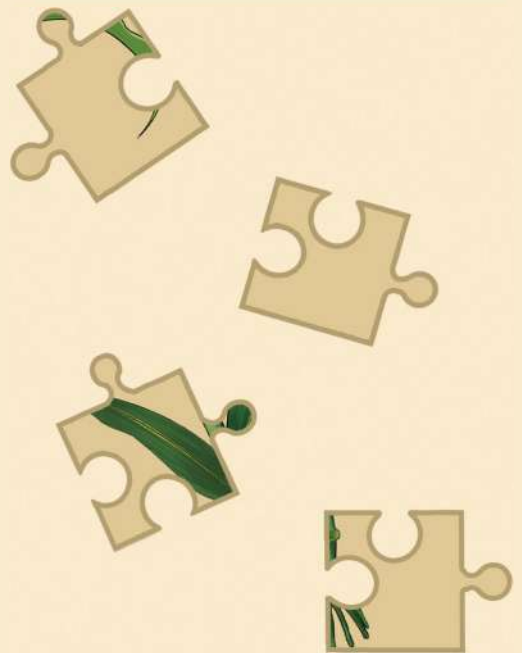


How do maize plants respond to nitrogen availability?

The spatial and temporal distribution of nitrogen within the canopy



Panpan Fan

Propositions

1. Detailed information of leaf traits is essential for a broad understand of maize canopy functioning.
(this thesis)
2. Selection for high yields in maize has not been associated with a more coordinated light and nitrogen distribution.
(this thesis)
3. Imitation is the first step towards scientific innovation.
4. Learning how to deal with uncertainty in field experiments is more useful than good planning.
5. Longer working hours and higher productivity cannot coexist.
6. Losing battles is ok.

Propositions belonging to the thesis, entitled

How do maize plants respond to nitrogen availability? The spatial and temporal distribution of nitrogen within the canopy.

Panpan Fan

Wageningen, November 20, 2023

**How do maize plants respond
to nitrogen availability?**

The spatial and temporal distribution
of nitrogen within the canopy

Panpan Fan

Thesis committee

Promotor

Prof. Dr N.P.R. Anten

Professor of Crop and Weed Ecology, Centre for Crop System Analysis
Wageningen University & Research

Co-promotors

Prof. Dr S.K. Li

Professor of Institute of Crop Science,
Chinese Academy of Agricultural Sciences, China

Prof. Dr J.B. Evers

Professor of Crop Physiology, Centre for Crop System Analysis
Wageningen University & Research

Other members

Prof. Dr L.M.F. Marcelis, Wageningen University & Research

Prof. Dr SV Archontoulis, Iowa State University, United States of America

Dr O Muller, Forschungszentrum, Jülich, Germany

Dr AGT Schut, Wageningen University & Research

This research was conducted under the auspices of the C.T. de Wit Graduate School
for Production Ecology and Resource Conservation (PE&RC).

**How do maize plants respond
to nitrogen availability?**
The spatial and temporal distribution
of nitrogen within the canopy

Panpan Fan

Thesis

submitted in fulfilment of requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 20 November 2023
at 11 a.m. in the Omnia Auditorium.

Panpan Fan

How do maize plants respond to nitrogen availability? The spatial and temporal distribution of nitrogen within the canopy, 160 pages

PhD thesis, Wageningen University, Wageningen, The Netherlands (2023)
With references, with summary in English

ISBN: 978-94-6447-920-1

DOI: <https://doi.org/10.18174/639856>

Abstract

Maize (*Zea mays* L.) is one of the most important cereal crops with high economic and social values. Considering the need to increase food production, it is important to investigate the determining factors that contribute to maize production in terms of agronomic management practices and crop breeding. The main aim was to explore the morphological and physiological trait responses of maize plants to N availability and to explore how these traits may have changed as a result of crop breeding. In this thesis, we focused on quantifying plant architecture, nitrogen and biomass distribution across leaf positions, stems, and other organs in maize.

The accumulation, partitioning, and reallocation processes of N at both the individual leaf and plant scales were quantified, examining their responses to varying N availabilities resulting from long-term N fertilizer treatments in a field experiment. The results revealed that a relatively larger fraction of grain N was derived from reallocation and a smaller fraction from the soil. When considering the vertical pattern of N reallocation across leaves in the canopy, we noted that a larger fraction of N in leaves was reallocated from lower leaves than from higher ones. However, in absolute terms, the largest amounts of N were reallocated from middle-canopy leaves compared to both top and bottom leaves. Additionally, plants exhibit phenotypic plasticity in response to these environmental changes. A trade-off strategy related to radiation capture and radiation-use efficiency (RUE) under nitrogen-deficient conditions was explored in maize plants. We conclude that the main strategy of maize to cope with low N is to maintain leaf area, mainly by increasing specific leaf area (SLA, cm^2/g) throughout the plant but only during the vegetative growth phase.

Additionally, the vertical distribution patterns of canopy architecture, light, and nitrogen within the maize canopy were also explored among cultivars released between 1950 and 2004. We found that the yield improvement from older to newer cultivars was strongly associated with changes in canopy architecture, resulting in increased light interception through higher leaf area index (LAI) and improved distribution of light in the canopy due to steeper leaf angles. Nevertheless, the coordination of light and nitrogen within the canopy did not differ among old and new cultivars. These results showed that higher yields of modern maize cultivars are not associated with coordinated light and N distribution within the canopy. This thesis also introduces a novel empirical equation for quantitatively describing the vertical distribution of leaf dimensions within the maize canopy. Compared with previous bell-shaped functions, the new equation reduces the number of parameters required to characterize the leaf morphology, simplifying numerical calculations. Furthermore, by analysing the char-

acteristics of the new equation with the growth and development of maize plants, it revealed that only four key leaf positions need to be considered to construct the new empirical equation, greatly simplifying data collection in the field.

The exploration of how maize plants respond to nitrogen availability in this thesis provides insights into the morphological and physiological traits of maize at the organ, plant, and canopy levels. These findings suggest that the appropriate N fertilizer can sustain high yields while reducing the environmental impact. Moreover, the goals of maize breeding should extend beyond achieving high yields to encompass high grain quality, specifically grain nitrogen concentration, aligning with production demands.

