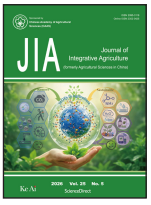
Journal of Integrative Agriculture 2026, 25(5): 1913–1926

KeAi CHINESE ROOTS GLOBAL IMPACT

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Plant growth retardant increases nitrogen utilization efficiency and harvest index in maize by optimizing the plant horizontal-vertical ratio and vascular bundle morphology

Qian Tang[1, 2*], Jianhong Ren[1*], Xinru Zhang[1], Cai Wu[1], Yarong Zhang[1], Dahong Bian[1], Guangzhou Liu[1], Yanhong Cui[1], Xiong Du[1], Chuang Wang[2#], Zhen Gao[1#]

[1] *State Key Laboratory of North China Crop Improvement and Regulation/Key Laboratory of Water-Saving Agriculture in North China, Ministry of Agriculture and Rural Affairs/Key Laboratory of Crop Growth Regulation of Hebei Province/College of Agronomy, Hebei Agricultural University, Baoding 071001, China*

[2] *College of Resources and Environment, Huazhong Agricultural University, Wuhan 430000, China*

Highlights

- Plant growth retardant (EC (an ethephon and cycocel compound)) improves nitrogen utilization efficiency and harvest index.
- The dry matter determines maize nitrogen uptake.
- EC and high-density planting can synergistically improve nitrogen use efficiency in maize.

Abstract

Improving nitrogen utilization efficiency is not only beneficial for increasing maize yield, but it can also mitigate the environmental impact of excessive nitrogen fertilizer use. Numerous studies have evaluated the impacts of plant growth retardants and plant density on plant lodging resistance and nitrogen uptake. However, the influence of plant growth retardants on nitrogen utilization efficiency (NUtE) under varying plant densities has been rarely reported. A field experiment conducted in 2020–2021 involved spraying EC (an ethephon and cycocel compound) at the 7th-leaf stage of maize with dosages of 0 (CK), 450, and 900 mL ha^{-1} at plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}. Compared to CK, the application of EC (especially the high dosage) significantly reduced plant height and dry matter, while increasing stem diameter, the plant horizontal-vertical ratio (PHVR, a new index which we define as the ratio of stem diameter of the basal first internode above the ground to plant height), and the number and area of vascular bundles. PHVR and vascular bundle morphology had significantly positive correlations with individual plant dry matter remobilization amount and its contribution to grain yield. Therefore, despite the reduced dry matter weight observed in the EC treatment, the greater dry matter remobilization enhanced the harvest index (HI). However, nitrogen uptake efficiency was not improved with the enhancement of PHVR and vascular bundle morphology, due to a reduction in dry matter accumulation. In contrast, the improved PHVR and vascular bundle were beneficial for accelerating nitrogen translocation, thus increasing NUtE significantly by 4.3–31.1% compared with CK across the plant densities. Increasing density simultaneously improved nitrogen uptake and utilization efficiency. Consequently, a high dosage application of EC under high density could not only significantly enhance lodging resistance but also improve NUtE and HI significantly by promoting the transport of dry matter and nitrogen.

Keywords: maize, vascular bundle, dry matter remobilization, nitrogen use efficiency, harvest index

Received 13 September 2024; Received in revised form 10 January 2025; Accepted 21 January 2025; Available online 20 February 2025
Qian Tang, E-mail: tangqian1998n@163.com; Jianhong Ren, E-mail: ndrenjianhong@163.com; #Correspondence Chuan Wang, E-mail: chuang.wang@mail.hzau.edu.cn; Zhen Gao, E-mail: gaozhenvision@163.com
* These authors contributed equally to this study.

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.02.037

1. Introduction

Exploring measures to increase yield and nitrogen use efficiency (NUE) is a globally recognized goal in maize production (Grassini *et al.* 2011; Zhang *et al.* 2015). Increasing planting density is a key way to increase yield (Testa *et al.* 2016; Zhang Y H *et al.* 2021). However, maize is highly susceptible to lodging under high planting density, due to inadequate canopy light and limited acquisition of nutrients, especially nitrogen (Deng *et al.* 2012; Xue *et al.* 2017). The occurrence of lodging reduces maize yield and increases the time and economic cost of harvesting (Ma *et al.* 2014; Wang *et al.* 2021). Thus, simultaneously improving yield and NUE in maize cultivation is a problem that agronomists and breeders have been dedicated to solving (Ciampitti and Vyn 2012).

Farmers usually apply excessive amounts of nitrogen fertilizer to alleviate the increased intraspecific competition under high planting density. Experimental results have shown that a modest increase in the nitrogen rate can reduce the maize lodging rate, thereby improving maize yield (Zhao *et al.* 2020; Liu *et al.* 2023). On the contrary, excess nitrogen fertilizer not only leads to lodging due to an increase in the height of the plant center of gravity, as well as reduced internode diameter and lignin content in stems (Ahmad *et al.* 2021), but it also causes environmental pollution. In addition, the excessive use of nitrogen fertilizer keeps the leaves in a perpetually green state (Antonietta *et al.* 2014; Li *et al.* 2021), which results in a high nitrogen uptake efficiency (NUpE) but a low nitrogen utilization efficiency (NUtE) and even yield loss (Lee and Tollenaar 2007; Ciampitti and Vyn 2012). Studies have indicated that the optimal N rate for an intensive maize system with smallholder farms ranges from 164 to 180 kg N ha^{-1} in the North China Plain (NCP) (Cui *et al.* 2013; Zhang *et al.* 2018). Moreover, increasing the plant density without extra N inputs can obviously improve the maize yield while also reducing nitrogen loss across China (Hou *et al.* 2020).

Spraying plant growth retardants is an effective regulatory measure under high planting density that have been proven to significantly improve maize stem lodging resistance and yield by reducing plant height, the center of gravity height, and the length of basal internodes, while increasing the diameter of basal internodes and enhancing rind penetration strength and the number and area of vascular bundles (Zhang *et al.* 2019; Gong *et al.* 2021). Plant growth retardants also alter nitrogen uptake and utilization. The application of ethylene at the V8 stage (when the 8th leaf collar is visible) reduces nitrogen uptake, but increases the NUtE (Zhang Y S *et al.* 2021). However, paclobutrazol, an inhibitor of gibberellin synthesis, does not have a significant impact on nitrogen uptake or utilization efficiency (Hütsch and Schubert 2021, 2022). It is essential to fully understand how plant growth retardants affect NUE, which is crucial for optimizing crop management to achieve high yield and high NUE under high planting density maize production.

NUE can be divided into NUpE and NUtE, which are affected by N uptake, allocation, and remobilization (Shao *et al.* 2024). Nitrogen uptake and allocation within the different vegetative organs before silking are crucial for establishing the canopy characteristics, thus determining assimilate production (Kamiji *et al.* 2014). N accumulation and remobilization after silking are closely related to leaf stay-green, photosynthesis, and nutrient uptake, which ultimately affect grain production (Lea and Azevedo 2006; Wang *et al.* 2014). In addition, the vascular system can facilitate the movement and translocation of nitrogen by transporting water and photoassimilates (Tian *et al.* 2021). A superior vascular bundle system of the ear internode and ear shank in maize can increase nitrogen translocation to the ear, improving NUtE (Liu *et al.* 2022). Plant growth retardant application reduces the leaf area index, thus reducing biomass accumulation and sink capacity (Tang *et al.* 2022), but it increases the vascular bundle area for the basal internodes of stems (Shi *et al.* 2016; Ye *et al.* 2016). Based on our previous results (Tang *et al.* 2022), maize grain yield can be significantly improved by increasing density under an optimal nitrogen application rate (187.5 kg ha^{-1}). We speculated that plant growth retardant application without increasing nitrogen input under high planting density would reduce nitrogen accumulation in maize due to reduced biomass accumulation, but it would increase NUtE due to the regulatory effect of plant growth retardants on stem and plant morphology. In this study, a field experiment with four planting densities and different concentrations of EC was conducted to: i) elucidate the effects of plant growth retardant on nitrogen uptake and nitrogen remobilization; and ii) investigate the relationships between vascular bundle and plant morphology with NUE. The results of this study may contribute to increasing NUtE in maize under a high planting density.

2. Materials and methods

2.1. Experimental site and design

The field experiments were conducted at the Hengshui Experimental Station of Hebei Academy of Agriculture and Forestry Sciences in 2020 and 2021. The soil characteristics and meteorological data were consistent with our previous study (Tang *et al.* 2022). A split-plot design with three replicates was adopted in this study. The main plots contained four planting densities (PD): 4.5, 6.0, 7.5, and 9.0 plants m^{-2} (D1, D2, D3, and D4); and the subplots contained three concentrations of EC (an ethephon and cycocel compound): 0 mL ha^{-1} EC, i.e., water control, 450 mL ha^{-1} EC, and 900 mL ha^{-1} EC (CK, T1, and T2). The EC was sprayed between 5:00 and 7:00 p.m. when the seventh leaf was fully expanded (V7). The maize variety 'Zhengdan 958' was used in this study. A specialist compound fertilizer for maize (750 kg ha^{-1}, N:P_2O_5:K_2O=25:8:12) was applied before manual sowing, at 187.5 kg N ha^{-1} (close to the optimal N rate for maize in the NCP). To guarantee the successful germination of maize, irrigation (750 m^3 ha^{-1}) was immediately conducted after manual sowing. The plot area was 80 m^2 (8 m×10 m). Pest, disease and weed management followed common practices.

2.2. Plant morphological characteristics and dry matter sampling

Three representative maize plants per plot were selected at the silking stage (R1) to measure plant height, ear height,

center of gravity height and the stem diameter of the basal first internode above the ground (Xu *et al.* 2017). Here we define a new parameter, the plant horizontal–vertical ratio (PHVR), to estimate the effects of EC on plant morphology. PHVR was calculated by the following formula:

PHVR (%)=Stem diameter of the basal first internode above ground/Plant height×100 (1)

At R1 and R6 (maturity stage), three maize plants were selected to determine dry matter accumulation. These samples were separated into stems (including sheath and tassel), leaves, and ears. At R6, the ears were further separated into ear cob, husk, and grains. All plant samples were oven-dried at 105°C for 30 min and then dried at 80°C to constant weight. The dry samples were weighed to determine dry matter accumulation, allocation and contribution to grains. Subsequently, the samples were ground with a grinder and passed through a 1 mm mesh sieve to determine N content, uptake, and remobilization.

