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To cite this article: Qiang Li, Fanlei Kong, Yawei Wu, Dongju Feng & Yuan Jichao (2020) Increasing nitrogen accumulation and reducing nitrogen loss with N-efficient maize cultivars, *Plant Production Science*, 23:3, 260-269, DOI: [10.1080/1343943X.2019.1710221](https://doi.org/10.1080/1343943X.2019.1710221)

To link to this article: <https://doi.org/10.1080/1343943X.2019.1710221>



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Published online: 03 Jan 2020.



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# Increasing nitrogen accumulation and reducing nitrogen loss with N-efficient maize cultivars

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## ABSTRACT

Many nitrogen-efficient (N-efficient) maize cultivars have been bred to meet increased food demand and allay urgent environmental concerns, but the effects of these cultivars on increasing N accumulation and reducing N loss in the maize system have not been reported. A two-year field pot experiment was conducted in Southwest China to evaluate the advantages of an N-efficient maize cultivar on N accumulation and N loss in 2014 and 2015. The results showed that the N-efficient maize cultivar ZH 311 had higher N uptake and accumulation ability than the N-inefficient maize cultivar XY 508, especially in the middle and late growth stages. ZH 311 maintained higher residual soil inorganic N in the appropriate growth stage, promoting soil N supply capability and enhancing N uptake while decreasing residual soil inorganic N at harvest and reducing the risk of N leaching. Higher nitrogen accumulation and residual soil inorganic N contributed to lower apparent N loss and apparent N loss efficiency for the N-efficient cultivar than for the N-inefficient cultivar. Moreover, N loss during the maize growth season mainly occurred before silking, and the N-efficient maize cultivar could effectively reduce N loss before silking because of its earlier N uptake ability. Overall, the selection of N-efficient maize cultivars is not only an important measure to enhance N use efficiency but also an important means to reduce the loss of N and environmental pollution, especially at low and medium N levels.

## ARTICLE HISTORY

Received 22 April 2019  
Revised 1 October 2019  
Accepted 14 November 2019

## KEYWORDS

Maize; N-efficient cultivar;  
N accumulation; soil mineral  
N; N loss

## 1 Introduction


Maize (*Zea mays* L.) is one of the most important cereal crops in the world, and it requires the largest portion of the grain crops planting areas in China (Jiang, 2013; Wang et al., 2014). Given the greatly decreasing farmland area and the limited potential of increasing the food planting area, the improvement of crop yield per unit area is an effective method to guarantee global food safety (Yan et al., 2016).

Nitrogen (N) is one of the most important limiting elements in natural ecosystems and in most agricultural systems (Chen et al., 2013), and N is closely correlated with agronomic characters (Zhang et al., 2015), leaf photosynthesis (Feng et al., 2015; Széles, Megyes & Nagy, 2012), biomass production and distribution (Ning, Zheng, Han, Jiang & Li, 2012; Zhang et al., 2014), and the panicle traits of crops (Jian et al., 2014). It has been proven that N fertilization is the most feasible method to improve maize yield per unit area (Chen et al., 2015; Zhang et al., 2016). However, to obtain high yields, farmers have become accustomed to

applying excessive amounts of synthetic N fertilizers, which has resulted in China becoming the largest consumer of N fertilizer in the world; China accounts for 9% of the world's arable land and consumes more than 30% of the world's N fertilizer (Chen et al., 2014; Shi, Zhang & Dong, 2013). Unreasonable N fertilization and excessive N fertilizer application might result not only in crop lodging, ripening delay, and poor yield and quality (Li et al., 2015; Ahmed, Humphreys, Salim & Chauhan, 2016) but also in low N use efficiency (Stamatiadis, Tsadilas, Samaras, Schepers & Eskridge, 2016), massive N loss (Qiao, Yang, Yan, Xue & Zhao, 2013), and environmental pollution that includes enhanced greenhouse gas emissions, surface-water eutrophication, and soil acidification and changes in biodiversity (Hamilton, Trimmer, Bradley & Pinay, 2016). In recent years, many methods have been proposed to reduce N loss, including N application models, site-specific N management plans, split applications to match the N needs of crops, and the development of high-efficiency N fertilizers (Hofmeier et al., 2015; Montemurro, Maiorana, Ferri & Convertini, 2006; Qiao

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 Supplemental data for this article can be accessed [here](#).

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et al., 2014; Sui et al., 2013). However, some of these approaches are difficult for farmers to implement due to higher labor and production costs.

Genetic variations in N uptake and grain yield per unit of N application have also been studied in different crops such as rice, wheat, oilseed, maize, and barley (Abdel-Ghani et al., 2013; Li et al., 2014; Singh, Agrawal, Agrawal, Singh & Singh, 2015; Ulas, Behrens, Wiesler, Horst & Erley, 2015). Technological developments in maize breeding have allowed breeders to develop N-efficient maize cultivars because they produce higher grain yields than N-inefficient cultivars with the same rate of N application (Chun, Mi, Li, Chen & Zhang, 2005; Coque & Gallais, 2010; Moll, Kamprath & Jackson, 1982; Pathan et al., 2015). The characteristics of N-efficient maize cultivars have been widely reported in recent years. Mu et al. (2015) found that the root length density, root surface area, and dry weight at the silking stage were 9.6–19.5% higher in an N-efficient cultivar than in an N-inefficient cultivar, while the root distribution pattern in the soil profile showed no significant differences between the two cultivars. Pathan et al. (2015) suggested that an N-efficient maize line induced larger changes in soil chemical properties and in the enzyme activity, soil microbial biomass and community structure than the N-inefficient maize line. Ma et al. (2014) demonstrated that N-efficient maize cultivars have higher root numbers and root density in deeper soil layers and more effective root distribution than N-inefficient maize cultivars, which lead to stronger nitrogen uptake ability. It is proposed that these properties enable N-efficient maize cultivars to alleviate the negative effects of using urea or ammonium-based N fertilizers on the environment. However, it is unknown what differences these two types of maize cultivar have on apparent N balance, N loss period and N loss rate.

