



## Development of a critical nitrogen dilution curve based on leaf dry matter for summer maize



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### ARTICLE INFO

#### Keywords:

Summer maize  
Critical nitrogen concentration  
Leaf dry matter  
Nitrogen management  
Nitrogen diagnosis

### ABSTRACT

Accurate diagnosis of nitrogen (N) fertilizer required for crop growth can serve as a guide for N management by improving N use efficiency and grain yields. The critical N concentration ( $N_c$ ), the minimum N required for maximal crop growth has been widely used to determine crop N status.  $N_c$  dilution curves have been determined in several crops including summer maize on plant dry matter (DM) basis, yet no attempt has been made to determine the  $N_c$  dilution curve on the basis of leaf dry matter (LDM) in summer maize. The present study aimed to determine a  $N_c$  dilution curve based on LDM for in-season assessment of crop N status in summer maize. Six field experiments were performed with four summer maize cultivars using varied N fertilizer rates ranging from 0 to 320 kg N ha<sup>-1</sup>. The leaf  $N_c$  curve was described by the equation:  $N_c = 3.45LDM^{-0.22}$ , when LDM ranged from 1.18 to 3.45 t ha<sup>-1</sup>. For LDM < 1.18 t ha<sup>-1</sup>, the constant  $N_c = 3.33\%$  was used. The newly developed curve effectively distinguishes N-limiting from non-N-limiting treatments under different environmental conditions. In the present study, the N nutrition index (NNI) ranged from 0.49 to 1.16 under different N rate treatments. The correlation between NNI and relative yield (RY) was a significantly positive, while the correlation between NNI and the agronomic N use efficiency (AE) was significantly negative. The newly developed leaf  $N_c$  dilution curve not only determines the crop N status, but also elucidates the yield and AE changes in response to different N rate treatments in summer maize. The projected results of the study will provide accurate N status diagnosis at critical growth stages and guidance for precision N management in summer maize, thus contributes towards the sustainability of intensive maize cropping systems in China.

### 1. Introduction

The continuous decline in the availability of land suitable for agriculture has increased the importance of maximizing crop yield per unit area in China (Ata-Ul-Karim et al., 2016a; Godfray et al., 2011). Supplementing crop growth with nitrogen (N) fertilizer application can significantly increase yields and improve economic returns. However, the excessive use of N fertilizer in intensive maize cropping systems of China has led to lower agronomic N use efficiency (AE), an increase in greenhouse gas emissions as well as soil and water pollution (Zhao et al., 2014; Zhu et al., 1997). Previous studies reported that the farmers usually apply approximately 263 kg N ha<sup>-1</sup> during the summer maize growth period in the North China Plain (Wang et al., 2014). In contrast, findings of several studies conducted across the North China Plain region demonstrated that the N application rate for summer maize cultivation could be reduced to 158 kg N ha<sup>-1</sup> without any reduction in

grain yield (Cui et al., 2008). The over application of N fertilizer in North China Plain has not only reduced farmers income by increasing cost of production but also polluted the groundwater in the region. The nitrate (NO<sub>3</sub>) concentration (50 mg NO<sub>3</sub>/L) in the region exceeds the World Health Organization's drinking water standard of 11.3 mg NO<sub>3</sub> N/L (Brauns et al., 2016). Therefore, optimizing the quantity of N required at each crop growth stage is critical for increasing grain yield, improving AE and environmental sustainability (Ata-Ul-Karim et al., 2017a).

The optimization of the crop N requirement during growth period is essential to accurately determine the crop N status. Several methods such as chlorophyll meter measurements (Ata-Ul-Karim et al., 2016b), dynamic monitoring of N in plant roots (Xu et al., 2012), remote sensing (Schlemmer et al., 2013), and direct measurement of plant N concentration (Plénet and Lemaire, 1999) have been used to determine the plant N status. Direct measurement of plant N concentration for N

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status diagnosis is based on the theory of critical N ( $N_c$ ) dilution, the minimum N concentration required for maximal growth (Ulrich, 1952). The plant  $N_c$  dilution curve can serve as an indicator of crop N status and can be derived by using the equation described by Greenwood et al. (1990):

$$N_c = a_c \text{ DM}^{-b} \quad (1)$$

where  $a_c$  is the  $N_c$  concentration for plant dry matter (DM) equal to  $1 \text{ t ha}^{-1}$  and  $b$  is the decline in  $N_c$  concentration with crop growth.

The  $N_c$  dilution curves have been determined in several crops, including winter wheat (Justes et al., 1994), spring maize (Plénet and Lemaire, 1999), rice (Ata-Ul-Karim et al., 2013), and winter barley (Zhao, 2014). Previous reports pointed out interspecies and intraspecies dissimilarities in the  $N_c$  curve (Justes et al., 1994), as well as between experimental sites (Greenwood et al., 1990). Classically used plant DM approach offers sufficient insight into the factors governing N uptake in crops (Ata-Ul-Karim et al., 2017b). Yet, the variations associated with the high degree of in-season spatial distribution of crop N status within the crop field, restrict the adaptation of plant DM approach in modern mechanized agricultural practices (Fitzgerald et al., 2010). Additionally, the previous studies showed that plant DM may not always be the most appropriate level of morphological aggregation at which useful weight/N relationships should be obtained, and the stress responses can change the bi-compartmental partitioning of DM among plant organs, thus affect the shape of the dilution curves (Ata-Ul-Karim et al., 2017b; Kage et al., 2002).