2.3. Vascular bundle number and area

At the tasseling stage (VT) in 2021, three plants per plot were selected to obtain the basal third internode above the ground. Cross sections of the internode were manually cut with razor blades and stored in Formalin-Aceto-Alcohol fixative (FAA, 38% formaldehyde:glacial acetic acid:70% alcohol=1:1:18). After staining with saffron, the slices were decolorized with a 50, 70, and 80% alcohol gradient for 3–8 s at each step. The stem microstructure, and numbers of large, small, and total vascular bundles were observed with a Zeiss Stemi 2000-C Stereo microscope (Carl Zeiss Inc., Ontario, CA, USA). In the individual big and small vascular bundles, phloem and xylem were observed with an Olympus BX53 microscope (Olympus China Co., Ltd., Beijing, China) and analyzed by ImageJ (version 1.49, NIH, USA) according to the methods given by He *et al.* (2005). Vascular bundles with a complete structure are big vascular bundles, and the rest are small vascular bundles. The number and area of vascular bundles, phloem and xylem were calculated as:

Total number of vascular bundles (TNVB)=Small vascular bundle number (SNVB)+Big vascular bundle number (BNVB) (2)

Small vascular bundle area (SAVB)=Single small vascular bundle area (SSAVB)×SNVB (3)

Big vascular bundle area (BAVB)=Single big vascular bundle area (SBAVB)×BNVB (4)

Total vascular bundle area (TAVB)=SAVB+BAVB (5)

Proportion of small vascular bundle area to total vascular bundle area, PSVB (%)=SAVB/TAVB×100 (6)

Proportion of big vascular bundle area to total vascular bundle area, PBVB (%)=BAVB/TAVB×100 (7)

2.4. Dry matter accumulation, allocation, remobilization and contribution to grains

The harvest index (HI) was calculated by dividing the grain weight per ear at R6 by the aboveground biomass per plant. Two metrics were used to assess the dry matter accumulation of different organs based on the measured dry matter weight (DM, kg ha^{-1}) (Chen *et al.* 2013).

Organ dry matter remobilization amount (DMR, kg ha^{-1}) was calculated as:

DMR=DM at R1−DM at R6 (8)

The contribution of organ dry matter remobilization to grain yield (CDMR, %) was calculated as:

CDMR (%)=DMR/Grain DM×100 (9)

2.5. Nitrogen content, uptake and remobilization

The nitrogen accumulation (N, kg ha^{-1}) levels of different organs were determined by dry matter weight and the nitrogen concentration measured by the SmartChem 200 automated discrete analyzer (AMS Alliance, Shenzhen, China) at 660 nm. Total N accumulation of all organs at R6 and R1 were marked as total nitrogen uptake amount (NUT, kg ha^{-1}) and Pre-silking N uptake (PreN, kg ha^{-1}), respectively. The plant and grain nitrogen allocations and NUE were calculated based on previous studies (Xu *et al.* 2012; Ciampitti and Vyn 2013; Chen and Vyn 2017):

NR (organ N remobilization amount, kg ha^{-1})=N at R1−N at R6 (10)

CNR (contribution of organ N remobilization to grain N, %)=NR/N_{grain}×100 (11)

PostN (post-silking N uptake, kg ha^{-1})=NUT−PreN (12)

VNRE (vegetative N remobilization efficiency, %)=NR/PreN×100 (13)

NHI (N harvest index, %)=N_{grain}/NUT×100 (14)

NUpE (N uptake efficiency, kg kg^{-1})=NUT/Nitrogen application rate (15)

NUtE (N utilization efficiency, kg kg^{-1})=GY/NUT (16)

PFPN (partial factor productivity of N, kg kg^{-1})=GY/Nitrogen application rate (17)

where GY is the grain yield, and the nitrogen application rate was 187.5 kg ha^{-1} in this study.

2.6. Statistical analyses

General linear modeling (GLM) was applied to analyze the influences of plant density, EC, year and their interactions on every variable using IBM SPSS Statistics 25.0 (SPSS, Inc., Chicago, IL, USA). Duncan's multiple comparison method (at the 0.05 level) was employed to determine the differences among treatments. The determination coefficients and *P*-values in this article were calculated by linear regression using SPSS 25.0. The histograms with error bars were plotted using SigmaPlot 14.0 (Systat Software, Inc., San Jose, CA, USA). The heat map of Pearson's coefficients was plotted in OriginLab Pro 2021 (OriginLab Corporation, Massachusetts, USA).

3. Results

3.1. Morphology of maize plants and vascular bundles

Regardless of planting density, spraying EC at V7 significantly reduced the plant height, ear height and center of gravity height (Fig. 1), whereas it markedly increased the diameter of the first basal internode (Fig. 2). Compared with CK, the plant height, ear height and center of gravity height were

reduced by 3.7, 10.9, and 11.6% under treatment T1 and by 6.7, 18.4, and 16.9% under treatment T2 averaged for the two years under all planting densities, respectively. The basal internode diameters of T1 and T2 were 4.1 and 6.5% higher than that of the control in both years (P<0.05). As shown in Fig. 3, year, PD, and EC, as well as the interaction between year and EC significantly affected PHVR. The PHVR values under treatments T1 and T2 were significantly elevated by 6.8 and 10.0% in comparison to CK in 2020, and by 9.6 and 18.4% in 2021, respectively. The PHVR of treatments D3 and D4 exhibited significant reductions compared to that of D1 in 2020, and the PHVR of treatment D4 was remarkably lower than D1 in 2021.

The total, small and big vascular bundle numbers and areas were significantly affected by PD, EC and PD×EC (except for PD×EC with the total and big vascular bundle areas) (Table 1; Fig. 4). Across all densities, compared to the CK, the total, small and big vascular bundle numbers in the third basal internode showed significant increases of 5.5, 5.7, and 5.3% under the T1 treatment, and 9.0, 7.6, and 11.2% under the T2 treatment, respectively. The average area of single small and big vascular bundles, as well as the total, small and big vascular bundle areas under T1 exhibited significant increments of 14.0, 21.8, 23.3, 19.0, and 27.4% compared with CK, and 26.2, 22.1, 34.5, 34.4, and 34.9% under treatment T2, respectively. In summary, the effects of EC on the proportion of small or big vascular bundle area varied among the different plant densities, and the proportion of small to total vascular bundle area was notably reduced with increasing plant density.

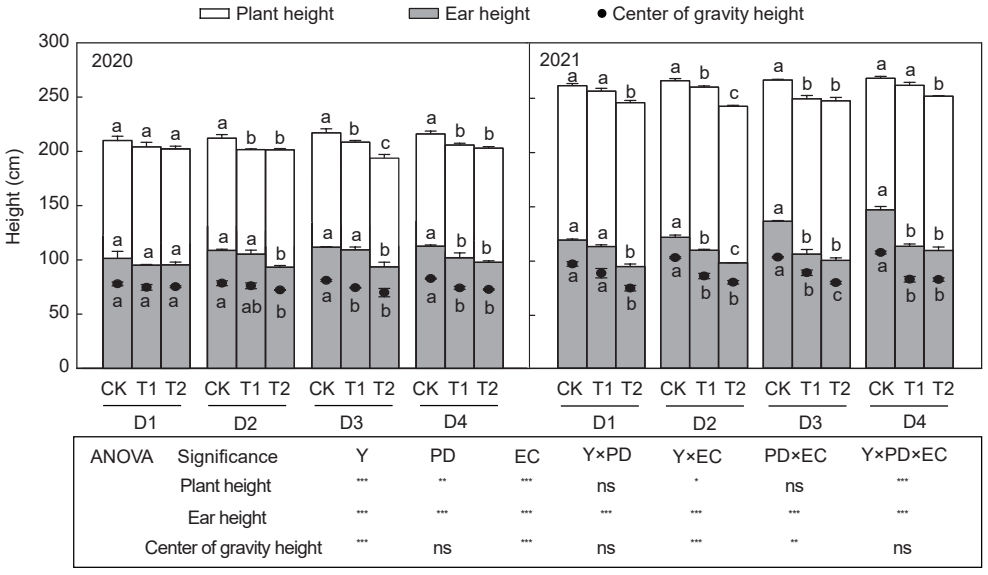


Fig. 1 Effects of plant growth retardant (EC) and plant density (PD) on the morphological structure of summer maize. Y, year. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively. Bars indicate the SD of the mean (n=3). Different lowercase letters indicate significant differences among EC treatments at the same plant density at P<0.05 according to Duncan's significant difference test. *, ** and *** indicate significant differences at the P<0.05, P<0.01 and P<0.001 probability levels, respectively; ns means non-significant.

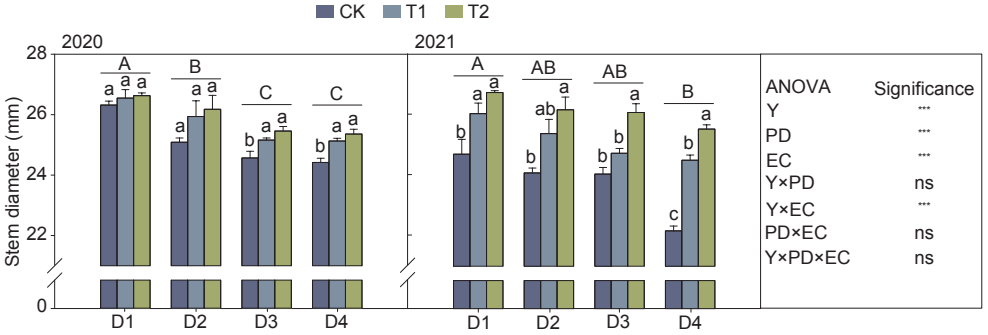


Fig. 2 Effects of plant growth retardant (EC) and plant density (PD) on the basal first internode stem diameter in 2020 and 2021. Y, year. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively. Bars indicate the SD of the mean (n=3). Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. *** indicates significant differences at the P<0.001 probability level; ns means non-significant.

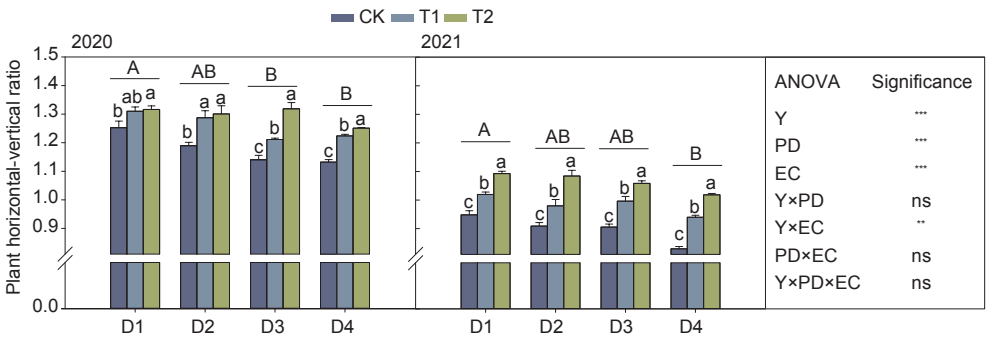


Fig. 3 Effects of plant growth retardant (EC) and plant density (PD) on the plant horizontal-vertical ratio (PHVR) in 2020 and 2021. Y, year. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively. ns means non-significant. Bars indicate the SD of the mean (n=3). Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. ** and *** indicate significant differences at the P<0.01 and P<0.001 probability levels, respectively.