To date, no research on the differences in N efficiency responses to N application between different maize cultivars has been reported in Southwest China. We preliminarily screened the N-efficient maize cultivar ZH 311 and N-inefficient cultivar XY 508 from the maize cultivars currently in production and application in Southwest China using indoor and outdoor experiments in the early period (Li et al., 2014; Li et al., 2015; Li et al., 2016) and then conducted a two-year field pot experiment near Jianyang City in the Mid-Sichuan Hilly Region, a main maize-producing region in Southwest China.

The objectives were to investigate whether N-efficient maize cultivars in the Mid-Sichuan Hilly Region can play an effective role in alleviating N losses from maize fields to the ecosystem and to identify the different effects of these two types of maize cultivars on apparent N balance, N loss period and N loss rate. The results of this study can provide useful information for understanding how N-efficient maize cultivars alleviate N loss in the fields and how to guide local farmers to implement the best N management practices according to the N loss characteristics of N-efficient and N-inefficient maize cultivars.

## 2 Materials and methods

### 2.1 Experimental materials

The N-efficient maize cultivar 'ZH 311' and the N-inefficient maize cultivar 'XY 508', screened in previous studies (Li et al. 2016; Li et al. 2014), were used as experimental materials in the present study. Both cultivars are major cultivars grown in Sichuan, and the two cultivars have similar growth periods of approximately 120 d. During the experimental period, the plant height and grain yield of the two cultivars were significantly different, and the data are presented in Figures S1 and S2.

### 2.2 Experimental design

A field pot experiment was conducted in 2014–2015 using soil collected from the top 20 cm of a field with long-term maize production in Jianyang, Sichuan Province, China. The soil was air-dried and passed through a 0.5-cm mesh; visible roots and organic residue were removed manually, and the soil was mixed thoroughly before use in the pot experiment. The soil properties of the tested soil are shown in Table 1 prior to the start of the pot experiment. The daily temperature and precipitation in Jianyang are shown in Figure 1.

The pot experiment included two maize hybrids, ZH 311 (which is an N-efficient maize cultivar provided by Sichuan Nongda Zhenghong Seed Co., Ltd.) and XY 508 (which is an N-inefficient maize cultivar provided by Pioneer Technology Co. Ltd.), and three rates of N application in 2014 (0, 150, and 300 kg N·ha<sup>-1</sup>) and four rates in 2015 (0, 150, 300, and 450 kg N·ha<sup>-1</sup>).

**Table 1.** Soil properties of the tested soil in 2014–2015.

Year	Organic matter (g·kg <sup>-1</sup> )	Total N (g·kg <sup>-1</sup> )	Total P (g·kg <sup>-1</sup> )	Total K (g·kg <sup>-1</sup> )	Alkali hydrolysable N (mg·kg <sup>-1</sup> )	Olsen-P (mg·kg <sup>-1</sup> )	Exchangeable K (mg·kg <sup>-1</sup> )	pH
2014	15.75	1.75	0.57	12.61	39.26	2.55	139.33	7.59
2015	13.30	1.56	0.40	8.25	36.34	2.27	128.50	8.16

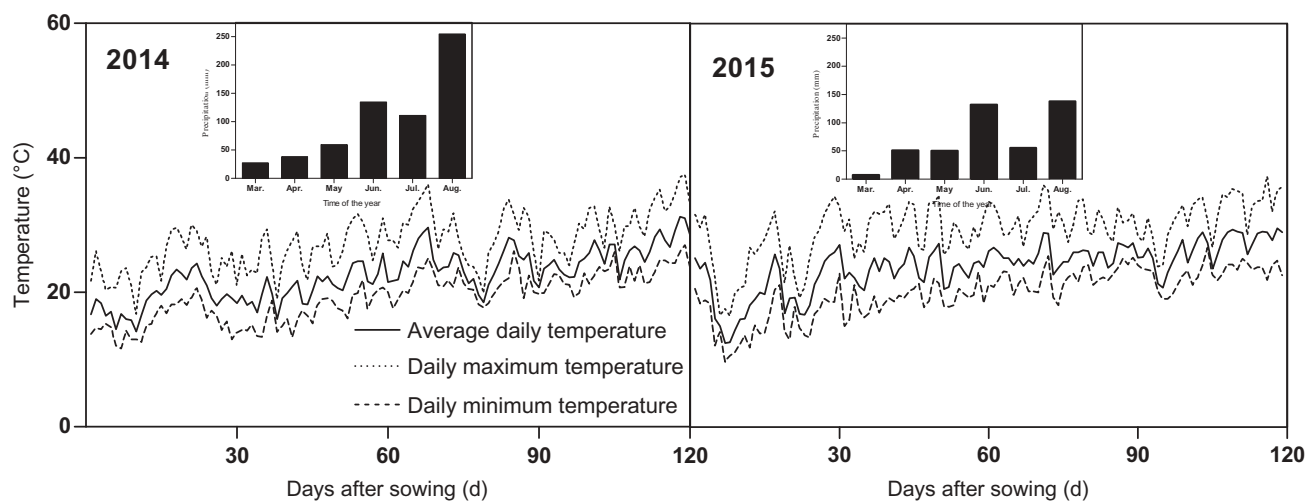


Figure 1. Climate data during the experimental period in Jianyang (2014–2015), China.

A randomized block design with three replicates was used, and each replicate included 20 plastic pots (both diameter and height were 30 cm); a total of 360 pots in 2014 and 480 pots in 2015 were used. Each plot was filled with 20 kg soil and buried in a 30 cm deep ditch and arranged in a wide-narrow row (1.4 m + 0.4 m) scheme with a plant density of 52,500 plants·ha<sup>-1</sup>. Seedlings with two fully expanded leaves were transplanted into plastic pots (two plants per pot). In all treatments, 72 kg·ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> as a single superphosphate (0–12.0–0) and 90 kg·ha<sup>-1</sup> of K<sub>2</sub>O as potassium chloride (0–0–63.1) were applied at the basal stage, whereas N fertilizer was equally split-applied (5:5) as urea (46.3–0–0) at the basal and large bell stages. Chemical products were used to control insects, diseases, and weeds to avoid yield loss.

