

# Effects of nitrogen fertilizer on root-shoot characteristics, grain yield and nitrogen use efficiency of different maize varieties in North China Plain

Xiangling Li<sup>1,2†</sup>, Liu Pan<sup>2†</sup>, Rui Wang<sup>1</sup>, Yiping Zuo<sup>1</sup>, Jian Wang<sup>1</sup>, Jinling Han<sup>1</sup>, Yuechen Zhang<sup>2\*</sup>, Baozhong Yin<sup>3\*</sup>

1. College of Agronomy and Biotechnology, Hebei Normal University of Science & Technology/Hebei Key Laboratory of Crop Stress Biology, Qinhuangdao 066004, Hebei, China;
2. College of Agronomy, Hebei Agricultural University/State Key Laboratory of North China Crop Improvement and Regulation, Baoding 071001, Hebei, China;
3. College of Plant Protection, Hebei Agricultural University/State Key Laboratory of North China Crop Improvement and Regulation, Baoding 071001, Hebei, China)

**Abstract:** Excessive nitrogen (N) fertilizer application in crop production has resulted in a series of environmental issues in North China Plain (NCP). Breeding N-efficient maize varieties is one of the effective strategies to improve the N use efficiency of maize and reduce environmental pollution. This study designed an experiment for two maize varieties ('Xian-Yu 335' [XY335] and 'Hua-Nong 138' [HN138]) with three N-level treatments (0 kg/hm<sup>2</sup>, 180 kg/hm<sup>2</sup>, 300 kg/hm<sup>2</sup>, i.e. N0, N180, N300). The aim of the study was to determine the response characteristics of maize canopy structure, root morphology, and anatomical structure, as well as N uptake and utilization of N fertilizer. Results showed that grain yield, N use efficiency, and N harvest index of XY335 were higher 5.20%-13.68%, 11.86%-19.11%, and 6.07%-3.33% than those of HN138, respectively. Compared with HN138, XY335 had a higher leaf area index and photosynthetic nitrogen-use efficiency. The root length, root length density, root absorption area, root absorption area, and root active absorption area of XY335 in each period were higher than that of HN138. The proportion of RCA of XY335 was significantly higher than that of HN138, which was 14.64% higher on average. In the future breeding process, the high N and low N double-high-efficiency varieties should pay more attention to the improvement of root cortex aeration tissue.

**Keywords:** maize, root, nitrogen accumulation, nitrogen use efficiency, yield

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## 1 Introduction

Nitrogen (N) is a key nutrient in the growth of most crops. The application of N fertilizers greatly promoted the increase of grain protein content, biomass, and yield of maize. However, excessive N application would reduce nitrogen use efficiency (NUE) and pose a potential threat to the ecological environment<sup>[1,2]</sup>. Breeding N-efficient maize varieties is one of the effective strategies to improve N use efficiency<sup>[3,4]</sup>. N efficient crops are

usually divided into three different types: (1) Crops could maintain normal growth and yield under low N level similar to those under normal N level, i.e. low N and high efficient (low N tolerant) varieties; (2) With the N concentration increases, crops had the potential to absorb more N and obtain a higher yield, which was high N-efficient varieties; (3) High N and low N double-high-efficient<sup>[5,6]</sup>. Since people are basically satisfied with food and clothing, it is urgent to solve environmental pollution and reduce production costs, research on the tolerance of crops to low N has become the focus. Therefore, popularizing low-N tolerant varieties has been considered an effective way to increase yield and reduce pollution.

The establishment of N-high-efficient maize (*Zea mays* L.) populations is a feedback process that unifies the photosynthesis of the aboveground and the water absorption and nutrients uptake by the roots of the underground. The strong root uptake promotes photosynthesis in the upper part of the ground and sufficient photosynthetic products will provide essential nutrients for the growth of the root system, which further constitutes a complete photosynthetic production system<sup>[7]</sup>. The function of the root system is to absorb water and nutrients from the soil, while the canopy layer synthesizes carbohydrates on the ground. The dependence and restrictive relationship between roots and shoots are mainly manifested in leaf area expansion and root elongation. The functional changes of the root and shoot changes determine the plant structure, and the growth and development status affects the

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**Biographies:** Xiangling Li, PhD, Lecturer, research interest: crop cultivation and physiology, Email: ncyfz2008@126.com; Liu Pan, PhD candidate, research interest: crop cultivation and physiology, Email: 523608222@qq.com; Rui Wang, MS candidate, research interest: maize cultivation and physiology, Email: wangrui10002022@163.com; Yiping Zuo, MS candidate, research interest: maize cultivation and physiology, Email: 3329887881@qq.com; Jian Wang, PhD, Lecturer, research interest: crop cultivation and physiology, Email: wangjian1989@live.cn; Jinling Han, MS, Professor, research interest: crop cultivation and physiology, Email: hanjinlingde@126.com.

† These authors contributed equally to this study.

\*Corresponding author: Yuechen Zhang, PhD, Professor, research interest: crop cultivation and physiology. College of Agronomy, Hebei Agricultural University, Baoding 071001, Hebei, China, Tel: +86-13503223858, Email: Zhangyc1964@126.com; Baozhong Yin, PhD, Professor, research interest: crop cultivation and physiology. College of Plant Protection, Hebei Agricultural University, Baoding 071001, Hebei, China. Tel: +86-13663121867, Email: Yinbaozhong@hebau.edu.cn.

functions of the root and shoot. If the root system is small and the uptake capacity is limited, the supply of sufficient water and nutrients in the aboveground part will be affected. On the other hand, some substances required by the root system are supplied by the synthesis of the aboveground part, then the aboveground plant promotes the growth of the root system. Under N stress, the substances absorbed by the root and synthesized by the shoot should be preferentially supplied to the growth of organs near the source<sup>[8,9]</sup>.

The plant absorbs water and nutrients in the soil through roots. The growth and distribution of roots in the soil directly affect the use efficiency of water and nutrients. Root morphology is closely related to physiological characteristics such as root uptake, assimilation, and root transport to the above-ground, among which root configuration plays a vital role<sup>[10-12]</sup>. The N application affects the number and size of node roots, and lateral roots of maize. Maize varieties with few crown roots usually could take root deeper in low N soils and have a strong ability to uptake N from deep soil<sup>[11-13]</sup>. In low-N conditions, N-efficient maize has more underground nodal roots and root length, which is conducive to absorbing more N from the soil<sup>[14]</sup>. Root anatomical structure affects root physiological function, and nutrient and water absorption<sup>[15]</sup>. The formation of aerenchyma in the root cortex can reduce the respiration consumption of the root and increase the NUE of maize under low N treatment<sup>[16]</sup>. Plant roots can perceive the surrounding environment and adjust accordingly for growth and development, thereby influencing the morphological and anatomical structures.