Table 1 Effects of plant growth retardant (EC) and plant density (PD) on vascular bundle number and area of the basal third internode above ground at the tasseling stage in 2021[1)]

PD[2)]	EC[3)]	TNVB	SNVB	BNVB	Average area of a single small vascular bundle (×10^{-3} mm^2)			Average area of a single big vascular bundle (×10^{-3} mm^2)			TAVB (mm^2)	SAVB (mm^2)	BAVB (mm^2)	PSVB (%)	PBVB (%)
					Sum	Phloem	Xylem	Sum	Phloem	Xylem					
D1	CK	708 b	432 b	276 b	70.8 a	2.4 a	7.3 a	85.0 b	8.1 a	14.6 a	54.0 b	30.5 a	23.5 c	56.5 a	43.5 c
	T1	716 b	436 b	280 b	72.0 a	2.5 a	7.3 a	97.2 a	7.9 a	13.3 a	58.6 ab	31.3 a	27.2 b	53.5 b	46.5 b
	T2	763 a	446 a	317 a	71.2 a	2.7 a	8.5 a	97.6 a	7.5 a	13.1 a	62.6 a	31.8 a	30.9 a	50.6 c	49.4 a
	Mean	729 A	438 A	291 AB	71.3 A	2.5 B	7.7 A	93.3 A	7.9 A	13.7 B	27.2 A	58.4 A	31.2 A	53.6 A	46.4 B
D2	CK	708 b	418 b	289 b	59.0 b	2.0 b	6.9 b	88.0 b	9.8 ab	10.3 b	50.2 b	24.7 b	25.5 c	49.2 a	50.8 a
	T1	713 b	421 b	292 b	72.9 a	4.3 ab	9.6 a	100.2 a	7.4 b	13.1 ab	59.9 a	30.6 a	29.3 b	51.0 a	49.0 a
	T2	747 a	428 a	320 a	73.7 a	4.6 a	10.2 a	104.6 a	12.3 a	16.9 a	64.9 a	31.5 a	33.4 a	48.5 a	51.5 a
	Mean	722 A	422 A	300 A	68.5 AB	3.7 A	8.9 A	97.6 A	9.8 A	13.4 B	29.4 A	58.3 AB	28.9 A	49.6 B	50.4 A
D3	CK	693 b	405 b	288 a	49.0 b	2.1 a	9.1 a	80.6 b	9.0 a	17.1 a	43.1 c	19.9 b	23.2 b	45.8 b	54.2 a
	T1	711 ab	420 ab	291 a	56.7 b	2.1 a	6.8 a	98.3 a	8.8 a	16.4 a	52.4 b	23.8 b	28.6 a	45.3 b	54.7 a
	T2	723 a	432 a	291 a	77.9 a	2.1 a	7.0 a	97.9 a	11.1 a	16.3 a	62.1 a	33.7 a	28.5 a	54.2 a	45.8 b
	Mean	709 A	419 A	290 AB	61.2 BC	2.1 B	7.6 A	92.3 A	9.6 A	16.6 A	26.8 AB	52.5 B	25.8 A	48.4 B	51.6 A
D4	CK	582 b	335 b	247 b	47.2 b	2.9 a	8.3 a	71.6 b	6.7 a	9.8 b	33.7 b	15.9 b	17.8 b	47.3 a	52.7 a
	T1	698 a	403 a	295 a	56.0 a	2.9 a	8.5 a	100.3 a	8.5 a	15.2 a	52.2 a	22.6 a	29.6 a	43.4 a	56.6 a
	T2	700 a	405 a	295 a	62.5 a	2.7 a	8.9 a	96.9 ab	10.7 a	15.2 a	53.9 a	25.3 a	28.6 a	47.2 a	52.8 a
	Mean	660 B	381 B	279 B	55.2 C	2.8 AB	8.6 A	89.6 A	8.6 A	13.4 B	25.3 B	46.6 C	21.3 A	45.9 B	54.1 A
ANOVA															
PD		***	***	***	***	*	ns	ns	ns	***	***	***	*	***	***
EC		***	***	***	***	ns	ns	***	*	**	***	***	***	ns	ns
PDÍEC		***	**	***	**	ns	*	ns	ns	**	ns	**	ns	**	**

[1)] TNVB, SNVB, and BNVB represent total, small, and big vascular bundle numbers, respectively; TAVB, SAVB, and BAVB represent total, small, and big vascular bundle areas, respectively; PSVB and PBVB represent the proportions of small and big to total vascular bundle area.
[2)] D1, D2, D3 and D4 indicate 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively.
[3)] CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively.
Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. *, ** and *** indicate significant differences at the P<0.05, P<0.01 and P<0.001 probability levels, respectively; ns means non-significant.

3.2. Dry matter accumulation, remobilization and harvest index

Year, PD, and EC had significant impacts on the dry matter weight of each organ at R1 and R6, and on pre-silking and post-silking dry matter accumulation (Table 2). Across all densities, the dry matter weight of stems and leaves at R1

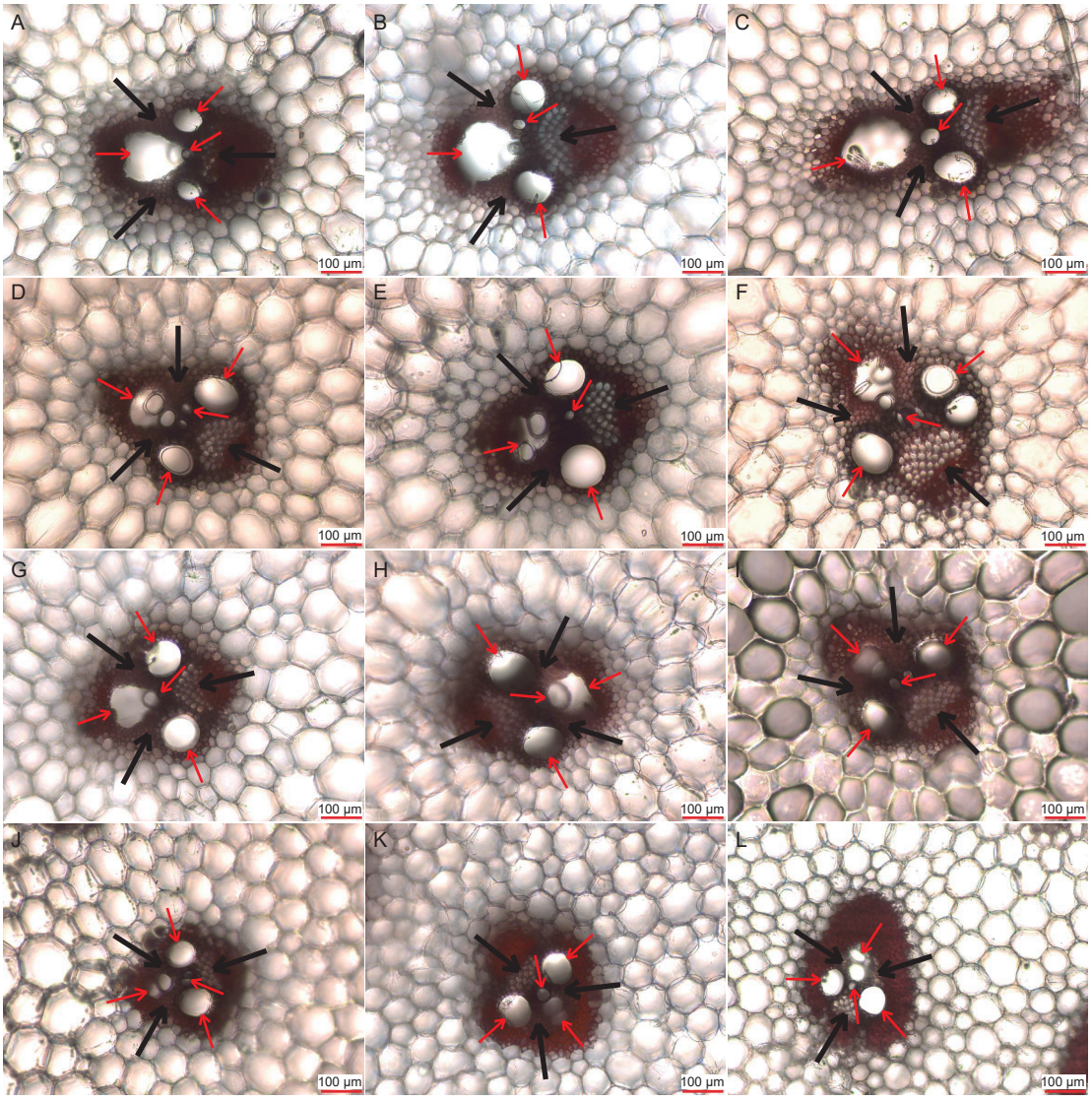


Fig. 4 Effects of plant growth retardant (EC) and plant density (PD) on the big vascular bundles of the basal third internode above ground at the tasseling stage of D1-CK (A), D1-T1 (B), D1-T2 (C), D2-CK (D), D2-T1 (E), D2-T2 (F), D3-CK (G), D3-T1 (H), D3-T2 (I), D4-CK (J), D4-T1 (K), and D4-T2 (L). Red arrows indicate xylem and black arrows indicate phloem. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively.

were significantly reduced by 7.1 and 11.3% under T1, and by 10.8 and 17.0% under T2 in 2020–2021, respectively. Similarly, stem dry matter weight at R6 was significantly reduced by 6.6 and 13.7%, and by 12.4 and 17.2% for leaves under T1 and T2 in both years, respectively, compared to CK. Under all T1 treatments, pre-silking and post-silking dry matter accumulation significantly declined by 8.4 and 5.3%, respectively; and by 12.8 and 9.7% for T2, compared to the control. With increasing density, the dry matter weight of maize was significantly increased.