### 2.3 Sampling and measurements

At the jointing stage (JS), large bell stage (LBS), silking stage (SS), filling stage (FS, 20 d after silking), and maturity stage (MS), two neighboring pots (4 plants) were sampled from each replicate, and the samples were divided into root, leaf lamina, stem plus sheath, and panicle. The fresh samples were oven-dried at 105°C for 60 min and then at 80°C until a constant weight was reached to determine the dry matter accumulation of the plants. The dried organ samples were milled and sifted through a 0.25-mm screen, and their Kjeldahl-N was determined.

For soil inorganic N (amount of inorganic N accumulation) analysis, soil samples were obtained from each plot before transplanting and at JS, LBS, SS, FS, and MS. According to the method of (Shi et al., 2013), fresh soil samples from the same

replicate were mixed, extracted immediately using 2 mol·L<sup>-1</sup> potassium chloride solution (soil solution ratio, 1:5), shaken at 25°C for 2 h, centrifuged at 3500 r min<sup>-1</sup> for 15 min, and then filtered. The content of NH<sub>4</sub>-N and NO<sub>3</sub>-N were analyzed by indophenol blue colourimetric and ultraviolet wavelength, respectively. Taken 200 nm and 228 nm as the measurement and reference wavelengths to determine NO<sub>3</sub>-N to eliminate the interference of NO<sub>2</sub>-N (Deng, Wang, Ren & Mei, 2014; Yan et al., 2016). Soil water content was determined by oven-drying at 105°C at each sampling stage.

### 2.4 Calculation methods (Deng et al., 2014; Yan et al., 2016)

$$\text{Naccumulation}(\text{gplant}^{-1}) = \text{Drymatter} \times \text{Nconcentration} \quad (1)$$

The amount of inorganic N accumulation (N min) of soil was calculated as follows:

$$\text{N min}(\text{kg ha}^{-1}) = 20 \times (1 - \text{soil water content before transplanting}) \times (1 + \text{soil water content at sampling stage}) \times \text{soil inorganic N (NH}_4 - \text{N and NO}_3 - \text{N) content at sampling stage} \quad (2)$$

The apparent N mineralization (ANM) during the maize growing season was calculated as the difference between N output (crop N accumulation + residual soil N min) and N input (initial soil N min) in the plots with no

N treatment. The apparent N loss (*N* loss) was calculated as follows:

$$\begin{aligned} \text{N loss (kg ha}^{-1}\text{)} = & \text{N supply initial soil N min} \\ & + \text{N fertilizer rate} + \text{ANM} \\ & - \text{N output residual soil N min} \\ & + \text{crop N accumulation} \end{aligned} \quad (3)$$

The apparent *N* loss in different growth stages and apparent *N* loss rate were calculated as follows:

$$\text{N loss}(t_1 \sim t_2) (\text{kg ha}^{-1}) = \text{Apparent N loss at } t_2 - \text{apparent N loss at } t_1 \quad (4)$$

$$\text{N loss rate}(t_1 \sim t_2) (\text{kg ha}^{-1}\text{d}^{-1}) = \frac{\text{N loss}(t_1 \sim t_2)}{(t_2 - t_1)} \quad (5)$$

## 2.5 Statistical analysis

The analyses were carried out on three replications. Statistical analysis was completed using the SPSS 18.0 software package for Windows. One-way analysis of variance (ANOVA) was used to determine whether the *N* treatments and cultivars had significant effects on *N* accumulation and residual soil *N* min at the 0.01 or 0.05 levels. The least significant difference (LSD) test at the 0.05 level was used to determine the differences between the means of each treatment.

## 3 Results

### 3.1 Nitrogen accumulation of maize

*N* fertilizer treatment, cultivar and their interaction significantly ( $p < 0.01$ ) affected the *N* accumulation of maize in the different growth stages (Table 2). The *N* accumulation of the *N*-efficient cultivar ZH 311 was

remarkably higher than that of the *N*-inefficient cultivar XY 508. ZH 311 was higher by 3.17, 7.10, 12.25, 14.98, and 20.57 kg·ha<sup>-1</sup> in 2014, and by 2.38, 5.91, 8.51, 13.95, and 17.20 kg·ha<sup>-1</sup> in 2015 at JS, LBS, SS, FS, and MS, respectively. Thus, with maize growth, the differences between the two maize cultivars on *N* accumulation increased. *N* fertilizer drastically increased maize *N* accumulation in the different stages, while the *N* effects of the two maize cultivars were significantly different. The *N*-efficient maize cultivar ZH 311 had higher *N* uptake and accumulation ability than the *N*-inefficient maize cultivar XY 508, especially in the middle and late growth stages. The *N* accumulations of both ZH 311 and XY 508 increased with increasing *N* level in each growth period within the range of treatments (0–300 kg N·ha<sup>-1</sup>) in 2014. However, in 2015, the *N* accumulation of the *N*-inefficient maize cultivar XY 508 increased with increasing *N* fertilizer level, and the highest value was observed at 450 kg N·ha<sup>-1</sup>, while the *N* accumulation of the *N*-efficient maize cultivar ZH 311 initially increased and then decreased with increasing *N* level, and the highest value was observed at 300 kg N·ha<sup>-1</sup>.

### 3.2 Residual soil inorganic N

The difference in residual soil inorganic *N* between ZH 311 and XY 508 was significant (Table 3). The residual soil inorganic *N* of ZH 311 was observably ( $p < 0.01$ ) higher than that of the *N*-inefficient cultivar XY 508 (except for LBS in 2014) by 6.03%, 6.94%, 6.98%, and 8.88% in 2014 and by 7.95%, 16.82%, 6.67%, 10.97%, and 10.59% in 2015 at JS, LBS, SS, FS, and MS, respectively. *N* fertilizer markedly increased the residual soil inorganic *N* of both cultivars, and the residual soil inorganic *N* of both cultivars increased with increasing *N* rate in each growth stage. The average of 5 growth stages over two years, the regression of the

Table 2. Nitrogen accumulation of maize in different growth stages (kg·ha<sup>-1</sup>).