Keywords: maize; yield; nitrogen availability; leaf rank; nitrogen reallocation; nitrogen deficiency; phenotypic plasticity; canopy architecture; light distribution; breeding selection; leaf profile

Table of contents

| | | |
|------------------|--|------------|
| Chapter 1 | General introduction | 9 |
| Chapter 2 | Nitrogen availability determines the vertical patterns of accumulation, partitioning, and reallocation of dry matter and nitrogen in maize | 29 |
| Chapter 3 | Plastic response of leaf traits to N deficiency in field-grown maize | 53 |
| Chapter 4 | Changes in the vertical distributions of light and nitrogen within the canopy during decades of maize breeding | 77 |
| Chapter 5 | A new empirical equation to describe the vertical leaf distribution profile of maize | 101 |
| Chapter 6 | General discussion | 125 |
| | Summary | 145 |
| | Acknowledgements | 153 |
| | About the Author | 156 |
| | List of Publications | 157 |
| | PE&RC Training and Education Statement | 158 |



CHAPTER 1

General introduction

1.1 Background

Maize (*Zea mays* L.) is one of the three most important cereals in the world and a major component of global and national (Chinese) food security (Hou et al., 2020; Zhang et al., 2013). Population growth and increasing demand for animal products continue to increase demand for maize as food, feed and fuel, and other industrial raw materials (Huang et al., 2002; Wheeler and Von Braum, 2013). Therefore, increasing maize production per unit area is crucial for future food security. Previous studies have demonstrated that increasing grain yield depends on genetic characteristics, morphological and physiological behaviour of plants, as well as its interaction with the environment (Sher et al., 2017; Welcker et al., 2022). The latter, the environment, in turn, can be modified through agronomic practices such as fertilizer application, irrigation or weeding. Both crop breeding and agronomic improvements in crop management have hence contributed to yield increases that have been achieved until now (Ci et al., 2011; Duvick, 2005). Considering the need to increase food production, it is important to investigate the determining factors that contribute to maize production in terms of agronomic management practices and crop breeding.

1.2 Agronomic management practices

Nitrogen (N) contributes to over 50% of the world's food production increase (Dimkpa et al., 2020; Zhang et al., 2015), as soils generally do not provide sufficient N to meet maize demands (van Grinsven et al., 2022; Zhai et al., 2022). Hence, N fertilization is used to achieve high and stable yields in maize production. The application of N fertilizer significantly influences the growth and development of maize plants. For example, increased N application can enhance the leaf area index (LAI, the amount of leaf area per unit soil area), delay leaf senescence, and thus extend the period over which leaves are photosynthetically active, thereby enhancing photosynthetic capacity of the canopy and ultimately increasing yields (Bonelli and Andrade, 2020; Li et al., 2022; Valentinuz et al., 2006; Yi et al., 2008). Increasing plant density is another viable strategy to increase production without increasing land use by enhancing grain yield per unit area (Hou et al., 2020; Sher et al., 2017). High plant density has been widely used to improve grain yields in maize, and the increased yield per unit area is primarily attributed to the optimized plant population rather than enhanced yield per individual plant (Assefa et al., 2018; Li et al., 2020). Higher planting density leads to a significant rise in LAI, aboveground dry matter (DM) accumulation, and grain yield per population of maize (Du et al., 2021; D. Zhang et al., 2020).

However, plant density should not be too high. As density increases, competition among maize plants also intensifies, leading to reduced acquisition of resources like light, water and nutrients per plant (Li et al., 2018; Sher et al., 2017). In dense plant populations, the close proximity of neighbouring plants can lead to shading, thereby restricting the amount of sunlight that reaches the lower leaves (Monsi and Saeki, 2005; Pierik and De Wit, 2014). This reduction in light availability consequently diminishes photosynthetic activity of these lower leaves, resulting in the premature senescence of these shaded leaves (Boonman et al., 2006; Legner et al., 2014). Furthermore, scarcity of N can trigger the onset of early leaf senescence as plants prioritize resource allocation towards vital organs or new growth (Gallais et al., 2007; Ning et al., 2013). There is thus an optimal plant density for maximal yield at which the positive effect of there being more plants per unit area just offsets the negative effect of yield per plant being lower. Previous studies have demonstrated that the application of N fertilizer can partially offset the negative effects caused by increased plant densities (Labra et al., 2020; Li et al., 2022; Wang et al., 2020). Specifically, adequate N availability promotes plant growth, leaf expansion, and overall productivity, and plants can partly overcome N limitations caused by high plant densities by providing additional N fertilization (Du et al., 2021; Li et al., 2018).

1.3 The roles of nitrogen in plant growth and development

Nitrogen (N) is a vital component of chlorophyll-embedding proteins, photosynthetic enzymes, and other proteins, closely influencing the structure and function of crops (Boomsma et al., 2009; Noor, 2017). Most of the nitrogen within leaves is utilized for photosynthesis, including amino acids involved in nitrogen metabolism enzymes and Rubisco (the enzyme that catalyses the carboxylase reaction that binds CO₂), a crucial participant in carbon metabolism (Field and Mooney, 1986; Mu et al., 2018). However, due to the potential profitability of N fertilizer application, farmers often apply excessive amounts in their pursuit of higher yields and productivity. Unfortunately, this excessive application of nitrogen can result in diminishing returns in terms of crop yields and nitrogen removal, leading to a decrease in nitrogen-use efficiency (NUE, defined as the amount of N harvested per unit nitrogen applied) (Zhang et al., 2015). Furthermore, over-application of N fertilizer contributes to various environmental issues such as soil acidification, water pollution, excessive losses of reactive nitrogen and greenhouse gas emissions (Hou et al., 2020; Noor, 2017), thereby importantly contributing to climate change (Zhang et al., 2015). Therefore, gaining a comprehensive understanding of the effects of nitrogen on plant growth and

development can provide a theoretical foundation for nitrogen management practices and precision agriculture, ultimately aiming to achieve high-yield and high-efficiency cultivation of maize. Several key aspects of growth and development that are affected by N are given in subsequent sections.

1.3.1 The response of leaf dynamics to N availability

Leaves are the primary organs that utilize light energy to synthesize carbohydrates through photosynthesis in plants. Hence, more leaf area typically contributes to more photosynthesis, growth and, in grain crops such as maize, more grain yield formation (Bonelli and Andrade, 2020; Li et al., 2022; Tollenaar and Lee, 2002). The amount of leaf area on a plant in turn, is determined by the rate of leaf growth and leaf area duration (LAD, i.e., the amount of time a leaf stays on a plant). Specifically, the rate and duration of leaf expansion determine the final leaf size. A faster rate of leaf expansion is crucial to achieving the optimal leaf area to cover the soil surface early in the season and maximise light interception (Tollenaar and Bruulsema, 1988). However, the canopy cover is affected by N availability (Bonelli and Andrade, 2020; Valentinuz et al., 2006). N promotes leaf elongation and expansive growth by influencing the rate of cell division or cell size (Carberry et al., 1993; Vos et al., 2005). Plants at limited N availability exhibit lower rates of leaf expansion compared to those with sufficient nitrogen (Muchow and Carberry, 1989; Van Oosterom et al., 2001). This results in smaller leaves, reduced LAI, decreased radiation interception, and ultimately lower yields (Li et al., 2022; Vos et al., 2005).

In addition leaf size in terms of area, the leaf lifespan plays crucial roles in determining leaf area duration (LAD) and dry matter accumulation, ultimately influencing crop yield (Carberry et al., 1993; Maddonni and Otegui, 1996). During leaf senescence, leaf chloroplasts disintegrate and chlorophyll content decreases (Kitonyo et al., 2018; Sadras et al., 2000; Vos et al., 2005), i.e., this is what makes senescent leaves lose their greenness. N is mobilized from aging leaves and transported to other plant tissues for reuse (Z. Liu et al., 2022). This remobilization of nitrogen enables plants to conserve and prioritize resources for essential physiological functions, such as seed development and growth of new leaves (Chen et al., 2015; Gallais et al., 2007). Intriguingly, in maize canopies leaves in the middle part of the canopy, tend to be larger than those higher or lower down in the canopy and also have the longest leaf life span (Li et al., 2022; Valentinuz et al., 2006). This is associated with the fact that these middle canopy leaves are the ones that are located closest to the ear and are thus the leaves that make the largest assimilate contribution to the seed production (Ciganda et al., 2008; Y. Li et al., 2019).