More than 95% of the dry matter of maize is synthesized by leaf photosynthesis, and the green leaf area affects the formation of photosynthetic capacity and economic yield<sup>[17,18]</sup>. The green leaf area and  $P_n$  are the key factors to determine the light utilization and yield formation. Increasing the  $P_n$  of maize, prolonging the effective photosynthesis period, and promoting photosynthate accumulation and transportation, are beneficial to increase grain weight and yield<sup>[19]</sup>. Previous studies have shown that the coordination of carbon and N metabolism, the slow decline of photosynthesis and the material production capacity of maize after anthesis are of great significance for effectively maintaining a high LAI and photosynthetic potential, and further increasing crop yields<sup>[20]</sup>. The optimum amount of N fertilizer makes the photosynthetic physiological indexes of the maize population reach the best state<sup>[21]</sup>. The N-efficient varieties have strong synergistic uptake and transportation characteristics of photosynthetic carbon (C) and N assimilation as well as C and N metabolism ability, which can meet the efficient utilization of photosynthetic C and N during the grain filling period<sup>[22]</sup>. In low N conditions, low N tolerant maize varieties maintain a higher  $P_n$  and obtain higher dry matter production capacity<sup>[4,23]</sup>.

In this previous experiments, the N tolerance ability of 20 maize varieties was evaluated, these varieties were divided into high N and low N double-high-efficient (EE) varieties, high nitrogen efficient varieties (HNE), low nitrogen efficient (LNE) varieties, and high N and low N double- low-efficient (NN) varieties. In the results, 'Xian-Yu 335' (XY335) was one EE variety and 'Hua-Nong 138' (HN138) was one NN variety<sup>[24]</sup>. In addition, maize varieties also showed significant differences in N metabolism enzyme activities, dry matter, etc<sup>[25]</sup>. However, the relationship between the shoot and root characteristics of different N-efficiency maize cultivars with yield under different N rates was still unclear. In this study, one EE variety (XY335) and one NN

variety (HN138) were used as the test material, to explore their leaf source and root morphological characteristics under the different N rates. This study could provide the theoretical reference and technical support for maize N-efficient breeding in North China Plain.

## 2 Materials and methods

### 2.1 Site description

The field experiments were conducted at the experimental station of Hebei Agricultural University (37°54'N, 115°12'E), Xinji City, Hebei Province, China, during the growing season in 2018. The annual means of air temperature and precipitation from 1980 to 2010 were 13.8°C and 516.4 mm, respectively. The soil texture for experiments was clay loam (ISSS Classification, International Soil Science Society), with a bulk density of 1.47 g/cm<sup>3</sup>, organic matter of 18.47 g/kg, total nitrogen of 1.25 g/kg, alkali-hydrolysable N of 91.55g/kg, extractable Olsen-P of 27.50 mg/kg, ammonium acetate extractable K of 145.79 mg/kg, and pH of 7.8.

### 2.2 Experimental design

The experiment adopted a split-plot design, the main plots were based on three N adoption levels, 0 kg N/hm<sup>2</sup> (N0), 180 kg N/hm<sup>2</sup> (N180), and 300 kg N/hm<sup>2</sup> (N300), and the sub-plots were based on two varieties, i.e. 'Xian-Yu 335' (XY335) and 'Hua-Nong 138' (HN138). Each treatment was replicated 3 times for a total of 18 plots. The area of each plot was 72 m<sup>2</sup>, with six rows of maize, the row length was 20 m, and the row spacing was 0.60 m. The density of maize was 67 500 strains/hm<sup>2</sup>. The N source fertilizer was urea (46% N), 50% was applied before seed sowing, and 50% in the bell mouth stage. 90 kg P<sub>2</sub>O<sub>5</sub>/hm<sup>2</sup> and 120 kg K<sub>2</sub>O/hm<sup>2</sup> were applied together with the seed fertilizer at one time before sowing. The seeds were sown on June 14th and harvested on October 7th, 2018. Irrigation, weed, and other management measures are the same as that in the conventional field.

### 2.3 Measurement

#### 2.3.1 Leaf area index

Three representative plants were calibrated, and the leaf length and width were measured at the silking, milking, and maturity stages, respectively. Single leaf area=length×width×0.75 leaf area index (LAI)=leaf area per plant×plants number per unit land area/unit land area.

#### 2.3.2 Net photosynthetic rate ( $P_n$ )

Five representative plants of each treatment were selected in the silking, milking, and maturity stage. The weather was clear and cloudless, using the LI-6400 XT (USA) portable photosynthesis system to control the light intensity to 1600  $\mu\text{mol}/\text{m}^2 \cdot \text{s}$ , select 50% of the leaf length at 10:00 am to test the  $P_n$  of the ear-leaf.

#### 2.3.3 Dry matter of root and shoots

Three representative plants were taken from each plot at the silking, milking, and maturity stages, and separated by roots, stems and sheaves, leaves, cobs, bracts, and grains. They were put into an oven at 105°C for 30 min and dried at 70°C to constant weight.

#### 2.3.4 Nitrogen content

The samples of dry matter of root and shoots were crushed and sieved into powder after drying, then boiled by the methods of H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>, and total N content was determined by AA3 continuous flow analyzer (Germany). Total nitrogen was measured using the standard Kjeldahl method<sup>[26]</sup>.

#### 2.3.5 Root morphology

The sampling times at silking and maturity stages, respectively, were divided from the top to down into five levels, i.e. 0-10 cm,

10-20 cm, 20-30 cm, 30-40 cm, and 40-50 cm (Figure1). An example of the root drilling method is shown in Figure1. For the soil samples, the "3D Monolith" root spatial sampling method<sup>[26]</sup> was adopted with a small cube in situ root-soil sampler, and the roots of three maize plants with the same growth and continuous position (planting row direction) were sampled<sup>[27]</sup>. With plants as the center, in the sampling soil profile, the direction parallel to the planting row is the *X*-axis direction, the direction perpendicular to the planting row is the *Y*-axis direction, and the direction of soil depth is the *Z*-axis direction. Soil block with a volume of 10 cm×10 cm×10 cm is taken as the sampling unit. The length of the *X*-axis was 130 cm, the *Y*-axis was 50 cm (the plant was located 25 cm in the center of the *Y*-axis), and the *Z*-axis was 50 cm. Remove all the roots from each soil block, clean the root surface and put it in a self-sealed bag, and take it back to the laboratory. The net root was taken for determination and analysis. The root samples were scanned into picture files by the Epson Perfection V700 root scanner. The total root length, the root surface area, and the root length density of each sample were determined by scanning (WinRhizo 3.1.2, Regent Instruments Inc, Quebec, Canada). Root length density ( $\text{cm}/\text{cm}^3$ ) = root length (cm)/soil volume ( $\text{cm}^3$ ).