Cultivar	Nitrogen rate	JS		LBS		SS		FS		MS	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
ZH 311	0 N	6.99 d	4.22 f	10.92 d	12.52 g	21.63 e	16.30 f	27.48 e	22.11 g	37.38 e	24.94 g
	150 N	27.29 b	32.64 d	55.43 c	42.24 e	78.54 c	75.57 d	99.25 c	93.37 e	120.15 c	108.13 e
	300 N	33.21 a	40.18 a	81.81 a	66.41 a	125.07 a	110.73 a	134.49 a	128.05 a	156.97 a	146.07 a
	450 N	–	33.36 c	–	56.69 c	–	101.36 b	–	123.15 b	–	138.64 b
	<b>Average</b>	<b>22.50 A</b>	<b>27.60 A</b>	<b>49.39 A</b>	<b>44.46 A</b>	<b>75.08 A</b>	<b>75.99 A</b>	<b>87.07 A</b>	<b>91.67 A</b>	<b>104.83 A</b>	<b>104.45 A</b>
XY 508	0 N	6.42 e	4.11 f	8.50 e	8.66 h	14.08 f	10.56 g	17.88 f	14.83 h	24.87 f	16.90 h
	150 N	24.10 c	29.15 e	54.05 c	34.66 f	73.79 d	66.16 e	90.74 d	77.01 f	99.49 d	83.32 f
	300 N	27.48 b	34.87 b	64.31 b	52.82 d	100.61 b	92.11 c	107.64 b	104.57 d	128.41 b	118.48 d
	450 N	–	32.78 d	–	58.07 b	–	101.08 b	–	114.49 c	–	130.10 c
	<b>Average</b>	<b>19.33 B</b>	<b>25.22 B</b>	<b>42.29 B</b>	<b>38.55 B</b>	<b>62.83 B</b>	<b>67.48 B</b>	<b>72.09 B</b>	<b>77.72 B</b>	<b>84.26 B</b>	<b>87.20 B</b>
F value	Cultivar (C)	496.3**	468.3**	132.8**	747.4**	182.9**	195.5**	213.5**	294.6**	518.2**	322.0**
	Nitrogen (N)	10,369.3**	18,860.6**	3732.4**	11,015.1**	3728.2**	4631.5**	3294.1**	3307.5**	5363.7**	3040.4**
	C × N	109.6**	125.8**	71.4**	212.2**	46.2**	40.1**	33.5**	21.4**	26.3**	29.3**

JS: jointing stage, LBS: large bell stage, SS: silking stage, FS: filling stage, MS: maturity stage. Data are presented as means of three replicates in each treatment. Different lowercase letters in column represent significant differences at  $p < 0.05$ . Different uppercase letters in column represent significant differences between cultivars. A summary of the ANOVA is presented with: no significant (ns), significant at  $p < 0.05$  (\*), very significant at  $p < 0.01$  (\*\*).



**Table 3.** Residual soil inorganic N in different growth stages (kg·ha<sup>-1</sup>).

Cultivar	Nitrogen rate	JS		LBS		SS		FS		MS	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
ZH 311	0 N	11.68 e	10.78 g	5.68 d	4.90 g	15.36 e	3.81 g	11.19 d	4.30 h	4.16 e	1.97 f
	150 N	45.12 c	34.99 e	7.80 b	16.88 e	51.13 c	41.42 d	26.02 c	19.53 e	5.21 d	4.06 e
	300 N	63.95 a	79.06 c	9.28 a	22.84 c	67.97 a	62.57 c	42.77 a	25.03 c	8.65 a	6.23 c
	450 N	–	90.44 b	–	29.20 a	–	87.56 a	–	32.90 a	–	8.21 a
	<b>Average</b>	<b>40.25 A</b>	<b>53.82 A</b>	<b>7.58 B</b>	<b>18.46 A</b>	<b>44.82 A</b>	<b>48.84 A</b>	<b>26.66 A</b>	<b>20.44 A</b>	<b>6.01 A</b>	<b>5.12 A</b>
XY 508	0 N	10.84 f	8.37 h	6.20 c	4.98 g	13.02 f	4.93 f	9.09 e	6.26 g	4.18 e	2.15 f
	150 N	43.60 d	28.61 f	7.85 b	13.76 f	49.07 d	32.14 e	26.03 c	15.48 f	6.74 b	4.36 de
	300 N	59.45 b	69.49 d	9.51 a	18.40 d	63.65 b	63.38 c	39.63 b	23.38 d	5.64 c	4.55 d
	450 N	–	92.95 a	–	26.05 b	–	82.69 b	–	28.56 b	–	7.45 b
	<b>Average</b>	<b>37.96 B</b>	<b>49.86 B</b>	<b>7.85 A</b>	<b>15.80 B</b>	<b>41.91 B</b>	<b>45.79 B</b>	<b>24.92 B</b>	<b>18.42 B</b>	<b>5.52 B</b>	<b>4.63 B</b>
F value	Cultivar (C)	989.6**	28.1**	13.3**	82.5**	62.5**	79.4**	15.3**	44.7**	69.3**	42.0**
	Nitrogen (N)	165,702.1**	1766.9**	722.8**	1067.2**	6908.1**	10,281.8**	1613.8**	1289.8**	872.0**	1021.2**
	C × N	239.1**	10.8**	3.4 <sup>ns</sup>	10.9**	3.8	52.7**	4.3	23.2**	518.0**	37.8**

JS: jointing stage, LBS: large bell stage, SS: silking stage, FS: filling stage, MS: maturity stage. Data are presented as means of three replicates in each treatment. Different lowercase letters in column represent significant differences at  $p < 0.05$ . Different uppercase letters in column represent significant differences between cultivars. A summary of the ANOVA is presented with: no significant (ns), significant at  $p < 0.05$  (\*), very significant at  $p < 0.01$  (\*\*).

difference in residual soil inorganic N between ZH 311 and XY 508 ( $y$ ) and the N application rate ( $x$ ) was  $y = -0.00003418x^2 + 0.01931x + 0.3668$  ( $R^2 = 0.9971^{**}$ ). The differences in residual soil inorganic N between ZH 311 and XY 508 first increased and then decreased with increasing N rate. The largest difference in residual soil inorganic N between ZH 311 and XY 508 was 3.09 kg·ha<sup>-1</sup> at 282.5 kg N ha<sup>-1</sup>. There was, therefore, a significant difference in residual soil inorganic N between the N-efficient cultivar ZH 311 and the N-inefficient cultivar XY 508.