Both leaf area and leaf lifespan are influenced by N availability. Higher N availability leads to increased leaf area, with a more pronounced effect observed in the upper leaves compared to the middle and lower leaves (Li et al., 2022; Valentinuz et al., 2006). Premature or excessive leaf senescence can lead to reduced photosynthetic capacity, nutrient deficiencies, and lower overall plant productivity (Dwyer and Stewart, 1986; Field, 1983; Li et al., 2009). The appropriate application of N fertilizer can enhance the activity of photosynthetic enzymes in maize leaves, increase chlorophyll and nitrogen content, and boost photosynthesis (Dreccer et al., 2000; Xiong and Flexas, 2021). This can also accelerate both vegetative and reproductive phenological growth, prolonging LAD and enhancing post-silking nutrient uptake and dry matter accumulation (Faverjon et al., 2019; R. Li et al., 2019; Yan et al., 2014). Therefore, it is crucial to ensure that sufficient N is available during critical growth stages, minimizing premature leaf senescence and optimizing plant performance.

1.3.2 The response of photosynthesis to N availability

Photosynthesis is the vital process through which plants convert light energy into chemical energy, and for most crops, approximately 95% of the harvested dry matter is a direct product of leaf photosynthesis (Reich et al., 1998). N availability significantly influences various physiological and biochemical processes related to photosynthesis, as N is an essential nutrient required for the synthesis of chlorophyll, enzymes, and other components involved in photosynthesis (Dreccer et al., 2000; Evans and Clarke, 2019). In the case of N-deficient plants, there is a reduction in chlorophyll content and ATP production, which hampers carbon assimilation and starch accumulation, as well as affecting enzyme activity. Consequently, these deficiencies lead to compromised photosynthetic rates and decreased plant productivity (Drouet and Bonhomme, 2004; Evans and Clarke, 2019). Individual leaves within a canopy can exhibit variations in their photosynthetic capacity due to factors such as leaf age, nitrogen content, and light availability. Leaves with higher photosynthetic capacity have a greater ability to fix carbon dioxide, contributing more to the overall canopy photosynthesis (Drouet and Bonhomme, 2004; Ellsworth and Reich, 1993).

Typically, the distribution of nitrogen within a canopy exhibits a vertical gradient, with higher concentrations observed in the upper layers where leaves receive greater light exposure and exhibit higher rates of photosynthesis (Archontoulis et al., 2011; Dreccer et al., 2000; Hirose and Werger, 1987). This vertical distribution of light and nitrogen is closely interconnected, and light availability plays a crucial role in determining the distribution of nitrogen within the canopy (Anten et al., 1995; Bertheloot et al., 2008;

Liu et al., 2020). It affects various factors such as leaf area, photosynthetic rates, and the processes of nitrogen uptake and assimilation (Archontoulis et al., 2011; Liu et al., 2020; Yu et al., 2022). The nitrogen distribution in the canopy often follows the light gradient. One of the underlying mechanisms is believed to be that relatively more illuminated leaves have higher transpiration rates, thus attract more root-produced cytokinin making those leaves stronger N sinks (Boonman et al., 2006). Conversely, nitrogen availability influences the vertical distribution of light by impacting leaf size, chlorophyll content, and photosynthetic capacity (Field and Mooney, 1983; Anten et al., 1996). However, the leaf nitrogen profile undergoes changes during crop development and is responsive to nitrogen availability. In the case of nitrogen-deficient plants, the leaf nitrogen profiles exhibit a more non-uniform and steeper distribution, which is associated with reduced leaf area and increased nitrogen reallocation from lower leaf layers (Evans and Clarke, 2019; Gallais et al., 2007; Gómez et al., 2019).

1.3.3 The response of grain yield formation to N availability

Dry matter (DM) accumulation and allocation to kernels are crucial factors that determine the final maize grain yield (Ning et al., 2013; Olmedo Pico and Vyn, 2021). Therefore, maintaining sufficient nitrogen supply is crucial to ensure optimal photosynthesis, canopy development, proper assimilate partitioning, and successful grain filling (Field, 1983; Liu et al., 2020; Mu et al., 2016). Typically, the grain yield increases as N availability increases, N-sufficient plants often exhibit higher harvest index (HI) compared to N-deficient plants, indicating that a greater proportion of the DM is directed towards harvestable yield (Noor, 2017; Ruiz et al., 2023). However, beyond the optimum N level, additional nitrogen inputs may have minimal or even negative effects on grain yield (Fernandez et al., 2020; Zhang et al., 2018). This is due to factors such as nutrient imbalances, environmental losses, salinity and increased susceptibility to diseases and lodging (Hou et al., 2012; Valentinuz and Tollenaar, 2006).

1.3.4 Phenotypic plasticity in response to N availability

Plant phenotypic plasticity is the capacity of plants of a given genotype to express different phenotypes in response to changes in environmental factors, such as climate, nutrient available or light (Liu et al., 2013; Lucas et al., 2019; Zhu, 2015). For instance, plants typically respond to a high plant density through internode elongation often leading to taller stature, lower ear position, and steeper leaf angle (Liu et al., 2017;

Sher et al., 2017), a set of responses collectively known as the shade-avoidance syndrome (Pierik and De Wit, 2014). In maize, the elongated internodes above the ear may improve the transmission of light, which improves the density tolerance of maize populations (Fournier and Andrieu, 2000; Tokatlidis and Koutroubas, 2004).

Under low nitrogen availability, plants often exhibit plastic responses to overcome N deficiency and maximize their resource acquisition, (Hikosaka, 2016; Lemaire et al., 2008). For instance, plants typically allocate relatively more resources to root growth and increase their root-to-shoot ratio in order to acquire more N (N. Zhang et al., 2020). To this end, maize plants can alter root architecture, by increasing the root surface area, elongation, or branching (R. Li et al., 2019; York et al., 2015). Specifically, plants can access deeper soil layers by elongating the roots, and explore larger soil volumes by more branches, in search of N (York et al., 2015; Yu et al., 2021). But as noted plants can also exhibit plasticity to changes in N availability in their above-ground structures e.g., through production of smaller leaves leading to lower LAI and leaves with lower nitrogen contents per unit area (specific leaf N content SLN) (Grindlay, 1997; Lemaire et al., 2008), and the response patterns to N deficiency differed among species (Massignam et al., 2011; Van Oosterom et al., 2001). Furthermore, plants exhibit adaptive responses to N availability by adjusting the structure and function of their chloroplasts. These adaptations include changes in thylakoid organization, grana stacking, and the expression of chloroplast proteins. Such modifications contribute to the optimization of light capture and energy utilization within the chloroplasts (Evans and Clarke, 2019; Stewart et al., 2003). Finally, as noted, plants typically expedite leaf senescence reallocating more N from older leaves to younger ones or to reproduction.