Figure 1 An example of the root drilling method to take root system of 0-50 cm soil layer in silking

### 2.3.6 Root physiology

The root segments at 0-20 cm depth were taken for root activity measurement by the TTC reduction method<sup>[27]</sup>, and the methylene blue adsorption was used to determine the root absorption area and active absorption area.

### 2.3.7 Root microstructure observation

The samples were taken from the third segment of the root at the silking stage, the root segments were cut 4-10 cm from the root base, and the samples were fixed with FAA fixation solution. Different alcohol gradient was used for dehydration, xylene gradient was transparent, and the biological tissue embedding machine (YD-6L, China) was used for embedding. Then use a paraffin microtome (Leica RM2255, Germany) for sectioning, the slice thickness was 10  $\mu\text{m}$ . The slices were double-stained with saffron and solid green and sealed with Canadian neutral gum for preservation. Then the slices were observed and photographed with an optical microscope (Olympus SZX10, Germany). Three slices were observed of each replicate and took the images for each slice were by photography software. The root cortex aeration tissue (RCA), the number of cortical cell layers, the thickness of cortical cells, the ratio of the cortex to root diameter, the central column diameter, and duct diameter were analyzed using the Image-Pro Plus 6.0 software (Germany).

### 2.3.8 Electron microscopic observation of root tip cells

The root tips were taken from the third segment of the root at

the silking stage, root segment was cut 4-10 cm from the base, fixed with 2.5% glutaraldehyde for 24 h, and stored in a refrigerator at 4°C. The test steps are as follows: 1) Root tips sample were dehydrated through different concentrations of 30%, 50%, 70%, 85%, 95%, and 100%; 2) The intermediate solution was replaced in two steps: the first step was soaked with a mixture of acetic acid (isoamyl acetate: ethanol = 1:1) for 10 min; the second step was soaked in isoamyl acetate for 10 min, shaking appropriately. 3) The replaced samples were transferred to the sample basket and put into the pre-cooled critical point dryer (using the British Quorum K850 critical point dryer). 4) Closed the cover, slowly injected the liquid  $\text{CO}_2$ , and all the samples were submerged and heat up to 15°C for 10 min, raising the temperature to 35°C to vaporize. After the liquid was vaporized, the gas was released slowly, and the sample was taken out after the gas was exhausted. 5) The sample was pasted with double-sided tape and coated with an ion sputtering instrument (108Auto ion sputtering instrument of Cresington, UK). 6) Finally, observation was carried out with scanning electron microscopy (Hitachi SU8010, Japan).

### 2.3.9 Yield and its components

In the final harvest, the cobs from the middle three rows of each plot were used for the yield estimate (moisture content 14%) by the methods<sup>[28,29]</sup>. The measured area of each plot was 20  $\text{m}^2$ , and the number of effective cobs was calculated. After artificial threshing, fresh grain weight and moisture were measured and converted to a yield of 14% moisture content. There were 20 cobs after air-dried were tested the number of rows per cob, number of grains per row, the number of grains per cob, and the thousand-grain weights.

### 2.3.10 Estimation of nitrogen use efficiency

Additionally, nitrogen use efficiency (NUE) and Nitrogen harvest index (NHI) were computed to explore the performance of nitrogen rates<sup>[28]</sup>.

Nitrogen use Efficiency (NUE, kg/kg) = grain yield/plant N content at maturity

Nitrogen harvest index (NHI, %) = grain N content/plant N content at maturity  $\times 100\%$

Leaf photosynthetic nitrogen use efficiency ( $\mu\text{mol CO}_2/\text{g N}\cdot\text{s}$ ) =  $P_N/\text{SLN}$

Specific leaf N (SLN;  $\text{g N}/\text{m}^2$ ) was calculated as N content divided by whole-leaf area.

## 2.4 Statistical analysis

The statistical difference of yield, root morphology (length, surface area), etc., were tested using the least significant difference (LSD) of one-way ANOVA (SPSS 19.0, IBM Co., New York, USA) at the  $p < 0.05$  probability level. All figures were constructed using SigmaPlot 10 (Systat Software Inc. San Jose, CA, USA). The images and the data of root anatomical slices were analyzed by Pro Plus 6.0 (Germany).

## 3 Results and discussion

### 3.1 Dry matter accumulation and accumulation rate

The shoot dry matter accumulation of XY335 was higher than HN138 (Table 1). Under N300 treatment, the dry matter accumulations of XY335 before and after silking were 8.01% and 18.99% higher than that of HN138, respectively. Under N180 and N0 treatment, those values were 1.43% and 15.06% higher than that of HN138, respectively. The dry matter accumulation rates of XY335 after silking under N300 and N180 treatments were 4.46% and 6.31% higher than that of HN138, respectively. It shows that

the dry matter accumulation ability of XY335 after silking was higher than that of HN138.

**Table 1 Matter accumulation and accumulation rate of two maize varieties under three nitrogen treatments before and after silking**

Nitrogen treatment	Varieties	Before silking		After silking	
		Dry matter accumulation /t·hm <sup>-2</sup>	Accumulation rate/%	Dry matter accumulation /t·hm <sup>-2</sup>	Accumulation rate/%
N300	XY335	158.14 <sup>a</sup>	43.74 <sup>ab</sup>	203.36 <sup>a</sup>	56.26 <sup>a</sup>
	HN138	146.41 <sup>b</sup>	46.14 <sup>a</sup>	170.91 <sup>b</sup>	53.86 <sup>b</sup>
N180	XY335	144.83 <sup>a</sup>	45.21 <sup>ab</sup>	175.56 <sup>a</sup>	54.79 <sup>a</sup>
	HN138	142.79 <sup>ab</sup>	48.46 <sup>a</sup>	151.87 <sup>b</sup>	51.54 <sup>b</sup>
N0	XY335	134.57 <sup>a</sup>	45.21 <sup>ab</sup>	163.10 <sup>a</sup>	54.79 <sup>a</sup>
	HN138	130.35 <sup>ab</sup>	47.11 <sup>a</sup>	146.33 <sup>b</sup>	52.89 <sup>b</sup>

Note: N300, N180, and N0 are treatments with N application rates of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively. The different small case letters indicate the differences between the two varieties within the same nitrogen treatment ( $p < 0.05$ ).