### 3.3 Apparent N balance

Due to low total N output (low crop N uptake and residual N min after harvest), the apparent N loss and apparent N loss rate during the entire growing season of the N-inefficient cultivar XY 508 was significantly ( $p < 0.01$ ) higher than that of the N-efficient cultivar ZH 311 (Table 4). Increasing N fertilizer not only increased N accumulation and residual N min after harvest but also drastically increased apparent N loss and apparent N loss efficiency at the same time. The two cultivars performed consistently in both years, and the linear regression equations for the apparent N loss ( $y$ ) and N fertilizer level ( $x$ ) of ZH 311 and XY 508 were  $y_{ZH\ 311} = -22.6630 + 0.73338x$  ( $R^2 = 0.9671^*$ ) and  $y_{XY\ 508} = -14.147 + 0.7394x$  ( $R^2 = 0.9881^{**}$ ) in 2015, respectively. With every 100 kg increase in N fertilizer, the apparent N loss increased 73.64 kg, and the ratio of apparent N loss and N supply (up to 43.15–73.67) increased with increasing N fertilizer. Regarding the cultivars, the ratio of apparent N loss and N supply of the N-inefficient maize cultivar XY 508 was clearly higher than that of the N-efficient cultivar ZH 311, and the results for the same cultivars at the same N levels in both years were largely the same.

### 3.4 N loss period

N fertilizer treatment, cultivar and their interaction significantly ( $p < 0.05$ ) affected apparent N loss in different periods of maize growth (Table 5). The apparent N loss of the N-inefficient cultivar XY 508 was higher than that of the N-efficient cultivar ZH 311, and that of XY 508 was higher by 13.05%, 24.04%, 5.53%, 15.35%, and 61.40% in 2014 and by 8.27%, 4.72%, 4.03%, 19.45%, and 26.91% in 2015 in sowing stage-JS, JS-LBS, LBS-SS, SS-FS, and FS-MS, respectively. The differences between the two maize cultivars in apparent N loss were lowest at JS-LBS in both years, and the highest differences were at FS-MS in both years. The differences in apparent N loss between ZH 311 and XY 508 mainly occurred at the later growth stages. Furthermore, the increased N rate drastically ( $p < 0.01$ ) increased apparent N loss in different periods of maize growth. The apparent N loss of ZH 311 increased with every 100 kg increase in N fertilizer, by 0.21, 0.02, 0.22, 0.05, and 0.04 kg·ha<sup>-1</sup> in 2014 and by 0.20, 0.09, 0.24, 0.08, and 0.01 kg·ha<sup>-1</sup> in 2015, while that of XY 508 was 0.23, 0.03, 0.23, 0.06, and 0.06 kg·ha<sup>-1</sup> in 2014 and was 0.22, 0.10, 0.25, 0.10, and 0.02 in 2015 at sowing-JS, JS-LBS, LBS-SS, SS-FS, and FS-MS, respectively. Therefore, the increase in apparent N loss of XY 508 with every 100 kg increase in N fertilizer was higher than that of ZH 311 in all the growth periods. That is, with the increased N rate, the pollution risk of planting XY 508 was much higher than that of planting ZH 311.

### 3.5 N loss rate

The highest apparent N loss rate was observed at LBS-SS in both years because of its high temperature and soil mineral N, while the lowest values were at JS-LBS and FS-MS in 2014 and 2015, respectively (Table 6).

Furthermore, the apparent N loss rate of the N-inefficient cultivar XY 508 in different periods was higher than that of the N-efficient cultivar ZH 311, and that of XY 508 was higher by 13.05% at sowing stage-JS, 24.04% at JS-LBS, 5.53% at LBS-SS, 15.34% at SS-FS, and 61.41% at FS-MS in 2014, and by 8.27%, 4.72%, 4.03%, 19.45%, and 26.91% in 2015, respectively. The differences between the two maize cultivars in apparent N loss rate at FS-MS were higher than those at the other periods. With increasing

N fertilizer, the apparent N loss rate in different periods significantly ( $p < 0.01$ ) increased, while the increases in ZH 311 and XY 508 were observably different. With every 100 kg increase in N fertilizer, the average over the 5 growth stages in the apparent N loss rate of ZH 311 increased by  $4.97 \text{ g}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$  in 2014 and by  $6.08 \text{ g}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$  in 2015. That of XY 508 increased by  $5.62 \text{ g}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$  in 2014 and by  $6.53 \text{ g}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$  in 2015. Moreover, every 100 kg increase in N fertilizer increased