All these responses will affect how things like grain yield will respond to fertilizer application. Understanding and quantifying plant plasticity in response to N availability is therefore crucial for optimizing agricultural management practices and improving crop productivity. Hence, in this thesis the plastic responses in leaf and canopy traits to differences in N availability in maize crops are explored. As explained in the next section, this understanding may also help breeding targets for nitrogen-efficient crop varieties.

1.4 The roles of genetic improvement in maize breeding

Maize breeding programs have historically focused on maximizing grain yields, but this singular emphasis has inadvertently influenced other secondary plant traits

(Duvick, 2005; Qin et al., 2016). The morphology of the maize plant and ear serves as an initial selection characteristic in breeding (G. Liu et al., 2022; Ma et al., 2022). For example, modern maize hybrids exhibit reduced plant height, ear height, and ear ratio, which enhances lodging resistance. They also have more erect leaves, enabling better light penetration into the canopy at high plant density, as well as smaller ears and kernels, facilitating rapid dehydration during late grain filling (Chen et al., 2016; Ci et al., 2011; Mueller et al., 2019). These morphological selection criteria favour light interception at the ear level and capitalize on higher plant densities to increase grain yield per unit field area. Furthermore, grain filling, the physiological process that determines yield attainment in cereal crops, plays a crucial role. The relatively longer grain-filling duration and higher grain-filling rate contributed to the yield improvement of maize hybrids through breeding as found for 50 hybrids released from 1964 to 2014 in China across multiple environments (Gao et al., 2023). However, despite significant genetic variability and heritability, leaf growth sensitivity, stomatal conductance, and water-use efficiency remained largely unaffected by selection (Welcker et al., 2022).

The N uptake by the crop and N utilization efficiency are two important components of NUE (Kant et al., 2011; Lammerts van Bueren and Struik, 2017). The maize breeding selection of yield as the primary objective can also affect N absorption and utilization over time. For instance, the new varieties of barley in Argentina exhibit higher physiological NUE compared to older varieties (Abeledo., 2008). Moreover, the stress resistance of maize plants enhanced over breeding history, enabling the new varieties to achieve higher yields under both low and high N availability conditions (Tollenaar., 2002; Gong., 2020). However, the N content in grain of modern varieties is lower than that of older varieties, which reflects the fact that modern varieties achieve higher yields for the same amount of N taken up from the soil (Yan et al., 2014). Lower grain N content in turn, implies lower grain protein content and thus in that sense a lower nutritional value. This N reduction in grain could be partly attributed to the stay-green characteristics of modern varieties, which as the name implies means that these varieties have slower senescence (leaves stay green). While this can favour canopy photosynthesis it also results in less N being reallocated to grains (Oosterom, 2010; Liu., 2021). Consequently, to achieve both high yield in terms of dry matter while maintaining a sufficient grain protein content it is important to carefully consider the balance between canopy photosynthesis and N reallocation.

1.5 Research objectives

The variation in plant architecture in response to different levels of N availability can lead to changes in the distribution of light within the maize canopy. As new upper leaves emerge and lower leaves senesce, the vertical distribution of light and nitrogen within the canopy undergo alterations over time. This dynamic process can influence the relationship between light and nitrogen, primarily due to the variation in plant architecture. Compared to older maize cultivars, newer cultivars demonstrate higher yields and possess additional secondary traits such as compact architecture and increased photosynthetic rates. By studying the differences between older and newer cultivars, valuable insights can be gained into the mechanisms responsible for the increases in yield potential that have been achieved through breeding under diverse crop management practices. These findings can contribute to a better understanding of how these cultivars leverage their characteristics to achieve improved yields but could also yield information about the potential to breed for a higher nitrogen-use efficiency in maize.

This thesis thus focuses on quantifying plant architecture, nitrogen and biomass distribution across leaf positions, stems, and other organs in maize. The main objective was to understand the spatial and vertical distribution of light and nitrogen in relation to N availability in terms of the morphological and physiological traits within the canopy, and how these traits may have changed by breeding over the past 54 years. The specific research objectives were:

- To quantify dry matter (DM) and nitrogen (N) accumulation, partitioning, and reallocation from vegetative to reproductive parts in maize, considering variations in soil N availability
- To investigate the strategies employed by maize plants to balance the trade-off between specific leaf nitrogen (SLN) and leaf area (LA), and the underlying traits during both vegetative and reproductive growth stages.
- To elucidate the extent to which selection for higher yields has influenced maize canopy architecture and the vertical distribution of light and nitrogen within the plant canopy.

1.6 Thesis outline

This thesis consists of six chapters, including this general introduction (Chapter 1), four research chapters (Chapters 2-5) and a general discussion (Chapter 6). The structure of this thesis is illustrated in Fig. 1.1.

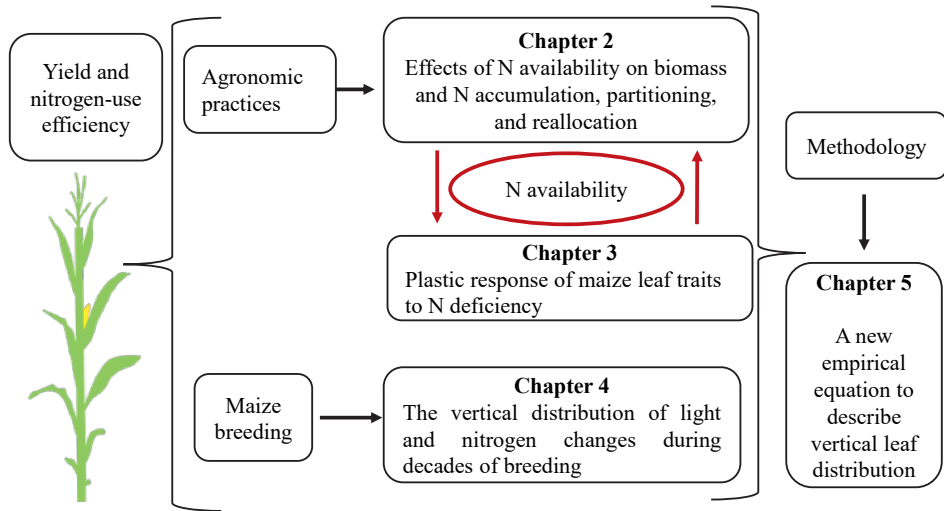


Fig. 1.1. Thesis outline. Agronomic practices and breeding are effective approaches to improve the yield and nitrogen-use efficiency. The agronomic practices such as N availability affect maize performance (Chapter 2), and maize plants respond to N availability through plasticity (Chapter 3). The selected plant traits by maize breeding depends on their performance (Chapter 4). Moreover, a new empirical equation to describe the vertical leaf distribution is presented in Chapter 5 to further address questions on maize N use and productivity using plant simulation models.