**3.2 Leaf area index and photosynthetic nitrogen-use efficiency in ear-leaf**

The formation of grain yield depends on the amount of dry matter accumulation, which the dry matter is produced by

**Table 2 Leaf area index (LAI), leaf net photosynthetic rate (Pn), and leaf photosynthetic nitrogen-use efficiency (PNUE) of two maize varieties under three different nitrogen treatments**

Nitrogen treatment	Varieties	LAI			Pn/ $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$			PNUE/ $\mu\text{mol CO}_2 \cdot \text{g}^{-1} \cdot \text{s}^{-1}$		
		Silking	Milking	Maturity	Silking	Milking	Maturity	Silking	Milking	Maturity
N300	XY335	4.74 <sup>a</sup>	4.52 <sup>a</sup>	3.91 <sup>a</sup>	41.73 <sup>a</sup>	34.23 <sup>a</sup>	26.50 <sup>a</sup>	13.73 <sup>a</sup>	15.14 <sup>a</sup>	13.69 <sup>a</sup>
	HN138	4.58 <sup>b</sup>	4.16 <sup>b</sup>	3.36 <sup>b</sup>	37.87 <sup>b</sup>	28.67 <sup>b</sup>	22.03 <sup>b</sup>	12.09 <sup>a</sup>	13.38 <sup>b</sup>	11.09 <sup>b</sup>
N180	XY335	4.14 <sup>a</sup>	3.92 <sup>a</sup>	3.64 <sup>a</sup>	36.27 <sup>a</sup>	28.03 <sup>a</sup>	23.70 <sup>a</sup>	12.94 <sup>a</sup>	12.59 <sup>ab</sup>	12.99 <sup>a</sup>
	HN138	4.09 <sup>b</sup>	3.74 <sup>b</sup>	3.07 <sup>b</sup>	33.37 <sup>b</sup>	25.83 <sup>b</sup>	19.10 <sup>b</sup>	10.63 <sup>ab</sup>	13.39 <sup>a</sup>	10.54 <sup>b</sup>
N0	XY335	4.04 <sup>a</sup>	3.41 <sup>a</sup>	2.81 <sup>a</sup>	32.67 <sup>a</sup>	27.53 <sup>a</sup>	23.33 <sup>a</sup>	11.64 <sup>a</sup>	13.24 <sup>a</sup>	12.35 <sup>a</sup>
	HN138	3.96 <sup>ab</sup>	3.25 <sup>b</sup>	2.27 <sup>b</sup>	30.63 <sup>b</sup>	24.63 <sup>b</sup>	17.20 <sup>b</sup>	9.33 <sup>b</sup>	11.45 <sup>b</sup>	10.71 <sup>b</sup>

Note: N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively. The different small case letters indicate the differences between the two varieties within the same nitrogen treatment ( $p < 0.05$ ).

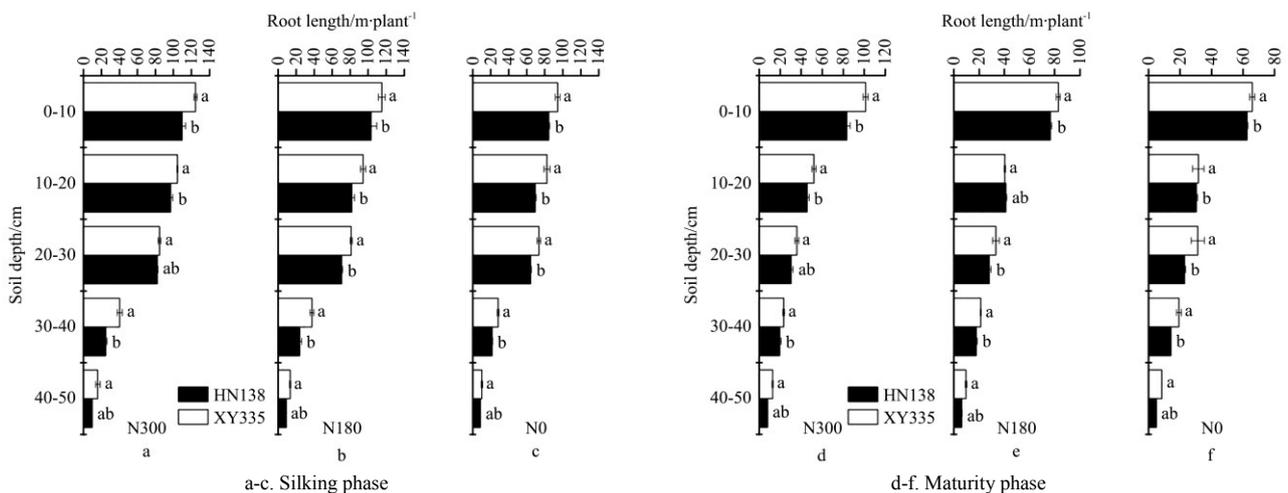
**3.3 Root length and root length density**

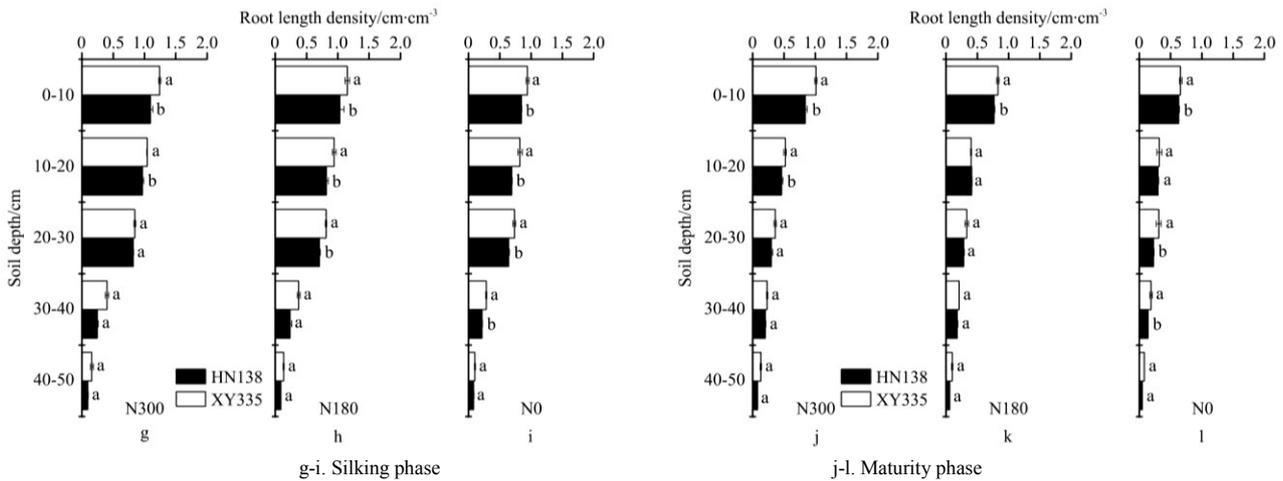
The root length and root length density of XY335 were significantly higher than HN138 (Figure 2). The proportion of the root length in the 0-20 cm soil layer of XY335 was higher than that of HN138, and the population root length density and root surface area of XY335 were also significantly larger than that of HN138 (Figure 2). The root surface area of the 0-20 cm soil layer accounted for more than 70% of the root surface area of the 0-