The crop  $N_c$  dilution theory has also been used to develop the  $N_c$  dilution curves on plant index bases (leaf, stem, spike and leaf area index) in rice (Yao et al., 2014a; Ata-Ul-Karim et al., 2017b), wheat (Yao et al., 2014b; Zhao, 2014; Zhao et al., 2016) and rapeseed (Weymann et al., 2016). The shapes of the curves on different plant index bases were similar to those on whole plant DM basis. The development of  $N_c$  dilution curve on different plant index bases will offer the possibility to investigate plant index responses to N deficiency or excess in more detail. The use of the different plant index and N concentration relationships in crop plants, rather than PDM alone, would offer greater benefit for improved understanding of the concept of  $N_c$  dilution curves in crops (Ata-Ul-Karim et al., 2017b). Detailed investigations on physiological processes of DM partitioning and N distribution in different plant organs during the crop growth period are imperative, in order to analyze options and limitations of improving N use efficiency and N management for maximal crop production.

There is a strong link between plant N concentration and its metabolic activities during the growth period. Metabolic activities at early growth stages are considered to be distributed throughout the plant body (due to lack of structural compartment) but as the plant growth proceeds, the metabolic activities mainly rely on metabolic components (leaves) rather the structural component (stems) (Justes et al., 1994). The N in the plant is thus initially proportional to the plant DM and then after progressive variations is become proportional to N dilution in metabolic compartments (Weymann et al., 2016). A recent study on rice indicated that due to the most efficient physiological use of N in leaves during crop growth period, the development of leaf  $N_c$  dilution curve can give better and in-depth understanding of crop N status (Ata-Ul-Karim et al., 2017b). Additionally, the real-time and non-destructive methods of monitoring crop growth in the field (e.g. remote sensing methods and digital photography) generally focus on the leaf canopy rather than the plants themselves (Rico-Garcia et al., 2009). Therefore, it is imperative to develop leaf dry matter (LDM) based  $N_c$  dilution curve to find out the most appropriate leaf N concentration (LNC) required to optimize metabolic activity, crop growth as well as to provide an extremely robust method for determining N status in summer maize crop.

The present study was aimed to develop a new  $N_c$  dilution curve based on LDM and to assess the reliability of the newly developed curve

for assessment of N nutrition status in summer maize. The projected results will provide a new strategy for N management in summer maize grown in the North China Plain.

## 2. Materials and methods

### 2.1. Experimental design

Six field experiments of varied N rates were conducted during 2015 and 2016 growing seasons at Xinxiang (35.2°N, 113.8°E) and Qinyang (35.1°N, 112.9°E). The distance between two experimental sites was approximately 80 km. The summer maize cultivars, N application rates, sowing and harvesting dates, as well as soil characteristics, are summarized in Table 1. During 2015 season (June to September), Xinxiang and Qinyang received 90.3 mm and 82.5 mm, 26.3 °C and 24.3 °C, 205.7 h and 191.4 h of average monthly precipitation, temperature, and sunshine hours, respectively, while during 2016 season (June to September), Xinxiang and Qinyang received 90.3 mm and 82.5 mm, 26.3 °C and 24.3 °C, 205.7 h and 191.4 h of average monthly precipitation, temperature, and monthly sunshine hours, respectively. Soil samples were collected from 0 to 20 cm soil layer before sowing summer maize crops. The samples were air-dried, sieved, and then used to measure organic matter (the Walkley-Black titration method, Nelson and Sommers, 1982), total N (traditional Kjeldahl method; Bremner and Mulvaney, 1982), Olsen-P (0.05 mol L<sup>-1</sup> NaHCO<sub>3</sub>; Olsen et al., 1954), NH<sub>4</sub>OAc-K<sup>+</sup> (1 mol L<sup>-1</sup> ammonium acetate at pH 7; van Reeuwijk, 1992). All field experiments were arranged in a randomized complete block design with three replicates. The size of each plot was 60m<sup>2</sup> (6 m × 10 m) in all the experiments conducted during 2015 and 2016 seasons. N fertilizer was applied before sowing (50%) and at the jointing stage (50%). All plots received adequate quantities of triple superphosphate (150 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium chloride (120 kg K<sub>2</sub>O ha<sup>-1</sup>) before sowing. Summer maize was over-seeded with hand planters and then thinned at the seedling stage to a planting density of 75,000 plants ha<sup>-1</sup> with a row spacing of 60 cm. Additional crop management practices were in accordance with local agriculture department recommendations. The summer maize was irrigated (60 mm) after sowing to ensure seed germination and uniform crop establishment. The rainfall during the crop growth period was enough to fulfill the crop water requirements without any supplemental irrigation. No obvious water, pest, or disease stress was observed during the growing seasons. The N fertilizer application was the only limiting factor.

### 2.2. Sampling and measurement

Five plants per plot were harvested at different crop developmental stages (elongation stage, bell stage, tasselling stage, anthesis stage and silking stage) to measure LDM and LNC. The sampling dates for each experiment are presented in Table 1.

For each sampling date, summer maize plants were severed at ground level and were separated into different organs (stem, leaf, and cob). Leaf samples were oven dried at 80 °C until a constant weight to measure the LDM (t ha<sup>-1</sup>). The dried samples were ground, passed through a 1-mm sieve and stored in plastic bags for chemical analysis. LNC was determined by using the micro-Kjeldahl method (Bremner and Mulvaney, 1982).

Grain yield was measured by harvesting whole plants manually from a 10 × 1.25 m<sup>2</sup> area of every plot. Harvested cobs were wet shelled and a grain subsample was dried at 55 °C until the constant weight. Grain yield was adjusted to 14% moisture. The relative yield (RY) was calculated by dividing the grain yield obtained for a particular N treatment by the highest yield among all treatments.

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