Cereal crops face a trade-off between the reallocation of dry matter (DM) and nitrogen (N) to support grain growth and maintain these resources in the canopy to support additional photosynthesis. For N this trade-off is of particular importance because of its relevance for both photosynthesis and grain protein content. In **Chapter 2**, the accumulation, partitioning, and reallocation of DM and N were quantified at the single leaf, canopy and plant scales and their response to different N availabilities created in a long-term N fertilizer field experiments. The N fertilizer management practices had been maintained since 2009, and our data were collected in the year 8 (2016) and 10 (2018) of the experiment. Notably, we also explored N and DM reallocation and its contribution to grain DM and N during the reproductive phase of a crop both at the whole plant and its vertical distribution across leaves in the canopy.

Crop canopy photosynthesis is the product of light capture, which is largely determined by LAI and photosynthesis per unit of intercepted light (so-called radiation-use efficiency, RUE) which is strongly determined by leaf content (SLN). When crop N availability is limiting, negative effects on canopy photosynthesis can either be due to reductions in light capture through a lower LAI or reductions in RUE through SLN (note that for a given amount of N in the canopy a higher LAI automatically entails

a lower SLN, there hence being a trade-off). **Chapter 3**, explores how maize deals with this trade-off, through responses to different N availabilities in (SLN) and, LAI, and their underlying traits during vegetative and reproductive growth. In order to do so, the same long-term N-fertilizer field experiment was conducted, and temporal and spatial distribution of SLA is also discussed in this study.

Breeding has increased yields during the past decades of breeding history, and the genetic yield improvement has been associated with changes in morphological and physiological traits. Further exploration of these associations can give insight into the mechanisms of yield improvements. However, to what extent yield-based selection indirectly affected canopy architecture, and the vertical distribution of light and nitrogen within the maize canopy has not been reported. In **Chapter 4**, the canopy architecture, the vertical distribution patterns of light and nitrogen, and grain yields of maize cultivars, released between 1950 and 2004, were hence quantified and analysed in this study.

The vertical distribution of leaf area is an important determinant of light capture in canopy. In order to further address questions on maize N use and productivity using plant simulation models, this distribution of leaf area needs to be well represented in such models. Therefore, we set out to make simple and robust mathematical descriptions of leaf dimensions (i.e., leaf length, leaf width, and leaf area) that can be implemented in plant models. In **Chapter 5**, the vertical distribution of leaf dimensions are described quantitatively by an empirical equation for which all parameters can be interpreted in a biological meaningful way. By analysing the characteristics of the equation combined with the growth and development of maize plants, four key leaf positions were need be used to build the equation.

In the General Discussion (**Chapter 6**), the main findings in this thesis are put in a broader context, and the information about the vertical distributions about canopy architecture, light and nitrogen distribution, and how they response to N availability within the canopy are discussed.

1.7 Study area

The basis for this thesis was a long-term field experiment with maize grown under different nitrogen (urea) fertilizer levels that was started in 2009 at the Gongzhuling Experimental Station (43°53'N, 124°81'E) of Chinese Academy of Agricultural Sciences, Jilin Province, Northeast China. This study was conducted in a long-term

N-fertilized (urea) field, in which the N fertilizer management practices had been maintained since 2009 (Fig. 1.2).



Fig. 1.2. The aerial view of the drone in the field experiment showing one replicate of the N fertilizer field maize experiment. N0, N1 and N2 denote the low, intermediate and high treatments respectively. This experiment has been run from 2009 and the data presented in this thesis reflect years 7-10 of the experiment.

The total N amount and application stage of the three N fertilizer management practices were as follows: no N fertilizer was applied throughout the whole growth period (N0); 150 kg N/ha was all applied as base fertilizer before sowing (N1), and 150 kg N/ha was applied as described for the N1 treatment, and an additional 150 kg N/ha split equally at V6 and silking stages (N2). The weeds, diseases, and pests were chemically controlled. The cultivars used in this study were Baihe (BH) released in 1950, Jidan 101 (JD101) in 1967, Zhongdan 2 (ZD2) in 1972, Yedan 13 (YD13) in 1998, Zhengdan 958 (ZD958) in 2000, and Xianyu 335 (XY335) in 2004 together spanning a 54-year range in release years. All of the cultivars are successful, widely-grown cultivars, representative of the elite germplasm of the period in which they were released. Individual cultivars were selected because they were known to be well-adapted to the agro-ecological conditions of the study area and seeds of proprietary parental lines were available, which allowed the creation of new F1 hybrid seeds.

References

- Anten, N. P. R., Hernandez, R., & Medina, E. M. (1996). The photosynthetic capacity and leaf Nitrogen concentration as related to light regime in shade leaves of a montane tropical forest tree, *Tetrochidium rubrivenium*. *Functional Ecology*, 491-500.
- Anten, N.P.R., Schieving, F., Werger, M.J.A., (1995). Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C3 and C4 mono- and dicotyledonous species. *Oecologia* 101, 504–513.
- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N.G., Struik, P.C., (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Assefa, Y., Carter, P., Hinds, M., Bhalla, G., Schon, R., Jeschke, M., Paszkiewicz, S., Smith, S., Ciampitti, I.A., (2018). Analysis of long term study indicates both agronomic optimal plant density and increase maize yield per plant contributed to yield gain. *Scientific Reports*, 8(1), 4937.
- Bertheloot, J., Martre, P., Andrieu, B., (2008). Dynamics of light and nitrogen distribution during grain filling within wheat canopy. *Plant physiology*, 148(3), 1707-1720.
- Bonelli, L.E., Andrade, F.H., 2020. Maize radiation use-efficiency response to optimally distributed foliar-nitrogen-content depends on canopy leaf-area index. *Field Crops Research*, 247, 107557.
- Boomsma, C.R., Santini, J.B., Tollenaar, M., Vyn, T.J., (2009). Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. *Agronomy Journal*, 101(6), 1426-1452.
- Boonman, A., Anten, N. P., Dueck, T. A., Jordi, W. J., van der Werf, A., Voeselek, L. A., & Pons, T. L. (2006). Functional significance of shade-induced leaf senescence in dense canopies: an experimental test using transgenic tobacco. *The American Naturalist*, 168(5), 597-607.
- Carberry, P. S., Hammer, G. L., & Muchow, R. C. (1993). Modelling genotypic and environmental control of leaf area dynamics in grain sorghum. III. Senescence and prediction of green leaf area. *Field Crops Research*, 33(3), 329-351.
- Chen, K., Camberato, J. J., Tuinstra, M. R., Kumudini, S. V., Tollenaar, M., & Vyn, T. J. (2016). Genetic improvement in density and nitrogen stress tolerance traits over 38 years of commercial maize hybrid release. *Field Crops Research*, 196, 438-451.
- Chen, Y., Xiao, C., Wu, D., Xia, T., Chen, Q., Chen, F., ... & Mi, G. (2015). Effects of nitrogen application rate on grain yield and grain nitrogen concentration in two maize hybrids with contrasting nitrogen remobilization efficiency. *European Journal of Agronomy*, 62, 79-89.
- Ci, X., Li, M., Liang, X., Xie, Z., Zhang, D., Li, X., ... & Zhang, S. (2011). Genetic contribution to advanced yield for maize hybrids released from 1970 to 2000 in China. *Crop science*, 51(1), 13-20.
- Ciganda, V., Gitelson, A., & Schepers, J. (2008). Vertical profile and temporal variation of chlorophyll in maize canopy: Quantitative “crop vigor” indicator by means of reflectance-based techniques. *Agronomy Journal*, 100(5), 1409-1417.
- Dimkpa, C. O., Fugice, J., Singh, U., & Lewis, T. D. (2020). Development of fertilizers for enhanced nitrogen use efficiency—Trends and perspectives. *Science of the Total Environment*, 731, 139113.
- Dreccer, M. F., Van Oijen, M., Schapendonk, A. H. C. M., Pot, C. S., & Rabbinge, R. (2000). Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. *Annals of Botany*, 86(4), 821-831.