photosynthesis<sup>[29,30]</sup>. Under each N treatment, the leaf area index (LAI) and photosynthetic rate (Pn) of XY335 were significantly higher than that of HN138 (Table 2). In this study, XY335 had a higher LAI and Pn under each N treatment and slowly decreased from milking to maturity. This may be due to the extended periods of high photosynthetic time was extended to ensure sufficient carbohydrate supply for grain filling<sup>[31]</sup>. However, HN138 had a lower LAI and Pn under each N treatment, decreased rapidly from milking to maturity, and with a short duration of high photosynthesis. Therefore, maintaining a high LAI and Pn after silking and prolonging leaf photosynthetic function period is important for biomass and grain yield increase.

The leaf photosynthetic nitrogen use efficiency (PNUE) of XY335 was significantly higher than that of HN138 during each stage (Table 2). XY335 had a higher PNUE, which is an important physiological mechanism for the higher NUE, that is, the proportion of photosynthetic nitrogen in leaves and the activity of photosynthetic enzymes affect PNUE<sup>[32]</sup>. Reasonable N application is beneficial to increase Pn and PNUE, but also increase grain yield<sup>[33,34]</sup>. Therefore, improving leaf photosynthetic nitrogen use efficiency plays an important effect in prolonging the photosynthetic function period, preventing the premature senescence of leaves under low N conditions, which could improve the material production capacity after silking.

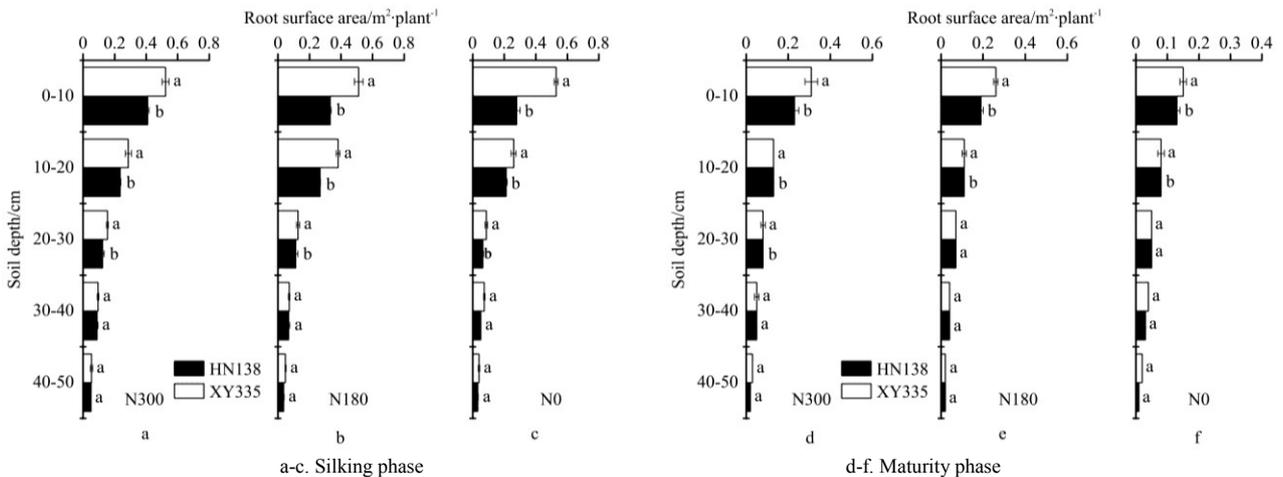
50 cm soil layer (Figure 3). As the main organ for nutrient uptake of maize roots affects the growth and development of the whole plant<sup>[35]</sup>. N uptake depends on the size and uptake of root. In this study, the XY335 had higher root length, root length density, and root surface area than that of HN138. The aboveground provides sufficient photosynthetic products for the root, which is conducive to the establishment of a good morphological structure of the root system and the maintenance of physiological functions.





Note: N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively. The different small case letters indicate the differences between two varieties within the same nitrogen treatment ( $p < 0.05$ ).

Figure 2 Root length and density in 0-50 cm soil profile of XY335 and HN138 under different nitrogen treatments at silking and maturity phases



Note: N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively. The different small case letters indicate the differences between two varieties within the same nitrogen treatment ( $p < 0.05$ ).

Figure 3 Root surface area in 0-50 cm soil profile of XY335 and HN138 under different nitrogen treatments at silking phase and maturity phase

### 3.4 Root biomass and ratio of root and shoot

The root dry matter of XY335 was significantly higher than that of HN138 ( $p < 0.05$ ) (Figure 4). Compared with the silking stage, the root dry matter of XY335 and HN138 at maturity stage decreased by 46.62% and 50.93%, respectively under N300 treatment, and the root dry matter was reduced by 47.41% and 44.58%, respectively under N180 treatment. The root and shoot ratio of XY335 was higher than HN138 at the silking stage, but there was no significant difference in the root and shoot ratio of the two varieties at the maturity stage. Previous studies have shown that N-efficient maize varieties had the largest range of root systems and dry matter weight<sup>[36]</sup>. The root is the main organ absorbing and transporting water and nutrients, and the root characteristics affect the growth and development of the aboveground and the formation of grain yield<sup>[37,38]</sup>. Root traits at silking stage had the closest relationship with NUE. The N-efficient inbred line T-213 was significantly higher than the inbred line Wu312 in the root length density, root surface area, and root dry matter<sup>[39]</sup>.

In this study, it was found that XY335 had larger root biomass, and the advantages were more obvious under low N conditions. The N uptake of XY335 plants was significantly higher than that of HN138. This was due to the fact that the population root length

density during the silking stage was large and the strong root system was conducive to improving plant N uptake. Therefore, it was concluded that the XY335 can improve the N uptake and utilization capacity of roots and achieve higher biomass and grain yield.