**Table 4.** Apparent N balance in soil-maize system in 2014–2015.

Cultivar	Nitrogen rate	Nitrogen input ( $\text{kg}\cdot\text{ha}^{-1}$ )			Nitrogen output ( $\text{kg}\cdot\text{ha}^{-1}$ )				Apparent N loss ( $\text{kg}\cdot\text{ha}^{-1}$ )	Apparent N loss efficiency (%)
		$N_{\text{min}}$ before sowing	Nitrogen fertilizer	Apparent N min	Total input	Crop N uptake	Residual N min after harvest	Total output		
2014	0 N	25.33	0	16.21	41.54	37.38 e	4.16 e	41.54 e	0.00 e	0.00 e
	150 N	25.33	150	16.21	191.54	120.15 c	5.21 d	125.36 c	66.17 d	34.55 d
	300 N	25.33	300	16.21	341.54	156.97 a	8.65 a	165.62 a	175.92 b	51.51 b
	<b>Average</b>	<b>25.33</b>	<b>150</b>	<b>16.21</b>	<b>191.54</b>	<b>104.83</b>	<b>6.01 A</b>	<b>110.84 A</b>	<b>80.70 B</b>	<b>28.69 B</b>
ZH 311	0 N	25.33	0	3.72	29.05	24.87 f	4.18 e	29.05 f	0.00 e	0.00 e
	150 N	25.33	150	3.72	179.05	99.49 d	6.74 b	106.24 d	72.81 c	40.67 c
	300 N	25.33	300	3.72	329.05	128.41 b	5.64 c	134.05 b	195.00 a	59.26 a
	<b>Average</b>	<b>25.33</b>	<b>150</b>	<b>3.72</b>	<b>179.05</b>	<b>84.26 B</b>	<b>5.52 B</b>	<b>89.78 B</b>	<b>89.27 A</b>	<b>33.31 A</b>
XY 508	0 N	–	–	–	–	518.2**	69.3**	536.1**	197.8**	87.9**
	Nitrogen (N)	–	–	–	–	5363.7**	872.0**	5575.3**	482.6**	4387.7**
	C × N	–	–	–	–	26.3**	518.0**	37.8**	84.1**	22.9**
	F value	Cultivar (C)	–	–	–	–	322.0**	42.0**	340.8**	226.7**
2015	0 N	17.27	0	9.63	26.91	24.94 g	1.97 f	26.91 g	0.00 f	0.00 g
	150 N	17.27	150	9.63	176.91	108.13 e	4.06 e	112.19 e	64.72 e	36.58 f
	300 N	17.27	300	9.63	326.91	146.07 a	6.23 c	152.30 a	174.61 c	53.41 d
	450 N	17.27	450	9.63	476.91	138.64 b	8.21 a	146.85 b	330.06 a	69.21 b
<b>Average</b>	<b>17.27</b>	<b>225</b>	<b>9.63</b>	<b>251.91</b>	<b>104.45</b>	<b>5.12 A</b>	<b>109.56 A</b>	<b>142.35 B</b>	<b>39.80 B</b>	
ZH 311	0 N	17.27	0	1.78	19.05	16.90 h	2.15 f	19.05 h	0.00 f	0.00 g
	150 N	17.27	150	1.78	169.05	83.32 f	4.36 de	87.67 f	81.38 d	48.14 e
	300 N	17.27	300	1.78	319.05	118.48 d	4.55 d	123.03 d	196.02 b	61.44 c
	450 N	17.27	450	1.78	469.05	130.10 c	7.45 b	137.55 c	331.50 a	70.68 a
<b>Average</b>	<b>17.27</b>	<b>225</b>	<b>1.78</b>	<b>244.05</b>	<b>87.20 B</b>	<b>4.63 B</b>	<b>91.83 B</b>	<b>152.23 A</b>	<b>45.06 A</b>	
XY 508	0 N	–	–	–	–	322.0**	42.0**	340.8**	226.7**	230.8**
	Nitrogen (N)	–	–	–	–	3040.4**	1021.2**	3297.7**	48,232.4**	7726.1**
	C × N	–	–	–	–	29.3**	37.8**	31.4**	31.4**	62.0**
	F value	Cultivar (C)	–	–	–	–	322.0**	42.0**	340.8**	226.7**

Data are presented as means of three replicates in each treatment. Different lowercase letters in column represent significant differences at  $p < 0.05$ . Different uppercase letters in column represent significant differences between cultivars. A summary of the ANOVA is presented with: no significant (ns), significant at  $p < 0.05$  (\*), very significant at  $p < 0.01$  (\*\*).

**Table 5.** Apparent N loss in different periods ( $\text{kg}\cdot\text{ha}^{-1}$ ).

Cultivar	Nitrogen rate	Sowing stage – JS		JS – LBS		LBS – SS		SS – FS		FS – MS	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
ZH 311	0 N	0.00 e	0.00 g	0.00 d	0.00 f	0.00 c	0.00 f	0.00 d	0.00 f	0.00 b	0.00 c
	150 N	21.26 d	22.37 f	4.00 c	10.93 e	28.95 b	19.82 e	6.08 c	10.40 e	2.77 b	1.20 c
	300 N	71.52 b	45.76 d	7.10 b	32.41 d	68.43 b	68.65 c	17.47 b	26.52 d	14.51 a	1.27 c
	450 N	–	116.21 a	–	40.32 b	–	124.66 b	–	39.18 b	–	9.69 a
<b>Average</b>	<b>30.93 B</b>	<b>46.08 B</b>	<b>3.70 B</b>	<b>20.92 B</b>	<b>32.46 B</b>	<b>53.28 B</b>	<b>7.85 A</b>	<b>19.03 B</b>	<b>5.76 B</b>	<b>3.04 A</b>	
XY 508	0 N	0.00 e	0.00 g	0.00 d	0.00 f	0.00 c	0.00 f	0.00 d	0.00 f	0.00 b	0.00 bc
	150 N	24.56 c	29.71 e	3.24 c	10.52 e	30.78 b	26.97 d	6.96 c	11.43 e	12.60 a	2.76 b
	300 N	80.33 a	58.12 c	10.53 a	34.31 c	71.99 a	67.57 c	20.19 a	33.15 c	15.29 a	2.87 b
	450 N	–	111.75 b	–	42.79 a	–	127.18 a	–	46.33 a	–	9.80 b
<b>Average</b>	<b>34.96 A</b>	<b>49.89 A</b>	<b>4.59 A</b>	<b>21.90 A</b>	<b>34.26 A</b>	<b>55.43 A</b>	<b>9.05 A</b>	<b>22.73 A</b>	<b>9.30 A</b>	<b>3.86 A</b>	
F value	Cultivar (C)	1165.5**	145.2**	5.2*	6.6*	5.1*	17.0**	3.8 <sup>ns</sup>	30.4**	10.5**	0.9 <sup>ns</sup>
	Nitrogen (N)	74,979.1**	23,882.3**	122.4**	2503.1**	2601.6**	11,331.8**	316.6**	810.5**	62.2**	25.9**
	C × N	472.5**	140.5**	60.6**	3.4 <sup>ns</sup>	1.7 <sup>ns</sup>	12.3**	1.7 <sup>ns</sup>	7.7**	8.4**	0.3 <sup>ns</sup>
	F value	Cultivar (C)	1165.5**	145.2**	5.2*	6.6*	5.1*	17.0**	3.8 <sup>ns</sup>	30.4**	10.5**