- Drouet, J. L., & Bonhomme, R. (2004). Effect of 3D nitrogen, dry mass per area and local irradiance on canopy photosynthesis within leaves of contrasted heterogeneous maize crops. *Annals of Botany*, 93(6), 699-710.
- Du, X., Wang, Z., Lei, W., & Kong, L. (2021). Increased planting density combined with reduced nitrogen rate to achieve high yield in maize. *Scientific Reports*, 11(1), 358.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy*, 86, 83-145.
- Dwyer, L. M., & Stewart, D. W. (1986). Effect of leaf age and position on net photosynthetic rates in maize (*Zea mays* L.). *Agricultural and forest meteorology*, 37(1), 29-46.
- Ellsworth, D. S., & Reich, P. B. (1993). Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia*, 96, 169-178.
- Evans, J. R., & Clarke, V. C. (2019). The nitrogen cost of photosynthesis. *Journal of Experimental Botany*, 70(1), 7-15.
- Faverjon, L., Escobar-Gutiérrez, A., Litrico, I., Julier, B., & Louarn, G. (2019). A generic individual-based model can predict yield, nitrogen content, and species abundance in experimental grassland communities. *Journal of Experimental Botany*, 70(9), 2491-2504.
- Fernandez, C. W., Ehlke, N., Sheaffer, C. C., & Jungers, J. M. (2020). Effects of nitrogen fertilization and planting density on intermediate wheatgrass yield. *Agronomy Journal*, 112(5), 4159-4170.
- Field, C. (1983). Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia*, 56, 341-347.
- Field, C., Mooney, H.A., (1986). The photoynthesis-nitrogen relationship in wild plants. In T. Givnish (ed) *On the Economy of Plant Form and Function* Cambridge, pp25-55.
- Field, C., & Mooney, H. A. (1983). Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. *Oecologia*, 56, 348-355.
- Fournier, C., & Andrieu, B. (2000). Dynamics of the elongation of internodes in maize (*Zea mays* L.). Effects of shade treatment on elongation patterns. *Annals of Botany*, 86(6), 1127-1134.
- Gallais, A., Coque, M., Le Gouis, J., Prioul, J. L., Hirel, B., & Quillere, I. (2007). Estimating the Proportion of Nitrogen Remobilization and of Postsilking Nitrogen Uptake Allocated to Maize Kernels by Nitrogen-15 Labeling. *Crop science*, 47(2), 685-691.
- Gómez, M. I., Magnitskiy, S., & Rodríguez, L. E. (2019). Nitrogen, phosphorus and potassium accumulation and partitioning by the potato group *Andigenum* in Colombia. *Nutrient Cycling in Agroecosystems*, 113, 349-363.
- Grindlay, D. J. C. (1997). REVIEW Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *The Journal of Agricultural Science*, 128(4), 377-396.
- Hikosaka, K. (2016). Optimality of nitrogen distribution among leaves in plant canopies. *Journal of Plant Research*, 129, 299-311.
- Hirose, T., & Werger, M. J. A. (1987). Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia*, 72, 520-526.
- Hou, P., Gao, Q., Xie, R., Li, S., Meng, Q., Kirkby, E. A., ... & Chen, X. (2012). Grain yields in relation to N requirement: Optimizing nitrogen management for spring maize grown in China. *Field Crops Research*, 129, 1-6.
- Hou, P., Liu, Y., Liu, W., Liu, G., Xie, R., Wang, K., ... & Li, S. (2020). How to increase maize production without extra nitrogen input. *Resources, Conservation and Recycling*, 160, 104913.

- Huang, J., Pray, C., & Rozelle, S. (2002). Enhancing the crops to feed the poor. *Nature*, 418(6898), 678-684.
- Kant, S., Bi, Y. M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of experimental Botany*, 62(4), 1499-1509.
- Kitonyo, O. M., Sadras, V. O., Zhou, Y., & Denton, M. D. (2018). Nitrogen supply and sink demand modulate the patterns of leaf senescence in maize. *Field Crops Research*, 225, 92-103.
- Labra, M. H., Struik, P. C., Calderini, D. F., & Evers, J. B. (2020). Leaf nitrogen traits in response to plant density and nitrogen supply in oilseed rape. *Agronomy*, 10(11), 1780.
- Lammerts van Bueren, E. T., & Struik, P. C. (2017). Diverse concepts of breeding for nitrogen use efficiency. A review. *Agronomy for Sustainable Development*, 37, 1-24.
- Legner, N., Fleck, S., & Leuschner, C. (2014). Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance. *Trees*, 28, 263-280.
- Lemaire, G., van Oosterom, E., Jeuffroy, M. H., Gastal, F., & Massignam, A. (2008). Crop species present different qualitative types of response to N deficiency during their vegetative growth. *Field Crops Research*, 105(3), 253-265.
- Li, J., Wu, M., Wang, K., Ming, B., Chang, X., Wang, X., ... & Li, S. (2020). Identifying ways to narrow maize yield gaps based on plant density experiments. *Agronomy*, 10(2), 281.
- Li, J., Xie, R. Z., Wang, K. R., Hou, P., Ming, B., Zhang, G. Q., ... & Li, S. K. (2018). Response of canopy structure, light interception and grain yield to plant density in maize. *The Journal of Agricultural Science*, 156(6), 785-794.
- Li, J., Yang, J., Fei, P., Song, J., Li, D., Ge, C., Chen, W., (2009). Responses of rice leaf thickness, SPAD readings and chlorophyll a/b ratios to different nitrogen supply rates in paddy field. *Field Crops Research*, 114(3), 426-432.
- Li, R., Liu, P., Dong, S., Zhang, J., & Zhao, B. (2019). Increased maize plant population induced leaf senescence, suppressed root growth, nitrogen uptake, and grain yield. *Agronomy Journal*, 111(4), 1581-1591.
- Li, Y., Ming, B., Fan, P., Liu, Y., Wang, K., Hou, P., ... & Xie, R. (2022). Quantifying contributions of leaf area and longevity to leaf area duration under increased planting density and nitrogen input regimens during maize yield improvement. *Field Crops Research*, 283, 108551.
- Li, Y., Song, H., Zhou, L., Xu, Z., & Zhou, G. (2019). Vertical distributions of chlorophyll and nitrogen and their associations with photosynthesis under drought and rewatering regimes in a maize field. *Agricultural and Forest Meteorology*, 272, 40-54.
- Liu, G., Hou, P., Xie, R., Ming, B., Wang, K., Xu, W., ... & Li, S. (2017). Canopy characteristics of high-yield maize with yield potential of 22.5 Mg ha⁻¹. *Field Crops Research*, 213, 221-230.
- Liu, G., Yang, Y., Liu, W., Guo, X., Xie, R., Ming, B., ... & Li, S. (2022). Optimized canopy structure improves maize grain yield and resource use efficiency. *Food and Energy Security*, 11(2), e375.
- Liu, T., Pan, Y., Lu, Z., Ren, T., & Lu, J. (2020). Canopy light and nitrogen distribution are closely related to nitrogen allocation within leaves in *Brassica napus* L. *Field Crops Research*, 258, 107958.