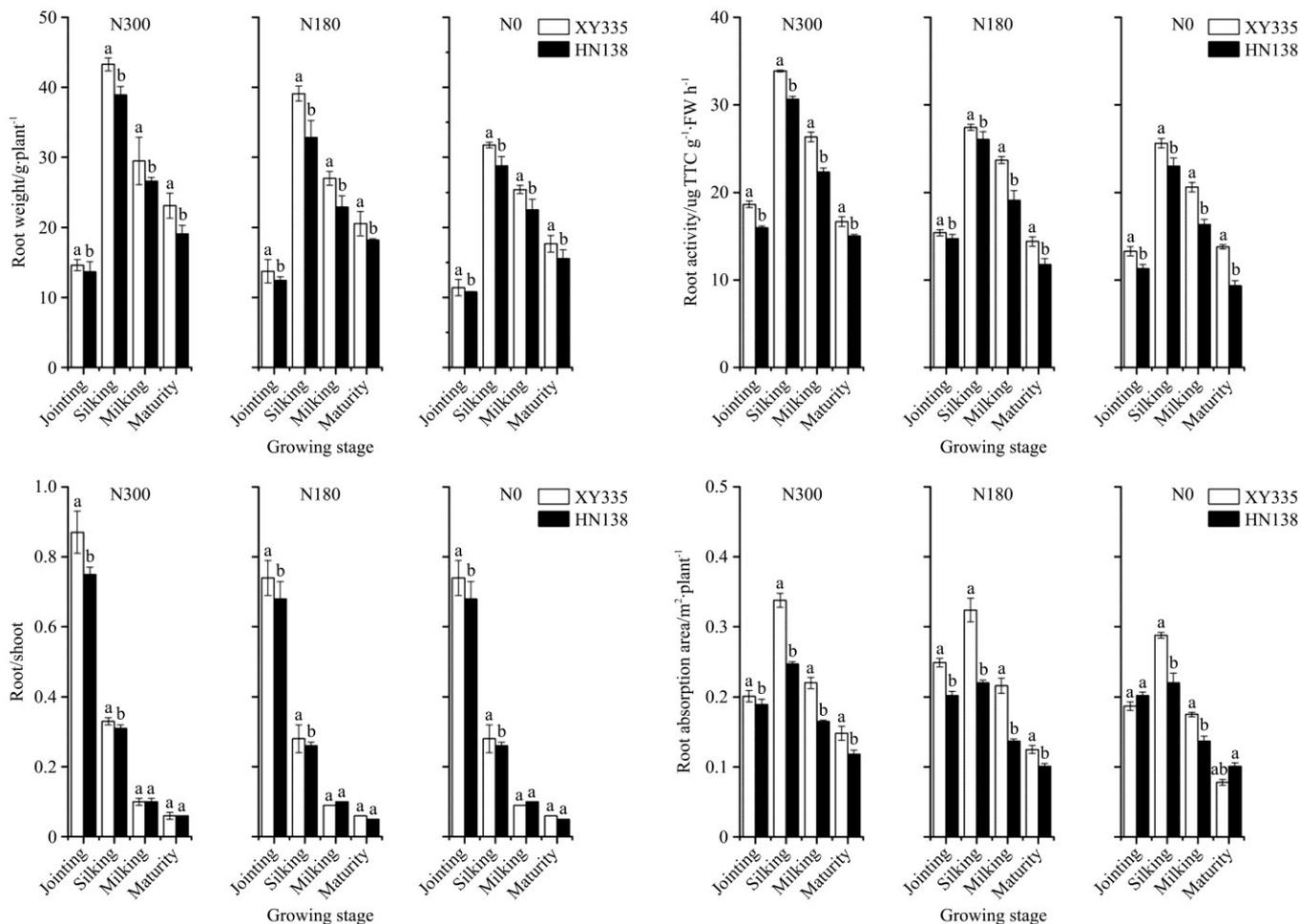
### 3.5 Root absorption area and root activity

The root vigor of XY335 was significantly higher than that of HN138 in all growth stages (Figure 4). Low N treatment reduced the root vigor of the two varieties. Compared with the silking stage, the root vigor of the two varieties at the maturity stage decreased by 50.81% and 50.98% under the N300 treatment, and the root vigor of the two varieties at the maturity stage decreased by 47.76% and 54.80% under the N180 treatment. Similarly, the root absorption area, the root absorption area, and the root active absorption area of XY335 in each period were significantly higher than that of HN138. Compared with the silking stage, the root absorption area of the maturity stage showed a decreasing trend. Under the N300 treatment, the root absorption area of XY335 and HN138 at the maturity stage decreased by 21.47% and 33.33%, respectively. Under the N180 treatment, the root absorption area at the maturity stage of XY335 and HN138 decreased to 33.33% and 37.78%, respectively.

In addition, it was found that the morphological structure and

spatial distribution of root system in XY335 were more reasonable than that of HN138. Furthermore, XY335 had a decrease in root dry weight, root length, and root surface area during the silking to maturity period, indicating it has a strong photosynthetic capacity and maintained a strong root function. The strong root system could absorb more water and uptake nutrients to feed the aboveground growth, the root shoot could be more coordinated<sup>[40]</sup>. XY335 has excellent root characteristics, reasonable spatial

distribution, high root vigor, and long duration, delays root senescence in the later stage, is conducive to absorbing more water and nutrients, and meets the requirements of N uptake during grain growth. The healthy growth of the aboveground also provided sufficient photosynthetic products for the root system, which is more conducive to the maintenance of the high value of root activity in the maturity stage, and ensuring the nutrient supply during grain filling stage.



Note: The different small case letters indicate the differences ( $p < 0.05$ ) between the varieties within the same nitrogen treatment and growing phase, the same letters indicate no differences. N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively.

