing the depletion of soil nitrogen due to maize cultivation. This approach supports the conservation of soil fertility and the long-term productivity of agricultural systems.

The study identified certain genotypes, including those from the Xianyu335 series, as superior in NUE under both nitrogen-deficient and sufficient conditions. These cultivars represent valuable genetic resources for breeding programs aimed at enhancing NUpE and NUE. Conversely, Xinyu9 and Xinyu80 were found to be less efficient in nitrogen utilization for grain dry matter production and yield, as well as in their response to nitrogen fertilization.

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This knowledge can aid in optimizing nitrogen fertilization strategies, enabling nitrogen-sensitive genotypes to achieve higher yields with reduced fertilizer inputs. Such targeted breeding and fertilization practices can lead to more efficient nitrogen use in agriculture, which is beneficial to both crop productivity and environmental sustainability.

Conclusions

The research revealed genotype-specific variations in NUpE, NUtE and NUE among maize cultivars under low and high nitrogen supply with drip irrigation. Aligning with findings from other crops, NUE differences were predominantly linked to NUpE over NUtE. Moreover, NUpE exhibited a significant positive correlation with dry matter yield and nitrogen uptake, while NUtE inversely correlated with grain nitrogen concentration.

This comparative analysis across maize genotypes under varying nitrogen conditions offers valuable insights for breeding nitrogen-efficient varieties and enhancing NUE. Notably, Xianyu 335 emerged as a top performer in terms of NUE under both nitrogen scenarios. Furthermore, these findings are instrumental for tailoring nitrogen management practices to the specific needs of different maize genotypes, optimizing fertilizer application to enhance NUE in varying nitrogen environments.

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Conflicts of interest. There were no conflicts of interest from the authors.

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