The leaf and stem dry matter remobilization amounts and the contribution to grain yield were significantly affected by year, PD, EC, and their interactions (Fig. 5). EC application significantly increased the absolute value of the dry matter remobilization amount and its contribution to grain at all densities in both years. Despite the negative dry matter remobilization amount of vegetative organs in 2020, its absolute magnitude still reflects the transport capacity of nutrients from the organs. Specifically, the absolute value of the leaf dry matter remobilization amounts of T1 and T2 were 30.8–228.0% and 39.3–258.9% higher than that of CK at each density in both years, respectively. Compared with CK, the absolute values of the contribution of leaf dry matter remobilization to grain yield under T1 and T2 were increased by 38.3–249.1% and 53.9–283.5% in both years, respectively. In addition, the absolute values of the stem dry matter remobilization amount of EC were significantly improved by 18.9 and 23.4% in 2020, and by 27.0 and 69.7% in 2021 under T1 and T2, respectively. The absolute values of the contribution of stem dry matter remobilization to grain yield under T1 and T2 were increased by 21.5 and 28.2% in 2020, and by 37.6 and 93.8% in 2021, respectively. The density analysis indicated that with increasing density, the dry weight remobilization amount and the contribution to grain yield exhibited trends of or decreasing or first increasing and then

Table 2 Effects of plant growth retardant (EC) and plant density (PD) on leaf, stem and grain dry matter weights per hectare at the silking and harvesting stages in 2020 and 2021[1)]

Year (Y)	PD[2)]	EC[3)]	DM_{R1L} (Mg ha^{-1})	DM_{R1S} (Mg ha^{-1})	DM_{R6L} (Mg ha^{-1})	DM_{R6S} (Mg ha^{-1})	DM_{R6G} (Mg ha^{-1})	PreDM (Mg ha^{-1})	PostDM (Mg ha^{-1})
2020	D1	CK	1.80 a	2.84 a	2.01 a	3.45 a	7.23 a	4.64 a	8.06 b
		T1	1.35 b	2.46 b	1.64 b	3.39 a	7.15 ab	3.81 b	8.37 ab
		T2	1.31 c	2.36 b	1.63 b	3.24 b	7.09 b	3.67 b	8.28 a
		Mean	1.49 D	2.55 D	1.76 D	3.36 D	7.16 D	4.04 D	8.24 D
	D2	CK	2.23 a	3.17 a	2.51 a	4.39 a	8.90 a	5.40 a	10.39 a
		T1	1.66 b	2.76 b	2.05 b	3.89 b	8.66 a	4.43 b	10.17 a
		T2	1.52 c	2.66 b	1.99 b	3.88 b	8.55 a	4.18 c	10.23 a
		Mean	1.80 C	2.87 C	2.18 C	4.05 C	8.70 C	4.67 C	10.26 C
	D3	CK	2.49 a	3.78 a	2.67 a	4.90 a	9.80 a	6.27 a	11.11 a
		T1	2.22 b	3.34 b	2.59 b	4.70 b	9.71 a	5.56 b	11.45 a
		T2	1.90 c	3.18 c	2.41 c	4.13 c	9.19 b	5.07 c	10.65 b
		Mean	2.20 B	3.43 B	2.56 B	4.58 B	9.57 B	5.63 B	11.07 B
	D4	CK	2.97 a	4.31 a	3.11 a	4.98 a	10.66 a	7.28 a	11.46 b
		T1	2.55 b	4.05 b	3.09 a	4.91 a	10.61 a	6.59 b	12.02 a
		T2	2.31 c	3.80 c	2.91 b	4.73 b	10.53 a	6.11 c	12.06 a
		Mean	2.61 A	4.05 A	3.04 A	4.87 A	10.60 A	6.66 A	11.85 A
2021	D1	CK	1.88 a	3.84 a	1.41 a	3.35 a	9.25 a	5.72 a	8.29 a
		T1	1.86 a	3.88 a	1.26 b	3.21 b	8.58 ab	5.74 a	7.31 b
		T2	1.73 b	3.78 a	1.10 c	2.77 c	8.05 b	5.51 a	6.41 c
		Mean	1.82 C	3.83 C	1.26 C	3.11 C	8.63 C	5.66 D	7.34 C
	D2	CK	1.83 a	3.42 a	1.55 a	2.98 a	8.27 a	7.01 a	10.06 a
		T1	1.77 a	3.42 a	1.30 b	2.95 a	7.76 b	6.93 a	9.09 b
		T2	1.63 b	3.37 a	1.10 c	2.79 b	7.11 c	6.67 b	8.01 c
		Mean	1.75 C	3.40 D	1.32 C	2.91 C	7.71 D	6.87 C	9.05 B
	D3	CK	2.84 a	5.36 a	2.51 a	4.73 a	11.59 a	8.20 a	10.64 a
		T1	2.52 b	4.60 b	1.87 b	3.95 b	10.38 b	7.12 b	9.09 b
		T2	2.50 b	4.62 b	1.84 b	3.74 c	10.07 b	7.12 b	8.53 c
		Mean	2.62 B	4.86 B	2.08 B	4.14 B	10.68 B	7.48 B	9.42 B
	D4	CK	2.95 a	5.89 a	2.78 a	5.37 a	13.05 a	8.84 a	12.35 a
		T1	2.91 a	5.78 a	2.45 b	4.89 b	11.88 b	8.69 a	10.53 b
		T2	2.87 a	5.33 b	2.38 b	4.20 c	11.85 b	8.20 b	10.23 b
		Mean	2.91 A	5.67 A	2.54 A	4.82 A	12.26 A	8.58 A	11.04 A
ANOVA									
Y			***	***	***	***	***	***	***
PD			***	***	***	***	***	***	***
EC			***	***	***	***	***	***	***
Y×PD			***	***	***	***	***	***	***
Y×EC			***	***	***	***	***	***	***
PD×EC			**	***	***	***	ns	***	ns
Y×PD×EC			***	***	***	***	ns	***	*

[1)] DM_{R1L} and DM_{R1S} represent leaf and stem dry matter weights at the silking stage; DM_{R6L}, DM_{R6S}, and DM_{R6G}, represent leaf, stem and grain dry matter weights at the harvesting stage; PreDM and PostDM, represent pre-silking and post-silking dry matter accumulation.
[2)] D1, D2, D3 and D4 indicate 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively.
[3)] CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively.
Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. *, ** and *** indicate significant differences at the P<0.05, P<0.01 and P<0.001 probability levels, respectively; ns means non-significant.

decreasing.

Year, PD, EC, and year×PD×EC each had a pronounced influence on HI in 2020–2021 (Fig. 6). HI decreased with an increase in density. Across all plant densities, the averages of HI under T1 and T2 increased by 2.4 and 4.0% compared to CK in 2020, and by 0.7 and 2.5% in 2021, respectively.

3.3. Nitrogen accumulation, remobilization, uptake and utilization

Year, PD, EC and year×PD had significant impacts on the N accumulation of different organs, organ N remobilization amounts and the contributions to grain N (except for EC and stem N content at R1 and the leaf N remobilization amount) (Table 3). The N accumulation of each organ increased with density, and EC application reduced the N accumulation to varying degrees in different densities. Averaged for all densities and years, the grain N accumulations of T1 and T2 at R6 were notably reduced by 14.5 and 24.2%, respectively, compared to the control. Compared with CK, the average stem N remobilization amount was significantly improved by

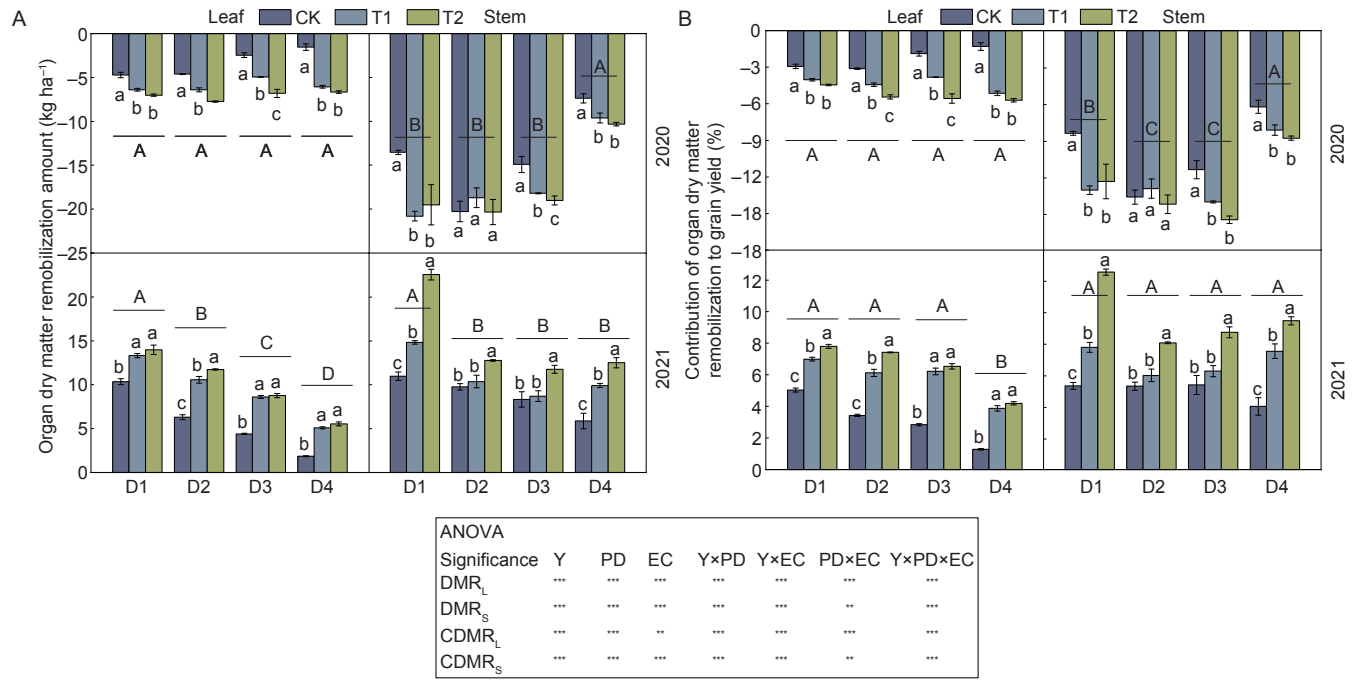


Fig. 5 Effects of plant growth retardant (EC) and plant density (PD) on leaf and stem dry matter remobilization amounts (A) and the contribution of leaf and stem dry matter remobilization to grain yield (B) in 2020 and 2021. DMR_L and DMR_S, leaf and stem dry matter remobilization amounts; $CDMR_L$ and $CDMR_S$, contributions of leaf and stem dry matter remobilization to grain yield; Y, year. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively. Bars indicate the SD of the mean (n=3). Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. ** and *** indicate significant differences at the P<0.01 and P<0.001 probability levels, respectively.