JS: jointing stage, LBS: large bell stage, SS: silking stage, FS: filling stage, MS: maturity stage. Data are presented as means of three replicates in each treatment. Different lowercase letters in column represent significant differences at  $p < 0.05$ . Different uppercase letters in column represent significant differences between cultivars. A summary of the ANOVA is presented with: no significant (ns), significant at  $p < 0.05$  (\*), very significant at  $p < 0.01$  (\*\*).

**Table 6.** Apparent N loss rate in different periods (kg·ha<sup>-1</sup>·d<sup>-1</sup>).

Cultivar	Nitrogen rate	Sowing stage – JS		JS – LBS		LBS – SS		SS – FS		FS – MS	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
ZH 311	0 N	0.00 e	0.00 g	0.00 d	0.00 f	0.00 d	0.00 f	0.00 d	0.00 f	0.00 b	0.00 b
	150 N	0.48 d	0.50 f	0.19 c	0.61 e	1.93 c	1.32 e	0.30 c	0.52 e	0.14 b	0.06 b
	300 N	1.63 b	1.02 d	0.34 b	1.80 d	4.56 b	4.58 c	0.87 b	1.32 d	0.73 a	0.06 b
	450 N	–	2.58 a	–	2.24 b	–	8.31 b	–	1.96 b	–	0.49 a
	<b>Average</b>	<b>0.70 B</b>	<b>1.03 B</b>	<b>0.18 B</b>	<b>1.16 B</b>	<b>2.16 B</b>	<b>3.55 B</b>	<b>0.39 A</b>	<b>0.95 B</b>	<b>0.29 B</b>	<b>0.15 A</b>
XY 508	0 N	0.00 e	0.00 g	0.00 d	0.00 f	0.00 d	0.00 f	0.00 d	0.00 f	0.00 b	0.00 b
	150 N	0.56 c	0.66 e	0.15 c	0.58 e	2.05 c	1.80 d	0.35 c	0.57 e	0.63 a	0.14 b
	300 N	1.83 a	1.29 c	0.50 a	1.91 c	4.80 a	4.50 c	1.01 a	1.66 c	0.76 a	0.14 b
	450 N	–	2.48 b	–	2.38 a	–	8.48 a	–	2.32 a	–	0.49 a
	<b>Average</b>	<b>0.79 A</b>	<b>1.11 A</b>	<b>0.22 A</b>	<b>1.22 A</b>	<b>2.28 A</b>	<b>3.70 A</b>	<b>0.45 A</b>	<b>1.14 A</b>	<b>0.46 A</b>	<b>0.19 A</b>
F value	Cultivar (C)	1131.2**	131.2**	5.1*	6.6*	5.0*	17.0**	3.5 <sup>ns</sup>	30.6**	11.0**	0.9 <sup>ns</sup>
	Nitrogen (N)	145,498.6**	22,722.0**	124.1**	2506.6**	2573.4**	11,440.2**	307.6**	807.0**	64.3**	25.8**
	C × N	471.7**	136.5**	58.5**	3.1 <sup>ns</sup>	1.7 <sup>ns</sup>	12.5**	1.7 <sup>ns</sup>	7.7**	8.6**	0.3 <sup>ns</sup>

JS: jointing stage, LBS: large bell stage, SS: silking stage, FS: filling stage, MS: maturity stage. Data are presented as means of three replicates in each treatment. Different lowercase letters in column represent significant differences at  $p < 0.05$ . Different uppercase letters in column represent significant differences between cultivars. A summary of the ANOVA is presented with: no significant (ns), significant at  $p < 0.05$  (\*), very significant at  $p < 0.01$  (\*\*).

the apparent N loss of XY 508 more than that of ZH 311, by 13.08% in 2014 and 7.40% in 2015, and the largest difference between ZH 311 and XY 508 was at FS-MS.

## 4 Discussion

The use of N fertilizer has been one of the most effective ways to close the 'yield gap' between the amount of maize production and the demand (Ning et al., 2012; Chen et al., 2015). In this study, both cultivars and N application rates have been discussed; our findings might allow increased N uptake in maize and reduce N loss from maize production to the environment in Sichuan, China.

### 4.1 N-efficient maize cultivar increase N accumulation

Because N accumulation is highly related to experimental conditions (especially soil fertility and meteorological factors) (Stamatiadis et al., 2016), N accumulation after jointing and total N accumulation were markedly higher in 2014 than in 2015 because of the high soil fertility and appropriate precipitation in the former year. Previous studies have demonstrated that the N accumulation of a crop mainly depends on the N rate and cultivar (Montemurro et al., 2006; Chen et al., 2015). N application was found to have a significant influence on N accumulation in this study, where the total N accumulation increased with an increase in N application (Table 2). Furthermore, the N-efficient maize cultivar ZH 311 took up significantly more N than the N-inefficient maize cultivar XY 508 during the maize growing season in both years (Table 2). Li et al. (2010) also found similar outcomes from other N-efficient maize cultivars (Xianyu 335 and Nongda 108, maize hybrids) and showed that N-efficient maize

cultivars could improve total N accumulation by increasing N accumulation and delaying leaf senescence after silking to produce more photosynthetic products. We also found that the difference between the N-efficient cultivar ZH 311 and the N-inefficient cultivar XY 508 on total N accumulation was significantly influenced by N fertilizer level and decreased with increasing N fertilizer level. That is, the ratios of total N accumulation in ZH 311 and XY 508 were negatively correlated with the level of N fertilizer, and the regression equation of the ratio of total N accumulation in ZH 311 and XY 508 ( $y$ ) and N fertilizer level ( $x$ ) was  $y = 1.4623 - 0.000863x$  ( $R^2 = 0.9728^*$ ), indicating that the advantages of the N-efficient cultivar are more prominent under low and medium N levels.