Figure 4 Root dry mater, ratio of root/shoot in 0-20 cm soil profile of XY335 and HN138 at four growth phases by three nitrogen treatments

### 3.6 Root anatomical structure

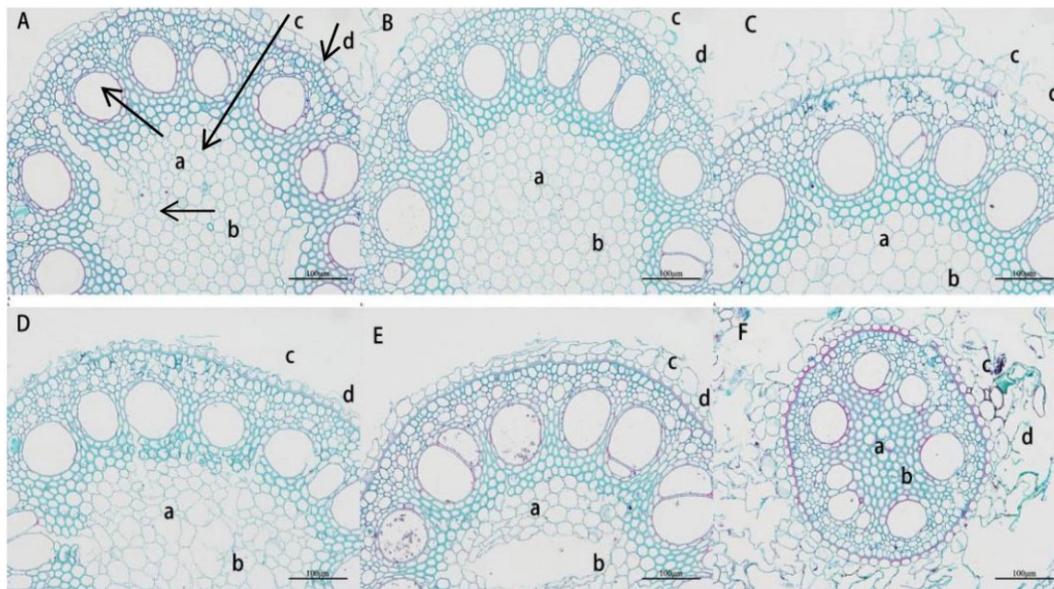
The thickness of root cortex cells was significantly different among the three N treatments (Figure 6). Compared with the N300 treatment, the diameter of the root ducts of XY335 and HN138 under the N180 treatment was reduced by 16.5% and 21.7%, respectively. The RCA is formed under the conditions of plant growth under environmental stress. Its formation can increase O<sub>2</sub> flow between the root cells of the plant, reduce the energy consumption of root respiration, and reduce the unnecessary nutrients of the plant under N deficiency condition. The proportion of RCA of XY335 was significantly higher than that of HN138, which was 14.64% higher on average (Table 3). The proportion of RCA in the two varieties under low N treatment increased significantly under higher N treatment, and XY335 and HN138 increased by 14.58% and 3.2%, respectively. The N180 treatment can promote the formation of RCA, and the adaptability of XY335 under low N treatment is stronger than that of HN138.

Comparison of root hair structure showed that there is no significant difference in the number of root hairs between XY335 and HN138 under high N treatment (Figure 5) and there are more root hairs in the root system of XY335 and the root hairs are arranged neatly, while the number of root hairs in HN138 root system is Less under low-N treatment. More root hairs help increase the root absorption area and help the root system absorb more nutrients from the soil.

The anatomical structure of the root system can also directly affect the physiological function of the root system and the absorption of nutrients and water<sup>[41]</sup>. The formation of RCA can reduce the respiratory consumption of the root system and increase the NUE of maize under low-N conditions<sup>[42]</sup>. Previous studies have found that among the anatomical characteristics of the root system, the formation of RCA, the increase in the number of root cortex cell layers, and the increase in root cortex cell size (CCS) can all reduce root respiration consumption in soil exploration<sup>[38]</sup>.

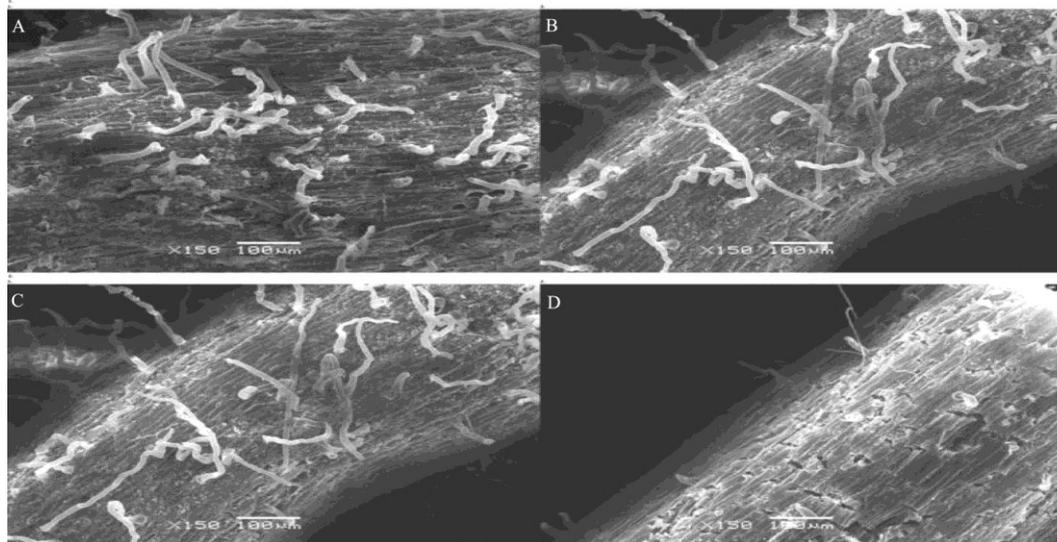
The proportion of RCA of modern maize varieties in the cross-sectional area of the root system has increased significantly, which reduces root respiration consumption and is conducive to an increase in yield<sup>[22]</sup>. This study found that the proportion of RCA

in XY335 increased significantly, especially under N180 treatment. The increase of RCA reduced the root respiration rate and the root metabolic consumption caused by the increase in root dry matter so that the plant growth could release more N.



Note: A, B, and C: N300, N180, and N0 treatment in XY335; D, E, and F: N300, N180 and N0 treatment in HN138. a: Stele b: Pulp cavity c: Vessel d: Cortex. N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively.

Figure 5 Microstructure differences of root in XY335 and HN138 under different nitrogen treatments



Note: A, B: N300 and N180 treatment in XY335; C, D: N300, N180 treatment in HN138. N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively.