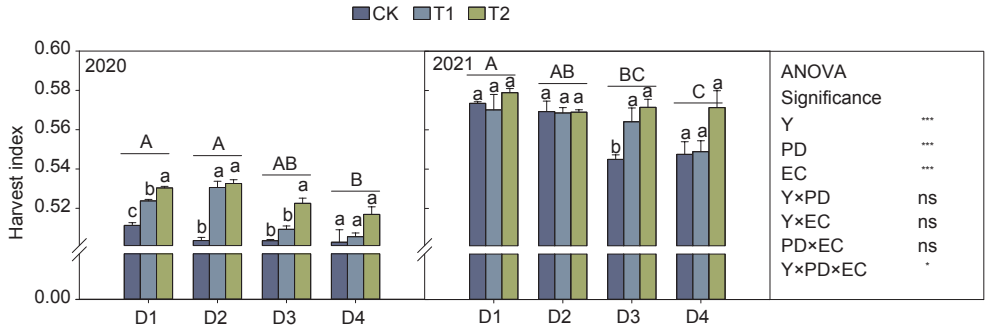


Fig. 6 Effects of plant growth retardant (EC) and plant density (PD) on the harvest index in 2020 and 2021. Y, year. D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively; CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively. Bars indicate the SD of the mean (n=3). Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. * and *** indicate significant differences at the P<0.05 and P<0.001 probability levels, respectively; ns means non-significant.

11.2% under T1 and 21.9% under T2. The contribution of the stem N remobilization amount to grain N had a significant improvement of 28.7% under T1, and further increased by 54.6% under T2.

Relevant indicators of nitrogen use were significantly influenced by year, PD, EC and year×PD, but EC did not have a significant impact on NHI, and year×PD did not significantly affect PFPN (Table 4). Compared with CK, the high dose of EC (T2) significantly reduced PostN and NUT by 18.5–68.3% and 11.5–26.9%, respectively. In comparison to the control, T1 and T2 exhibited significant increases in vegetative N remobilization efficiency (VNRE) across all densities, which rose by 13.8 and 23.4% in 2020, and by 47.8 and 54.0% in 2021, respectively. Compared with CK, the NUpE of T1 and T2 were markedly diminished by 12.5 and 19.8% across all densities, while the NUtE of T1 and T2 were increased by 10.0 and 16.2%. In both years, PFPN at each density experienced significant declines of 3.8 and

Table 3 Effects of plant growth retardant (EC) and plant density (PD) on leaf and stem N contents, remobilization amounts, and the contribution of N remobilization to grain N in 2020 and 2021[1)]

Year (Y)	PD[2)]	EC[3)]	N_{R1L} (kg ha^{-1})	N_{R1S} (kg ha^{-1})	N_{R6L} (kg ha^{-1})	N_{R6S} (kg ha^{-1})	N_{R6G} (kg ha^{-1})	NR_L (kg ha^{-1})	NR_S (kg ha^{-1})	CNR_L (%)	CNR_S (%)
2020	D1	CK	35.0 a	31.3 a	34.5 a	16.3 a	102.9 a	0.5 a	15.0 a	0.5 a	14.6 a
		T1	31.7 b	30.6 a	30.4 b	16.0 a	84.8 b	1.3 a	14.6 a	1.4 a	17.4 a
		T2	32.8 ab	29.4 a	30.4 b	13.6 a	79.3 b	2.4 a	15.8 a	2.9 a	19.9 a
		Mean	33.2 D	30.4 D	31.8 D	15.3 A	89.0 B	1.4 A	15.1 D	1.6 A	17.3 C
	D2	CK	45.8 a	36.3 a	44.4 a	19.6 a	127.7 a	1.3 a	16.8 a	1.0 a	13.1 b
		T1	38.6 b	33.8 a	36.1 b	13.2 b	98.2 b	2.4 a	20.5 a	2.5 a	20.9 ab
		T2	35.8 b	35.5 a	35.9 b	11.7 b	92.6 b	–0.1 a	23.8 a	0.0 a	26.0 a
		Mean	40.0 C	35.2 C	38.8 C	14.8 A	106.2 A	1.2 A	20.4 C	1.2 A	20.0 BC
	D3	CK	50.4 a	43.8 a	48.8 a	19.2 a	120.8 a	1.6 a	24.6 b	1.4 a	21.0 a
		T1	49.7 a	43.3 a	47.6 a	18.2 a	112.5 a	2.1 a	25.0 ab	1.8 a	22.4 a
		T2	45.7 b	41.9 a	46.5 a	13.3 b	100.2 a	–0.8 a	28.6 a	–0.8 a	28.7 a
		Mean	48.6 B	43.0 B	47.7 B	16.9 A	111.2 A	1.0 A	26.1 B	0.8 A	24.0 AB
	D4	CK	61.8 a	46.4 b	58.4 a	20.4 a	105.7 a	3.4 a	26.0 b	3.0 a	22.3 b
		T1	58.7 a	50.0 a	59.7 a	18.6 ab	93.5 ab	–1.0 ab	31.3 a	–1.0 ab	29.1 a
		T2	53.3 b	48.5 a	57.0 a	14.5 b	66.1 b	–3.7 b	34.0 a	–3.6 b	33.7 a
		Mean	57.9 A	48.3 A	58.4 A	17.9 A	88.4 AB	–0.4 A	30.4 A	–0.5 A	28.4 A
2021	D1	CK	55.9 a	40.4 b	23.0 a	23.1 a	111.3 a	32.9 a	17.3 b	29.5 c	15.5 c
		T1	55.2 a	40.9 b	21.5 a	21.9 b	102.3 b	33.6 a	19.0 b	32.9 b	18.6 b
		T2	52.8 a	42.8 a	18.7 b	18.7 c	86.4 c	34.1 a	24.0 a	39.4 a	27.8 a
		Mean	54.6 D	41.4 D	21.1 D	21.2 A	100.0 C	33.5 D	20.1 D	33.9 B	20.7 B
	D2	CK	72.7 a	48.3 a	32.4 a	25.5 a	135.9 a	40.2 a	22.8 a	29.7 b	16.8 b
		T1	70.9 ab	49.6 a	27.3 b	22.0 a	115.4 b	43.6 a	27.6 a	37.8 a	23.9 a
		T2	66.8 b	49.2 a	24.3 b	24.2 a	105.5 c	42.5 a	25.0 a	40.4 a	23.7 a
		Mean	70.1 C	49.1 C	28.0 C	23.9 A	118.9 B	42.1 C	25.1 C	36.0 AB	21.5 B
	D3	CK	81.3 a	54.3 a	37.3 a	19.1 a	139.2 a	44.0 a	35.2 a	31.6 b	25.2 b
		T1	78.9 a	52.1 a	29.9 b	16.2 a	109.7 b	49.0 a	35.9 a	44.7 a	32.7 ab
		T2	79.1 a	52.4 a	30.5 b	14.5 a	105.1 b	48.6 a	37.9 a	46.1 a	36.2 a
		Mean	79.8 B	52.9 B	32.6 B	16.6 B	118.0 B	47.2 B	36.3 B	40.8 A	31.4 A
	D4	CK	92.0 a	65.8 a	36.3 a	29.4 a	156.1 a	55.7 a	36.5 b	35.7 a	23.4 c
		T1	93.8 a	63.7 a	38.4 a	21.7 ab	138.3 b	55.4 a	42.0 ab	40.1 a	30.5 b
		T2	93.5 a	62.4 a	38.9 a	14.7 b	122.5 c	54.6 a	47.6 a	44.6 a	38.9 a
		Mean	93.1 A	64.0 A	37.9 A	21.9 A	139.0 A	55.2 A	42.0 A	40.1 A	30.9 A
ANOVA											
Y			***	***	***	***	***	***	***	***	***
PD			***	***	***	**	***	***	***	*	***
EC			***	ns	***	***	***	ns	***	***	***
Y×PD			***	***	***	***	***	***	***	***	*
Y×EC			ns	ns	ns	ns	ns	ns	ns	***	ns
PD×EC			ns	ns	***	*	ns	ns	ns	ns	ns
Y×PD×EC			ns	**	*	ns	ns	ns	ns	ns	ns

[1)] N_{R1L} and N_{R1S} represent the N contents of leaves and stems per hectare at the silking stage; N_{R6L}, N_{R6S} and N_{R6G} represent the N contents of leaves, stems and grains per hectare at the harvesting stage; NR_L and NR_S represent the N remobilization amounts of leaves and stems; CNR_L and CNR_S represent the contributions of leaf and stem N remobilization to grain N, respectively.
[2)] D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively.
[3)] CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively.
Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. *, ** and *** indicate significant differences at the P<0.05, P<0.01 and P<0.001 probability levels, respectively; ns means non-significant.

6.8% under treatments T1 and T2, respectively. In addition, with an increase in density, the NUT, PreN, VNRE, NUpE, NUtE, and PFPN were significantly increased, while PostN was not significantly different. However, NHI was significantly reduced with density increases.