- Liu, Y., Xie, R., Hou, P., Li, S., Zhang, H., Ming, B., ... & Liang, S. (2013). Phenological responses of maize to changes in environment when grown at different latitudes in China. *Field Crops Research*, 144, 192-199.
- Liu, Z., Sha, Y., Huang, Y., Hao, Z., Guo, W., Ke, L., ... & Mi, G. (2022). Efficient nitrogen allocation and reallocation into the ear in relation to the superior vascular system in low-nitrogen tolerant maize hybrid. *Field Crops Research*, 284, 108580.
- Lucas, F. T., Borges, B. M. M. N., & Coutinho, E. L. M. (2019). Nitrogen fertilizer management for maize production under tropical climate. *Agronomy Journal*, 111(4), 2031-2037.
- Ma, D. ling, Xie, R. zhi, Yu, X. fang, Li, S. kun, & GAO, J. L. (2022). Historical trends in maize morphology from the 1950s to the 2010s in China. *Journal of Integrative Agriculture*, 21(8), 2159-2167.
- Maddoni, G. A., & Otegui, M. E. (1996). Leaf area, light interception, and crop development in maize. *Field Crops Research*, 48(1), 81-87.
- Massignam, A. M., Chapman, S. C., Hammer, G. L., & Fukai, S. (2012). Effects of nitrogen supply on canopy development of maize and sunflower. *Crop and Pasture Science*, 62(12), 1045-1055.
- Monsi, M., & Saeki, T. (2005). On the factor light in plant communities and its importance for matter production. *Annals of botany*, 95(3), 549-567.
- Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G. (2018). Dynamic remobilization of leaf nitrogen components in relation to photosynthetic rate during grain filling in maize. *Plant Physiology and biochemistry*, 129, 27-34.
- Muchow, R. C., & Carberry, P. S. (1989). Environmental control of phenology and leaf growth in a tropically adapted maize. *Field Crops Research*, 20(3), 221-236.
- Mueller, S. M., Messina, C. D., & Vyn, T. J. (2019). Simultaneous gains in grain yield and nitrogen efficiency over 70 years of maize genetic improvement. *Scientific Reports*, 9(1), 9095.
- Ning, P., Li, S., Yu, P., Zhang, Y., & Li, C. (2013). Post-silking accumulation and partitioning of dry matter, nitrogen, phosphorus and potassium in maize varieties differing in leaf longevity. *Field Crops Research*, 144, 19-27.
- Noor, M. A. (2017). Nitrogen management and regulation for optimum NUE in maize—A mini review. *Cogent Food & Agriculture*, 3(1), 1348214.
- Olmedo Pico, L. B., & Vyn, T. J. (2021). Dry matter gains in maize kernels are dependent on their nitrogen accumulation rates and duration during grain filling. *Plants*, 10(6), 1222.
- Pierik, R., & de Wit, M. (2014). Shade avoidance: phytochrome signalling and other above-ground neighbour detection cues. *Journal of experimental botany*, 65(11), 2815-2824.
- Qin, X., Feng, F., Li, Y., Xu, S., Siddique, K. H., & Liao, Y. (2016). Maize yield improvements in China: past trends and future directions. *Plant Breeding*, 135(2), 166-176.
- Reich, P. B., Ellsworth, D. S., & Walters, M. B. (1998). Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology*, 12(6), 948-958.
- Ruiz, A., Trifunovic, S., Eudy, D. M., Sciarresi, C. S., Baum, M., Danalatos, G. J., ... & Archontoulis, S. V. (2023). Harvest index has increased over the last 50 years of maize breeding. *Field Crops Research*, 300, 108991.
- Sadras, V. O., Echarte, L., & Andrade, F. H. (2000). Profiles of leaf senescence during reproductive growth of sunflower and maize. *Annals of Botany*, 85(2), 187-195.

- Sher, A., Khan, A., Cai, L. J., Ahmad, M. I., Asharf, U., & Jamoro, S. A. (2017). Response of maize grown under high plant density; performance, issues and management-a critical review. *Adv. Advances in Crop Science and Technology*, 5(3), 1-8.
- Stewart, D. W., Costa, C., Dwyer, L. M., Smith, D. L., Hamilton, R. I., & Ma, B. L. (2003). Canopy structure, light interception, and photosynthesis in maize. *Agronomy Journal*, 95(6), 1465-1474.
- Tokatlidis, I. S., & Koutroubas, S. D. (2004). A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crops Research*, 88(2-3), 103-114.
- Tollenaar, M., & Bruulsema, T. W. (1988). Efficiency of maize dry matter production during periods of complete leaf area expansion. *Agronomy Journal*, 80(4), 580-585.
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field crops research*, 75(2-3), 161-169.
- Valentinuz, O. R., & Tollenaar, M. (2006). Effect of genotype, nitrogen, plant density, and row spacing on the area-per-leaf profile in maize. *Agronomy Journal*, 98(1), 94-99.
- van Grinsven, H. J., Ebanyat, P., Glendining, M., Gu, B., Hijbeek, R., Lam, S. K., ... & Ten Berge, H. F. (2022). Establishing long-term nitrogen response of global cereals to assess sustainable fertilizer rates. *Nature Food*, 3(2), 122-132.
- Van Oosterom, E. J., Carberry, P. S., & Muchow, R. C. (2001). Critical and minimum N contents for development and growth of grain sorghum. *Field Crops Research*, 70(1), 55-73.
- Vos, J. P. E. L., Van Der Putten, P. E. L., & Birch, C. J. (2005). Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Research*, 93(1), 64-73.
- Wang, X., Wang, G., Turner, N. C., Xing, Y., Li, M., & Guo, T. (2020). Determining optimal mulching, planting density, and nitrogen application to increase maize grain yield and nitrogen translocation efficiency in Northwest China. *BMC plant biology*, 20(1), 1-21.
- Welcker, C., Spencer, N. A., Turc, O., Granato, I., Chapuis, R., Madur, D., ... & Tardieu, F. (2022). Physiological adaptive traits are a potential allele reservoir for maize genetic progress under challenging conditions. *Nature Communications*, 13(1), 3225.
- Wheeler, T., & Von Braun, J. (2013). Climate change impacts on global food security. *Science*, 341(6145), 508-513.
- Xing, G. A. O., Li, Y. X., Yang, M. T., Li, C. H., Song, Y. C., Wang, T. Y., ... & Shi, Y. S. (2023). Changes in grain-filling characteristics of single-cross maize hybrids released in China from 1964 to 2014. *Journal of Integrative Agriculture*, 22(3), 691-700.
- Xiong, D., & Flexas, J. (2021). Leaf anatomical characteristics are less important than leaf biochemical properties in determining photosynthesis responses to nitrogen top-dressing. *Journal of Experimental Botany*, 72(15), 5709-5720.
- Yan, P., Yue, S., Qiu, M., Chen, X., Cui, Z., & Chen, F. (2014). Using maize hybrids and in-season nitrogen management to improve grain yield and grain nitrogen concentrations. *Field Crops Research*, 166, 38-45.
- Yi, Z., Wang, P., Tao, H., Zhang, H., & Shen, L. (2008). Effects of types and application rates of nitrogen fertilizer on the development and nitrogen utilization of summer maize. *Frontiers of Agriculture in China*, 2, 44-49.
- York, L. M., Galindo-Castañeda, T., Schussler, J. R., & Lynch, J. P. (2015). Evolution of US maize (*Zea mays* L.) root architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress. *Journal of Experimental Botany*, 66(8), 2347-2358.