Except for the yield-increasing effect and ammonia volatilization loss, the level of residual mineral N in the soil is the most direct reflection of whether N fertilization is reasonable (Hamilton et al., 2016; Qiao et al., 2013). Previous studies have shown that N application drastically increases mineral N accumulation in the topsoil after harvest and that higher residual mineral N enhances soil N supply capacity, potentially migrates to the subsoil and leads to nitrate pollution of groundwater (Qiao et al., 2014; Yan et al., 2016). N fertilization was found to drastically increase residual soil inorganic N in each stage in our study, and mineral N accumulation increased with increased N application (Table 3). Moreover, the residual soil inorganic N of the N-efficient maize cultivar ZH 311 was significantly higher than that of the N-inefficient maize cultivar XY 508 in the appropriate growth stage, while that of the N-inefficient maize cultivar was higher than that of the N-efficient maize cultivar ZH 311 at MS under the low N treatment (150 N). These results indicate that the N-efficient maize cultivar ZH 311 could increase residual soil inorganic N at the appropriate growth stage, promoting soil N supply



capability and enhancing N uptake while decreasing residual soil inorganic N at harvest and reducing the risk of N leaching.

#### 4.2 N-efficient maize cultivar reduces N loss

According to Yan et al. (2016) and Deng et al. (2014), N balance requires the quantification of both N inputs (including initial soil  $N_{\text{min}}$ , N mineralization, N fertilizer, and artificially provided N) and N outputs (including crop N uptake, N loss, and N immobilization) during the crop growing season and is widely used to evaluate the sustainability or efficiency of N applications, as well as the ability to maintain soil fertility in crop systems. Wang et al. (2014) reported that apparent N loss increased significantly with increasing N application rate and was consistent with our study. Notably, apparent N loss is greatly related to environmental conditions and field management practices, and the apparent  $N_{\text{min}}$ , crop N uptake and apparent N loss were noticeably higher in 2014 than in 2015 because of the high soil fertility and excessive precipitation. Compared to the N-inefficient maize cultivar XY 508, the N-efficient maize cultivar ZH 311 drastically increased the total N output by enhancing crop N uptake, which decreased the apparent N loss by 10.62% in 2014 and 6.94% in 2015 (Table 4). Nevertheless, the differences between the two maize cultivars in apparent N loss first increased and then decreased with increasing N application rate, suggesting that the N-efficient maize cultivar could effectively reduce apparent N loss compared with the N-inefficient maize cultivar and that the advantage of the N-efficient maize cultivar ZH 311 with respect to N uptake and apparent N loss was more obvious under low and medium N treatments. The loss of N can easily cause environmental pollution, so the selection of an N-efficient maize cultivar is important not only to enhance N use efficiency but also to reduce the loss of N and environmental pollution.

Previous studies have shown that N loss occurred more readily after fertilization events and at the early stages of crop growth due to the large proportion of N fertilizer input and the relatively slow N uptake by plants at the early stages (Chen et al., 2015). In this study, it was found that 80.09%~83.14% and 82.72%~84.50% of N loss occurred before the silking stage in 2014 and in 2015 (Table 5), respectively. Furthermore, the apparent N loss rate before silking was clearly higher than that after silking, indicating that apparent N loss mainly occurred before silking because all N fertilizer was applied before silking in Sichuan, and the period before silking is a relatively slow growth stage in the maize growth season. In this study, the apparent N loss and apparent N loss rate in each period of the N-efficient maize

cultivar ZH 311 were noticeably lower than that of the N-inefficient maize cultivar XY 508, while the difference between the two maize cultivars in N loss at sowing stage-JS was significantly higher than that at the other stages. These results were in agreement with Chen et al. (2015), who reported that N-efficient rice cultivars showed significant reductions in N loss via  $\text{NH}_3$  volatilization,  $\text{N}_2\text{O}$  emission, leaching and runoff compared with N-inefficient rice cultivars, and that the difference between the two cultivars in N loss mainly comes from the higher ability of the N-efficient cultivar to uptake more N at the early growth stages. Therefore, the N loss during the maize growth season mainly occurred before silking, and the N-efficient maize cultivar could effectively reduce N loss before silking because of its earlier N uptake ability. We researched the differences between N-efficient and N-inefficient maize cultivars on apparent N loss under different N fertilizer rates; N loss pathways, such as  $\text{NH}_3$  volatilization,  $\text{N}_2\text{O}$  emission, leaching and runoff, require further research.

## 5 Conclusion

The N-efficient maize cultivar ZH 311 had higher N uptake and accumulation ability than the N-inefficient maize cultivar XY 508, especially in the middle and late growth stages. ZH 311 maintained higher residual soil inorganic N in the appropriate growth stage, promoting soil N supply capability and enhancing N uptake while decreasing residual soil inorganic N at harvest and reducing the risk of N leaching. Higher nitrogen accumulation and residual soil inorganic N contributed to a lower apparent N loss and apparent N loss efficiency for the N-efficient cultivar than for the N-inefficient cultivar. Moreover, the N loss during the maize growth season mainly occurred before silking, and the N-efficient maize cultivar could effectively reduce N loss before silking because of its earlier growth N uptake ability. It was concluded that the selection of N-efficient maize cultivars is not only an important measure to enhance N use efficiency but also an important means to reduce the loss of N and environmental pollution, especially at low and medium N levels.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This study was funded by the Special Fund for Agro-scientific Research in the Public Interest [20150312705], the Ministry of Science and Technology of the People's Republic of China

[2012BAD04B13], and the Science and Technology Department of Sichuan Province [2014NZ0040].

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