3.4. Correlation analysis

Significantly positive correlations were observed between PHVR and the different parameters of vascular bundles (Fig. 7). Dry matter remobilization of the total plant, including leaves and stems (DMR_p) and the contribution to grain yield ($CDMR_p$), and HI were highly significantly positively correlated with various indicators of vascular bundles. Similarly, N remobilization of the total plant, including leaves and stems (NR_p), had significant positive correlations with all vascular bundles indicators, while the contribution to grain N (CNR_p) was only significantly positively correlated with the big vascular bundle indicators. NUtE showed significantly positive correlations with the numbers and areas of all vascular bundles, except for the small and individual

Table 4 Effects of plant growth retardant (EC) and plant density (PD) on maize plant N uptake, remobilization and utilization efficiency related traits in 2020 and 2021[1)]

Year (Y)	PD[2)]	EC[3)]	NUT (kg ha^{-1})	PreN (kg ha^{-1})	PostN (kg ha^{-1})	VNRE (%)	NHI (%)	NUpE (kg kg^{-1})	NUtE (kg kg^{-1})	PFPN (kg kg^{-1})
2020	D1	CK	153.7 a	66.4 a	87.3 a	23.3 a	66.9 a	0.82 a	59.6 b	48.8 a
		T1	131.3 b	62.3 a	69.0 b	25.5 a	64.5 a	0.70 b	65.9 a	46.0 b
		T2	123.3 b	62.2 a	61.2 b	29.1 a	64.3 a	0.66 b	68.3 a	44.9 b
		Mean	136.1 C	63.6 D	72.5 A	26.0 A	65.2 A	0.73 C	64.6 B	46.6 D
	D2	CK	191.7 a	82.1 a	109.6 a	22.0 b	66.6 a	1.02 a	57.2 b	58.4 a
		T1	147.6 b	72.3 b	75.3 b	31.6 a	66.6 a	0.79 b	72.0 a	56.7 ab
		T2	140.2 b	71.3 b	68.9 b	33.2 a	66.0 a	0.75 b	74.9 a	55.9 b
		Mean	159.8 B	75.2 C	84.6 A	28.9 A	66.4 A	0.85 B	68.0 AB	57.0 C
	D3	CK	188.8 a	94.2 a	94.6 a	27.8 a	63.7 a	1.01 a	66.5 a	66.2 a
		T1	178.4 a	93.0 a	85.4 a	29.2 a	63.0 a	0.95 ab	65.7 a	62.5 b
		T2	160.0 a	87.6 b	72.4 a	31.7 a	62.6 a	0.85 b	69.4 a	59.2 c
		Mean	175.7 AB	91.6 B	84.1 A	29.6 A	63.1 B	0.94 AB	67.2 AB	62.6 B
	D4	CK	195.2 a	108.2 a	87.0 a	27.2 a	59.6 a	1.04 a	67.5 b	70.3 a
		T1	186.0 ab	108.6 a	77.4 ab	27.8 a	57.9 a	0.99 ab	69.6 ab	69.0 a
		T2	172.8 b	101.9 b	70.9 b	29.8 a	58.6 a	0.92 b	74.7 a	68.7 a
		Mean	184.7 A	106.2 A	78.4 A	28.2 A	58.7 C	0.98 A	70.6 A	69.3 A
2021	D1	CK	157.4 a	96.3 a	61.2 a	52.1 b	70.7 a	0.84 a	64.2 b	53.9 a
		T1	145.7 b	96.0 a	49.7 b	54.8 b	70.2 a	0.78 a	65.4 b	50.8 ab
		T2	123.8 c	95.6 a	28.3 c	60.7 a	69.7 a	0.66 b	75.6 a	49.8 b
		Mean	142.3 C	96.0 D	46.4 A	55.9 B	70.2 A	0.76 C	68.4 B	51.5 D
	D2	CK	193.8 a	121.0 a	72.8 a	52.1 b	70.1 a	1.03 a	63.4 c	65.5 a
		T1	164.7 b	120.6 a	44.2 b	59.1 a	70.1 a	0.88 b	66.1 b	58.1 b
		T2	153.9 c	116.0 a	37.9 b	58.2 a	68.5 a	0.82 c	69.6 a	57.2 b
		Mean	170.8 B	119.2 C	51.6 A	56.5 B	69.6 A	0.91 B	66.4 B	60.3 C
	D3	CK	195.6 a	135.6 a	59.9 a	58.4 b	71.2 a	1.04 a	67.8 b	70.7 a
		T1	155.9 b	131.1 a	24.8 b	64.8 a	70.4 a	0.83 b	80.9 a	67.3 ab
		T2	150.1 b	131.5 a	18.6 b	65.8 a	70.0 a	0.80 b	82.6 a	66.1 b
		Mean	167.2 B	132.7 B	34.4 A	63.0 A	70.5 A	0.89 B	77.1 A	68.0 B
	D4	CK	221.8 a	157.8 a	64.0 a	58.5 a	70.4 a	1.18 a	62.7 b	74.2 a
		T1	198.5 b	157.5 a	41.0 b	61.7 a	69.7 a	1.06 b	74.4 ab	78.3 a
		T2	176.2 c	155.9 a	20.3 c	65.7 a	69.6 a	0.94 b	76.3 a	71.7 a
		Mean	198.8 A	157.1 A	41.7 A	61.9 A	69.9 A	1.06 A	71.2 AB	74.7 A
ANOVA										
Y			**	***	***	***	***	*	***	***
PD			***	***	**	***	***	***	***	***
EC			***	***	***	***	ns	***	***	***
Y×PD			***	***	***	*	***	***	***	ns
Y×EC			ns	ns	*	ns	ns	ns	ns	ns
PD×EC			ns	ns	ns	ns	ns	ns	ns	*
Y×PD×EC			*	ns	ns	ns	ns	**	***	*

[1)] NUT, total N uptake; PreN, pre-silking N uptake; PostN, post-silking N uptake; VNRE, vegetative N remobilization efficiency; NHI, N harvest index; NUpE, N uptake efficiency; NUtE, N utilization efficiency; PFPN, partial factor productivity of N.
[2)] D1, D2, D3, and D4 indicate plant densities of 4.5, 6.0, 7.5, and 9.0 plants m^{-2}, respectively.
[3)] CK, T1, and T2 indicate water control, 2 mL L^{-1} EC (ethephon:cycocel=3:1), and 4 mL L^{-1} EC, respectively.
Different lowercase and capital letters indicate significant differences among EC treatments at the same plant density and different plant densities, respectively, at P<0.05 according to Duncan's significant difference test. *, ** and *** indicate significant differences at the P<0.05, P<0.01 and P<0.001 probability levels, respectively; ns means non-significant.

small vascular bundle areas (Fig. 7). However, NUpE was significantly negatively correlated with all the parameters of vascular bundles. In addition, the indicators related to the small and total vascular bundles were also significantly negatively correlated with PFPN.

The correlation analysis revealed that pre- and post-silking dry matter accumulation and pre-silking N accumulation were very significantly positively correlated with NUpE in 2020 and 2021 (Fig. 8). Therefore, the determination of N uptake efficiency resulted from the combined effects of pre- and post-silking N accumulation. The correlation analysis revealed that PHVR was significantly positively correlated not only with the absolute value of dry matter remobilization per plant and its contribution to grain yield, but also with harvest index and NUtE (Fig. 9). Therefore, we believe that the increase in dry matter remobilization and its contribution to grain yield led to an increase in the harvest index, ultimately enhancing NUtE.

4. Discussion

4.1. hanges in dry matter determine nitrogen uptake

Dry matter accumulation is the material foundation for maize

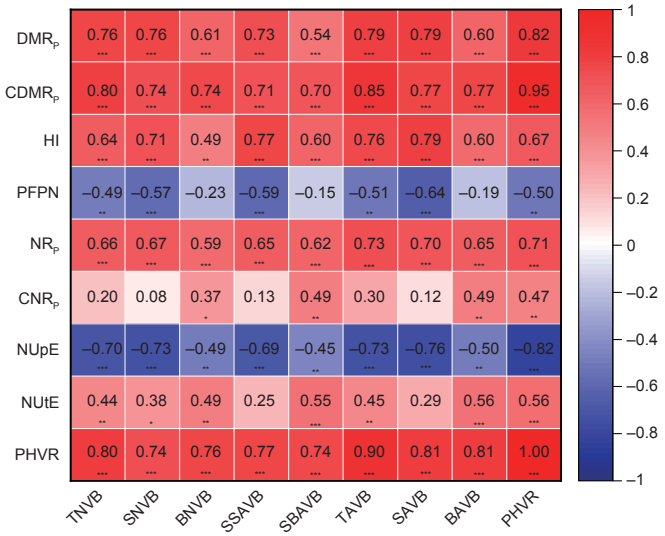


Fig. 7 Relationships between TNVB, SNVB, BNVB, SSAVB, SBAVB, TAVB, SAVB, BAVB, PHVR and DMR$_P$, with CDMR$_P$, HI, PFPN, NR$_P$, CNR$_P$, NUpE, NUtE, and PHVR in 2021 (n=36). TNVB, SNVB and BNVB indicate total, small and big vascular bundle numbers; SSAVB and SBAVB indicate single small and big vascular bundle areas; TAVB, SAVB and BAVB indicate total, small and big vascular bundle areas. DMR$_P$, total dry matter remobilization per plant; CDMR$_P$, contribution of DMR$_P$ to grain yield; HI, harvest index; PFPN, partial factor productivity of N; NR$_P$, total N remobilization per plant; CNR$_P$, contribution of NR$_P$ to grain yield; NUpE, N uptake efficiency; NUtE, N utilization efficiency; PHVR, plant horizontal-vertical ratio. Numbers represent Pearson's correlation coefficient R. − represents a negative correlation between the two indicators. *, ** and *** indicate significant differences at P<0.05, P<0.01 and P<0.001, respectively.

yield formation. Higher biomass is usually accompanied by higher leaf area and photosynthetic capacity, which allows maize to convert absorbed nitrogen into organic nitrogen compounds more effectively (Wei *et al.* 2015). Moreover, higher biomass typically indicates stronger metabolic activity, which can provide more energy (such as ATP) to drive the active uptake of nitrogen (Xu *et al.* 2012; Kamiji *et al.* 2014; Bloom 2015). The compound EC, which is composed of ethylene and cycocel, has been reported to inhibit cell division and expansion by synthesizing the ethylene precursor ACC (Saiz-Fernández *et al.* 2017), thus resulting in less leaf growth and dry matter accumulation (Tang *et al.* 2022). Our results showed that EC treatments reduced biomass. Some studies reported that reduced biomass accumulation indicates an insufficient photosynthetic supply capacity and reduced N uptake, which drive more assimilate and N remobilization from the vegetative organs to the grain (Zhang Y S *et al.* 2021; Tang *et al.* 2022). This study also indicated that EC reduced N uptake but increased N remobilization (Table 3), and the greater transportation capability of vascular bundles would promote N uptake (Xu *et al.* 2017). However, a negative correlation between vascular bundle area and N uptake was observed in this study. This suggested that biomass plays a dominant role in N uptake while vascular bundles do not. In addition to the requirements of aboveground growth and metabolism (Xu *et al.* 2012), root morphology and vitality also determine nitrogen absorption (Liu *et al.* 2017). As discussed above, EC reduced the leaf area and biomass, thus reducing the nitrogen requirement. In addition, reduced maize root biomass was observed under ethephon treatment (Xu *et al.*