Figure 6 Microstructure differences of root in XY335 and HN138 under different nitrogen treatments assessed by scanning

**Table 3 Root micro-structures of two maize varieties at silking phase by different nitrogen treatments**

Nitrogen treatment	Varieties	Cortex cell layers No	Thickness of the cortex/ $\mu\text{m}$	Ratio of root cortex aeration tissue (RCA)/%	Ratio of the cortex to the root diameter/%	Central column / $\mu\text{m}$	Vessel diameter /mm
N300	XY335	4 <sup>b</sup>	49.86 <sup>b</sup>	28.32 <sup>a</sup>	8.31 <sup>b</sup>	546.78 <sup>a</sup>	66.82 <sup>a</sup>
	HN138	8 <sup>a</sup>	82.33 <sup>b</sup>	24.62 <sup>b</sup>	14.41 <sup>a</sup>	515.98 <sup>b</sup>	66.38 <sup>a</sup>
N180	XY335	10 <sup>a</sup>	82.69 <sup>b</sup>	32.45 <sup>a</sup>	12.40 <sup>b</sup>	413.56 <sup>a</sup>	55.80 <sup>a</sup>
	HN138	12 <sup>a</sup>	129.77 <sup>a</sup>	25.41 <sup>b</sup>	26.41 <sup>a</sup>	376.19 <sup>b</sup>	51.95 <sup>b</sup>
N0	XY335	7 <sup>a</sup>	70.05 <sup>b</sup>	40.27 <sup>a</sup>	7.08 <sup>b</sup>	511.67 <sup>a</sup>	62.15 <sup>a</sup>
	HN138	8 <sup>a</sup>	134.37 <sup>a</sup>	36.21 <sup>b</sup>	29.36 <sup>a</sup>	208.60 <sup>b</sup>	41.47 <sup>b</sup>

Note: N300, N180, and N0 are treatments with N application rates of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are ‘Xian-Yu 335’ and ‘Hua-Nong 138’, respectively. The different small case letters indicate the differences ( $p < 0.05$ ) between the varieties within the same nitrogen treatment.

### 3.7 Nitrogen use Efficiency and N harvest index

The plants N accumulation of XY335 was significantly higher than that of HN138 (Table 4). The N accumulation of XY335 and

HN138 plants under N180 treatment was 33.9% and 42.5% lower compared with N300 treatments, respectively. The NUE and NHI of XY335 were significantly higher than that of HN138. Under

N300 treatment, the NUE and NHI of XY335 were 11.86% and 6.07% higher than HN138, respectively. Under N180 treatment, The NUE and NHI of XY335 were increased by 19.11% and 3.33% compared with that of HN138.

### 3.8 Grain yield and ear characteristics

The grain yield of XY335 was significantly higher than that of HN138 (Table 5). Compared with the N300 treatments, the grain

yields of XY335 and HN138 under N180 treatments decreased by 9.65% and 18.31%, respectively. Under N300 and N180 treatments, the numbers of grains per ear of XY335 and HN138 were 5.20% and 13.68% higher than those of HN138. These results indicated that the higher storage capacity of XY335 ensures a higher number of grains per ear, which was beneficial to grain yield.

**Table 4 N contents, nitrogen use efficiency (NUE), and nitrogen harvest index (NHI) in two maize varieties under three N treatments**

Nitrogen treatment	Varieties	Plant N contents/g·plant <sup>-1</sup>	Grain N contents/g·plant <sup>-1</sup>	NUE/kg·kg <sup>-1</sup>	NHI/%
N300	XY335	3.79 <sup>a</sup>	2.96 <sup>a</sup>	62.68 <sup>a</sup>	78.14 <sup>a</sup>
	HN138	3.05 <sup>b</sup>	2.05 <sup>b</sup>	59.80 <sup>b</sup>	73.40 <sup>b</sup>
N180	XY335	2.63 <sup>a</sup>	2.03 <sup>a</sup>	72.59 <sup>a</sup>	75.93 <sup>a</sup>
	HN138	2.21 <sup>b</sup>	1.69 <sup>b</sup>	70.25 <sup>b</sup>	73.40 <sup>a</sup>
N0	XY335	2.01 <sup>a</sup>	1.53 <sup>a</sup>	79.25 <sup>a</sup>	74.43 <sup>a</sup>
	HN138	1.74 <sup>b</sup>	1.24 <sup>b</sup>	72.24 <sup>b</sup>	72.45 <sup>b</sup>

Note: The different small case letters indicate the differences ( $p < 0.05$ ) between the varieties within the same nitrogen treatment. N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are 'Xian-Yu 335' and 'Hua-Nong 138', respectively.

**Table 5 Yield and its composition of two maize varieties under three nitrogen treatments**

Nitrogen rate	Varieties	Grain yield/t·hm <sup>-2</sup>	Ears/10 <sup>4</sup> ·hm <sup>-2</sup>	Kernel numbers per ear	1000-grain weight/g	Shoot biomass/t·hm <sup>-2</sup>	Harvest index
N300	XY335	12.33 <sup>a</sup>	6.31 <sup>a</sup>	521.27 <sup>a</sup>	375.3 <sup>a</sup>	22.79 <sup>a</sup>	0.54 <sup>a</sup>
	HN138	11.03 <sup>b</sup>	6.18 <sup>b</sup>	495.52 <sup>b</sup>	360.1 <sup>b</sup>	19.62 <sup>b</sup>	0.56 <sup>a</sup>
N180	XY335	11.14 <sup>a</sup>	5.94 <sup>a</sup>	523.63 <sup>a</sup>	357.9 <sup>a</sup>	19.05 <sup>a</sup>	0.58 <sup>a</sup>
	HN138	9.01 <sup>b</sup>	5.75 <sup>b</sup>	460.62 <sup>b</sup>	352.9 <sup>a</sup>	16.32 <sup>b</sup>	0.55 <sup>a</sup>
N0	XY335	9.82 <sup>a</sup>	5.78 <sup>a</sup>	495.28 <sup>a</sup>	343.7 <sup>ab</sup>	17.20 <sup>a</sup>	0.57 <sup>a</sup>
	HN138	8.18 <sup>b</sup>	5.39 <sup>b</sup>	433.25 <sup>b</sup>	351.2 <sup>a</sup>	14.91 <sup>b</sup>	0.55 <sup>a</sup>

Note: N300, N180, and N0 are treatments with N application rate of 300, 180, and 0 kg N/hm<sup>2</sup>, respectively. XY335 and HN138 are 'Xian-Yu 335' and 'Hua-Nong 138', respectively. The different small case letters indicate the differences ( $p < 0.05$ ) between the varieties within the same nitrogen treatment.

## 4 Conclusions

The high N and low N double-high-efficient (EE) varieties XY335 had a higher leaf area index, photosynthetic nitrogen-use efficiency and total root length, population root length density, and root activity. The proportion of root cortex aeration tissue and duct diameter of XY335 were also significantly higher than those of HN138. These results suggested that XY335 with harmonious root and shoot structure can realize the synergistic improvement of yield and N use efficiency. In the future breeding process, the high N and low N double-high-efficiency varieties should pay more attention to the improvement of root cortex aeration tissue.

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