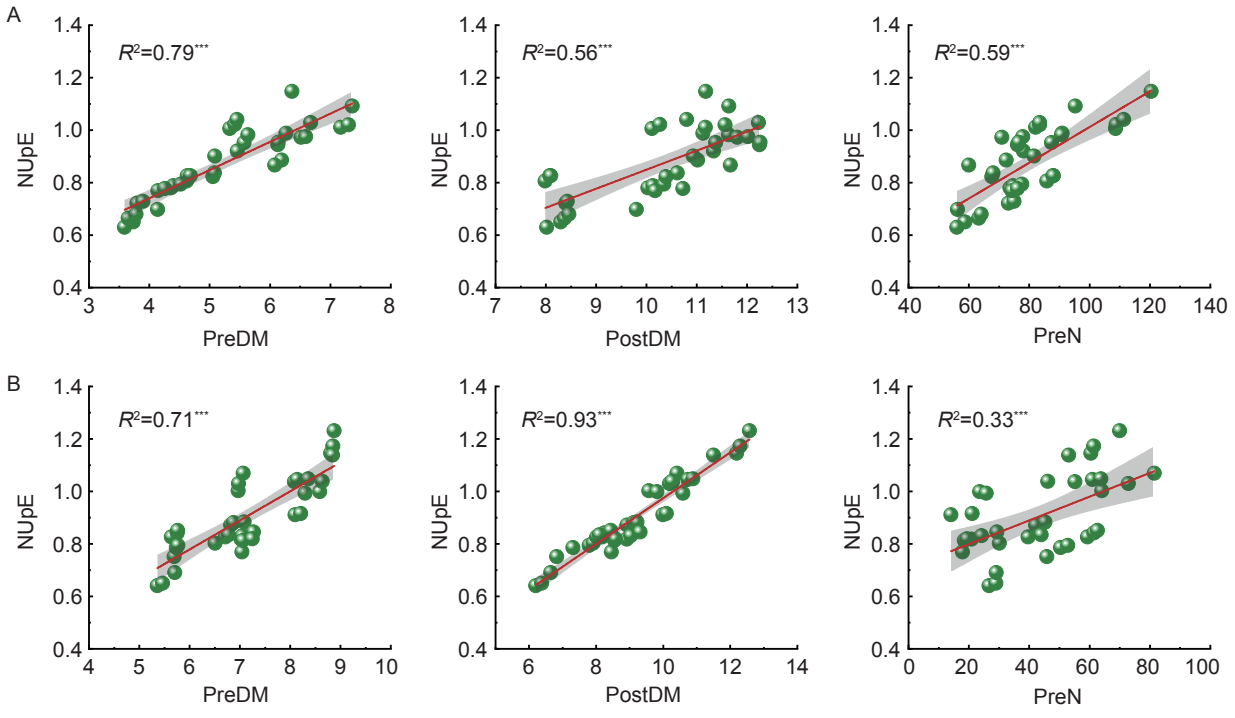


Fig. 8 Relationships between PreDM, PostDM, PreN, and NUpE in 2020 (A) and 2021 (B). PreDM, pre-silking dry matter accumulation; PostDM, post-silking dry matter accumulation; PreN, pre-silking N uptake; NUpE, N uptake efficiency. The gray bands represent the 95% confidence intervals. R^2 indicates the coefficient of determination. *** indicates significant difference at P<0.001.

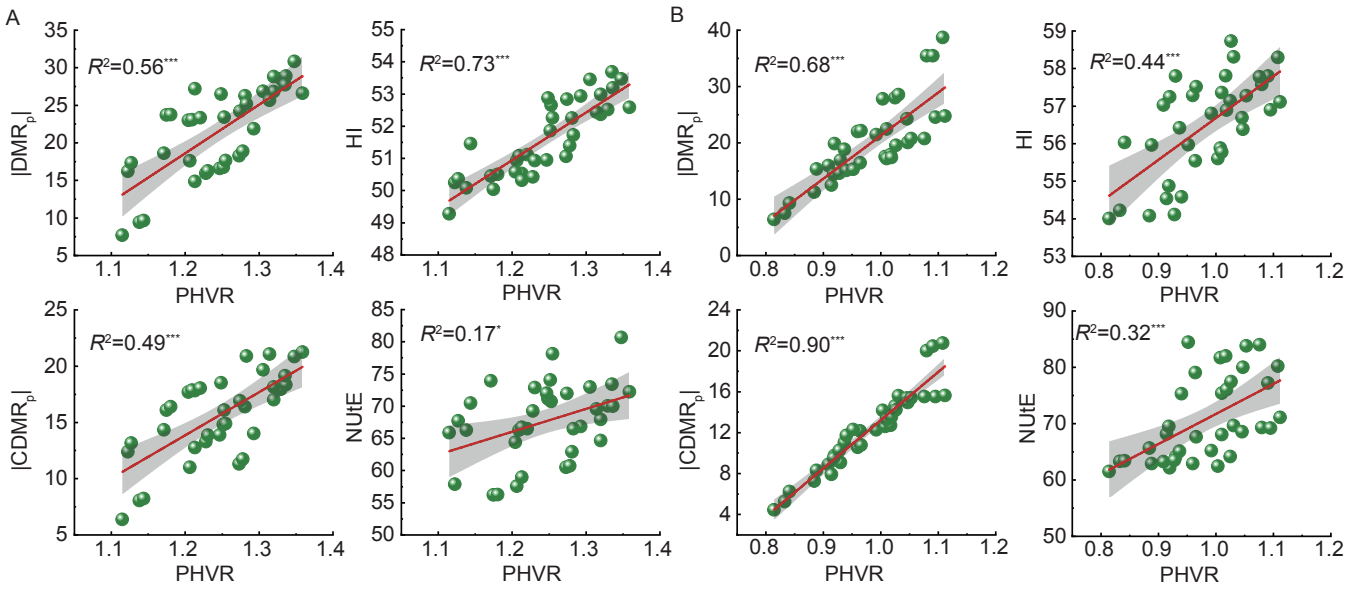


Fig. 9 Relationships between PHVR with |DMR$_p$|, |CDMR$_p$|, HI, and NUtE in 2020 (A) and 2021 (B). PHVR, plant horizontal-vertical ratio; |DMR$_p$|, absolute value of the total dry matter remobilization per plant; |CDMR$_p$|, absolute value of the contribution of DMR$_p$ to grain yield; HI, harvest index; NUtE, N utilization efficiency. R^2 indicates the coefficient of determination. The gray bands represent the 95% confidence intervals. * and *** indicate significant differences at P<0.05 and P<0.001, respectively.

2014). We concluded that root and shoot biomass limited the nitrogen uptake.

Increasing plant density generally leads to higher resource use efficiency, resulting in higher biomass accumulation and grain yield (Zhang Y H *et al.* 2021). Under relatively low nitrogen application and dense planting conditions, biomass and nitrogen accumulation and distribution to grains were higher, thus a high NUE and yield of maize can be achieved (Ciampitti and Vyn 2013). The grain yield in our previous study (Tang *et al.* 2022), as well as biomass and N uptake in the present study were found to increase with increasing density under a nitrogen application rate of 187.5 kg N ha^{-1}. These findings suggested that the nitrogen supply was sufficient for the maize requirement under high density (9.0 plants m^{-2}; Table 2). Note that the nitrogen input was not increased, but biomass and N accumulation did increase, which also demonstrated that biomass determines N uptake.

4.2. Optimized maize morphology improves NUtE and harvest index

Vascular bundles play important roles in matter transport (Shane *et al.* 2000), mechanical support, and the coordination of source–sink flow (Xu *et al.* 2017). Larger areas and numbers of vascular bundles are more conducive to the efficient transport of water and nutrients (Liu *et al.* 2022). Spraying EC, especially a high concentration of EC, significantly increased the areas and numbers of vascular bundles (Table 1). Correlation analysis showed that the relevant indicators of vascular bundles were significantly positively correlated with the total dry matter remobilization, N remobilization and NUtE (Fig. 7). In other words, EC increased the transportation capability of vascular bundles, which contributed to increasing NUtE. However, as a "flow" organ, the vascular bundles play a crucial role in facilitating assimilate (including nitrogen) absorption and transport. They do not have the ability to determine the processes of nitrogen absorption and transport on their own. In this study, we defined a new indicator, PHVR, to represent maize morphology. Interestingly, the vascular bundles were significantly positively correlated with PHVR. Moreover, PHVR had a significant positive correlation with NUtE. These findings suggested that PHVR can serve as a more easily accessible indicator for evaluating NUtE than vascular bundles. As a result, EC application significantly enhanced NUtE by improving PHVR and vascular bundle structure.

Greater intraspecific competition will weaken individual plant growth under a high planting density, i.e., thinner stems and greater plant height. In this study, reduced areas and numbers of vascular bundles and PHVR were observed under high plant density. However, increasing the density ensures biomass accumulation of the population, thus leading to greater nitrogen uptake and utilization efficiency. Therefore, negative correlations between PFPN and NUpE with vascular bundles were observed.

The harvest index reflects the proportion of assimilates allocated to the grains and vegetative organs in crops, and it also indicates that the flow of photosynthetic products from "source" organs to "sink" organs is smooth. Our results also showed that EC application significantly improved HI, and HI was significantly positively correlated with vascular bundle indicators and PHVR (Fig. 9). Raun and Johnson (1999) have also indicated that a greater HI is the key to high NUE. In conclusion, the increase of NUtE is inextricably linked to an increase in HI and they affect each other.

4.3. Interaction of EC and planting density on NUE

Biomass was significantly affected by the interactive effect of EC and density. In other words, the adverse effect of EC on

biomass was smaller under high-density conditions, which was conducive to achieving a higher yield under high-density planting. EC application significantly improved NUE, but the interaction between EC and density had no significant effect on NUE, which indicates that EC can increase NUE at any density. Considering the positive effects of increasing density on biomass accumulation, nitrogen absorption and utilization efficiency, spraying EC under high-density conditions is recommended to increase biomass, yield and NUE. It is also generally believed that a greater nitrogen supply is needed to increase the yield under high planting density. However, our previous research found that the yield under high density (9.0 plants m^{-2}) still increased with a nitrogen application rate of 187.5 kg ha^{-1} (Tang *et al.* 2022). Greater NUE was observed in this study, where the experimental conditions were the same as in the previous study. Thus, we believed that appropriately increasing the planting density without adding to the nitrogen input can improve yield and enhance NUE. Of course, further research on the interactions among density, EC, and nitrogen application is still needed.

5. Conclusion

Spraying EC at V7 reduced dry matter accumulation and nitrogen uptake in maize. However, increasing planting density could enhance nitrogen uptake and utilization efficiency even though nitrogen input was not increased. Furthermore, EC not only reduced the risk of lodging but also improved stem vascular bundles and PHVR, thus increasing NUtE and HI. Therefore, high planting density in combination with a plant growth retardant can synergistically improve NUtE and ultimately increase the yield potential of maize.

Acknowledgements

This work was supported by the National Key R&D Program of China (2023YFD2301500).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

References

Ahmad I, Ahmad S, Yang X N, Meng X P, Yang B P, Liu T, Han Q F. 2021. Effect of uniconazole and nitrogen level on lodging resistance and yield potential of maize under medium and high plant density. *Plant Biology*, **23**, 485–496.

Antonietta M, Fanello D D, Acciaresi H A, Guiamet J J. 2014. Senescence and yield responses to plant density in stay green and earlier-senescing maize hybrids from Argentina. *Field Crops Research*, **155**, 111–119.

Bloom A J. 2015. Photorespiration and nitrate assimilation: A major intersection between plant carbon and nitrogen. *Photosynthesis Research*, **123**, 117–128.

Chen K R, Vyn T J. 2017. Post-silking factor consequences for N efficiency changes over 38 years of commercial maize hybrids. *Frontiers in Plant Science*, **8**, 1737.

Chen X, Chen F, Chen Y, Gao Q, Yang X, Yuan L, Zhang F, Mi G. 2013. Modern maize hybrids in Northeast China exhibit increased yield potential and resource use efficiency despite adverse climate change. *Global Change Biology*, **19**, 923–936.

Ciampitti I A, Vyn T J. 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crops Research*, **133**, 48–67.

Ciampitti I A, Vyn T J. 2013. Grain nitrogen source changes over time in maize: A review. *Crop Science*, **53**, 366–377.

Cui Z, Chen X, Zhang F. 2013. Development of regional nitrogen rate guidelines for intensive cropping systems in China. *Agronomy Journal*, **105**, 1411–1416.

Deng J M, Zuo W Y, Wang Z Q, Fan Z X, Ji M F, Wang G X, Ran J Z, Zhao C M, Liu J Q, Niklas K J, Hammond S T, Brown J H, 2012. Insights into plant size-density relationships from models and agricultural crops. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 8600–8605.

Gong L S, Qu S J, Huang G M, Guo Y L, Zhang M C, Li Z H, Zhou Y Y, Duan L S. 2021. Improving maize grain yield by formulating plant growth regulator strategies in North China. *Journal of Integrative Agriculture*, **20**, 622–632.

Grassini P, Thorburn J, Burr C, Cassman K G. 2011. High-yield irrigated maize in the Western U.S. Corn Belt: I. On-farm yield, yield potential, and impact of agronomic practices. *Field Crops Research*, **120**, 142–150.

He Q P, Dong S T, Gao R Q. 2005. Relationship between development of spike vascular bundle and sink capacity of ear and kernel in maize (*Zea mays* L.). *Acta Agronomica Sinica*, **31**, 995–1000. (in Chinese)

Hou P, Liu Y, Liu W, Liu G, Xie R, Wang K, Ming B, Wang Y, Zhao R, Zhang W. 2020. How to increase maize production without extra nitrogen input. *Resources*, *Conservation and Recycling*, **160**, 104913.

Hütsch B W, Schubert S. 2021. Can nutrient-utilization efficiency be improved by reduced fertilizer supply to maize plants treated with the plant growth regulator paclobutrazol? *Journal of Agronomy and Crop Science*, **207**, 884–900.

Hütsch B W, Schubert S. 2022. Grain yield, harvest index, water-use efficiency and nitrogen partitioning to grain can be improved by application of the plant growth regulator paclobutrazol to maize plants with reduced N supply. *Journal of Agronomy and Crop Science*, **209**, 1–12.

Kamiji Y, Pang J, Milroy S P, Palta J A. 2014. Shoot biomass in wheat is the driver for nitrogen uptake under low nitrogen supply, but not under high nitrogen supply. *Field Crops Research*, **165**, 92–98.

Lea P J, Azevedo R A. 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. *Annals of Applied Biology*, **149**, 243–247.

Lee E A, Tollenaar M. 2007. Physiological basis of successful breeding strategies for maize grain yield. *Crop Science*, **47**, S202–S215.

Li G H, Cheng G G, Lu W P, Lu D L. 2021. Differences of yield and nitrogen use efficiency under different applications of slow release fertilizer in spring maize. *Journal of Integrative Agriculture*, **20**, 554–564.

Liu G Z, Yang Y S, Guo X X, Liu W M, Xie R Z, Ming B, Xue J, Wang K R, Li S K, Hou P. 2023. A global analysis of dry matter accumulation and allocation for maize yield breakthrough from 1.0 to 25.0 Mg ha^{-1}. *Resources Conservation and Recycling*, **188**, 106656.

Liu Z, Sha Y, Huang Y W, Hao Z H, Guo W Q, Ke L H, Chen F J, Yuan L X, Mi G H. 2022. Efficient nitrogen allocation and reallocation into the ear in relation to the superior vascular system in low-nitrogen tolerant maize hybrid. *Field Crops Research*, **284**, 108580.

Liu Z, Zhu K, Dong S, Liu P, Zhao B, Zhang J. 2017. Effects of integrated agronomic practices management on root growth and development of summer maize. *European Journal of Agronomy*, **84**, 140–151.

Ma D L, Xie R Z, Liu X, Niu X K, Hou P, Wang K R, Lu Y L, Li S K. 2014. Lodging-related stalk characteristics of maize varieties in China since the 1950s. *Crop Science*, **54**, 2805–2814.

Raun W R, Johnson G V. 1999. Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, **91**, 357–363.

Saiz-Fernández I, De Diego N, Brzobohatý B, Muñoz-Rueda A, Lacuesta M. 2017. The imbalance between C and N metabolism during high nitrate supply inhibits photosynthesis and overall growth in maize (*Zea mays* L.). *Plant Physiology and Biochemistry*, **120**, 213–222.

Shane M W, McCully M E, Canny M J. 2000. The vascular system of maize stems revisited: Implications for water transport and xylem safety. *Annals of Botany*, **86**, 245–258.

Shao H, Wu X, Chi F, Zhu F, Liu J, Duan J, Shi W, Xu Y, Mi G. 2024. How

does increasing planting density affect nitrogen use efficiency of maize: A global meta-analysis. *Field Crops Research*, **311**, 109369.
Shi D Y, Li Y H, Zhang J W, Liu P, Zhao B, Dong S T. 2016. Effects of plant density and nitrogen rate on lodging-related stalk traits of summer maize. *Plant, Soil and Environment*, **62**, 299–306.
Testa G, Reyneri A, Blandino M. 2016. Maize grain yield enhancement through high plant density cultivation with different inter-row and intra-row spacings. *European Journal of Agronomy*, **72**, 28–37.
Tang Q, Ren J H, Du X, Niu S D, Liu S S, Wei D J, Zhang Y R, Bian D H, Cui Y H, Gao Z. 2022. Reduced stem nonstructural carbohydrates caused by plant growth retardant had adverse effects on maize yield under low density. *Frontiers in Plant Science*, **13**, 1035254.
Tian Q L, He L H, Shuang L, Wu L, Fei D, Wei Z, Zhong X Y, Ren W J. 2021. *Indica* rice restorer lines with large sink potential exhibit improved nutrient transportation to the panicle, which enhances both yield and nitrogen-use efficiency. *Journal of Integrative Agriculture*, **20**, 1438–1456.
Wang K R, Xie R Z, Ming B, Hou P, Xue J, Li S K. 2021. Review of combine harvester losses for maize and influencing factors. *International Journal of Agricultural and Biological Engineering*, **14**, 1–10.
Wang Z G, Gao J L, Ma B L. 2014. Concurrent improvement in maize yield and nitrogen use efficiency with integrated agronomic management strategies. *Agronomy Journal*, **106**, 1243–1250.
Wei S S, Wang X Y, Zhang J W, Liu P, Zhao B, Li G, Dong S T. 2015. The role of nitrogen in leaf senescence of summer maize and analysis of underlying mechanisms using comparative proteomics. *Plant Science*, **233**, 72–81.
Xu C L, Gao Y B, Tian B J, Ren J H, Meng Q F, Wang P. 2017. Effects of EDAH, a novel plant growth regulator, on mechanical strength, stalk vascular bundles and grain yield of summer maize at high densities. *Field Crops Research*, **200**, 71–79.
Xu G H, Fan X R, Miller A J. 2012. Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, **63**, 153–182.
Xue J, Xie R Z, Zhang W F, Wang K R, Hou P, Ming B, Gou L, Li S K. 2017. Research progress on reduced lodging of high-yield and -density maize. *Journal of Integrative Agriculture*, **16**, 2717–2725.
Xu X, Li X Y, Zhang Z F, Zhou X, Xia K, Gan L J. 2014. Effects of mixture compound of ethephon and glycinebetaine on the growth and development of maize. *Journal of Maize Science*, **22**, 71–75. (in Chinese)
Ye D L, Zhang Y S, Al-Kaisi M M, Duan L S, Zhang M C, Li Z H. 2016. Ethephon improved stalk strength associated with summer maize adaptations to environments differing in nitrogen availability in the North China Plain. *Journal of Agricultural Science*, **154**, 960–977.
Zhang W Q, Fan Y L, Zhang S, Lu B S, Duan L S, Li Z H, Tan W M. 2019. Waxy maize yield in response to a novel plant growth regulator and plant density. *International Journal of Agriculture and Biology*, **22**, 304–312.
Zhang X, Davidson E A, Mauzerall D L, Searchinger T D, Dumas P, Shen Y. 2015. Managing nitrogen for sustainable development. *Nature*, **528**, 51–59.
Zhang Y, Wang H, Lei Q, Luo J, Lindsey S, Zhang J, Zhai L, Wu S, Zhang J, Liu H, Ren T, Liu H. 2018. Optimizing the nitrogen application rate for maize and wheat based on yield and environment on the Northern China Plain. *Science of the Total Environment*, **618**, 1173–1183.
Zhang Y H, Xu Z G, Li J, Wang R. 2021. Optimum planting density improves resource use efficiency and yield stability of rainfed maize in semiarid climate. *Frontiers in Plant Science*, **12**, 2461.
Zhang Y S, Wang Y B, Liu C R, Ye D L, Ren D Y, Li Z H, Zhang M C. 2021. Ethephon reduces maize nitrogen uptake but improves nitrogen utilization in *Zea mays* L. *Frontiers in Plant Science*, **12**, 3183.
Zhao Y N, Huang Y F, Li S, Chu X, Ye Y L. 2020. Improving the growth, lodging and yield of different density-resistance maize by optimizing planting density and nitrogen fertilization. *Plant Soil and Environment*, **66**, 453–460.

Executive Editor-in-Chief Shaokun Li
Managing Editor Ning Wang