- Yu, N., Ren, B., Zhao, B., Liu, P., & Zhang, J. (2022). Optimized agronomic management practices narrow the yield gap of summer maize through regulating canopy light interception and nitrogen distribution. *European Journal of Agronomy*, 137, 126520.
- Yu, Y., Qian, C., Gu, W., & Li, C. (2021). Responses of root characteristic parameters and plant dry matter accumulation, distribution and transportation to nitrogen levels for spring maize in northeast China. *Agriculture*, 11(4), 308.
- Zhai, J., Zhang, Y., Zhang, G., Tian, M., Xie, R., Ming, B., ... & Li, S. (2022). Effects of nitrogen fertilizer management on stalk lodging resistance traits in summer maize. *Agriculture*, 12(2), 162.
- Zhang, D., Sun, Z., Feng, L., Bai, W., Yang, N., Zhang, Z., ... & Zhang, L. (2020). Maize plant density affects yield, growth and source-sink relationship of crops in maize/peanut intercropping. *Field Crops Research*, 257, 107926.
- Zhang, F., Chen, X., & Vitousek, P. (2013). An experiment for the world. *Nature*, 497(7447), 33-35.
- Zhang, N., Van Westreenen, A., Evers, J. B., Anten, N. P., & Marcelis, L. F. (2020). Quantifying the contribution of bent shoots to plant photosynthesis and biomass production of flower shoots in rose (*Rosa hybrida*) using a functional-structural plant model. *Annals of botany*, 126(4), 587-599.
- Zhang, T. A., Chen, H. Y., & Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbes. *The ISME journal*, 12(7), 1817-1825.
- Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, 528(7580), 51-59.
- Zhu, J. (2015). Plant plasticity in intercropping: mechanisms and consequences. Wageningen University and Research.



CHAPTER 2

Nitrogen availability determines the vertical patterns of accumulation, partitioning, and reallocation of dry matter and nitrogen in maize

Panpan Fan^{1,2}, Bo Ming¹, Jochem B Evers², Yaoyao Li¹,
Shaokun Li¹, Ruizhi Xie¹, Niels P.R. Anten²

¹Key Laboratory of Crop Physiology and Ecology, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

²Center for Crop Systems Analysis, Wageningen University & Research, P.O.Box 430, 6700 AK Wageningen, the Netherlands

This chapter has been published in *Field Crops Research*, 297, 108927

Abstract

Context or problem: The reallocation of dry matter (DM) and nitrogen (N) from vegetative tissues to the grains are critical for both yield quantity and protein content of cereal crops. However, it is unclear to which extent the dynamics in DM and N reallocation depend on N availability, and how individual leaves within the maize canopy respond to different N availabilities in terms of these processes.

Objective or research question: This study aimed to quantify DM and N accumulation, partitioning, and reallocation from vegetative to reproductive parts in maize in relation to soil N availability.

Methods: A long-term N fertilizer trial was conducted in Jilin province, Northeast China, growing maize at three N fertilizer levels (low N availability, N0; intermediate N availability, N1; and high N availability, N2). The accumulation, partitioning, and reallocation of DM and N were quantified at the whole-plant, organs, and single-leaf scales in 2015 and 2016.

Results: Although both post-silking DM accumulation and post-silking N uptake increased in response to higher N availability, 8.3-38.8% of grain N still needed to be reallocated from vegetative organs with a larger fraction coming from leaves (10.5-36.5%) than from stems (4.4-11.6%). This dependency of grain N on N reallocation from vegetative parts increased at lower soil N availability. Furthermore, the vertical patterns of reallocated leaf DM and reallocated leaf N changed with N availabilities. While in general leaves in the middle part of the canopy tended to reallocate more DM and N to the grains than leaves from the upper or lower canopy parts, these most-contributing leaves were higher in the canopy at low than at high N availability.

Conclusions and implications: Grain N relies on reallocation more than does grain DM. At lower N availability, more grain DM and grain N need to be reallocated from vegetative organs. Furthermore, the leaf ranks from which most DM and N were reallocated, gradually increased from the bottom to upper ranks as N availability decreased. Together, our results on the dynamics of N and DM uptake and reallocation during the reproductive phase are important for plant and crop models that require these processes for accurate predictions of maize performance.

Keywords: Dry matter; Leaf N content; Leaf rank; N availability; Reallocation

2.1 Introduction

Yields in cereal crops are determined both by dry matter (DM) accumulation through photosynthesis and by the reallocation of photosynthates from vegetative organs to grain during the reproductive stage (Olmedo Pico and Vyn, 2021; Yang et al., 2021). Meanwhile, maize grain quality is associated with grain protein content, which is closely linked to grain nitrogen (N) content (Cliquet et al., 1990; Kumar et al., 2019). Grain N content in turn is the sum of N uptake from soil and N reallocation during the reproductive phase. Hence, understanding the accumulation, partition, and reallocation processes of DM and N is essential to meet the grain demand for both high yield and high quality.

Grain DM production is contributed by leaf area, photosynthesis duration and photosynthetic rates per unit leaf area (Li et al., 2022; Wang et al., 2018). Many studies pointed out that increasing the total DM accumulation or the proportion of post-silking DM is vital for achieving higher grain DM since more than half of the total aboveground DM was accumulated through photosynthesis at the post-silking stage (Cliquet et al., 1990; Liu et al., 2022; Wei et al., 2019). Grain DM may come from either current assimilation allocated directly to grains or from the reallocation of assimilates derived from pre-silking photosynthesis that were temporarily stored in vegetative organs (Liu et al., 2017; Molla et al., 2019). However, the relative contributions of photo-assimilations and reallocated DM to grain may differ between maize genotype and depends on soil N availability (Chen et al., 2015; Evans, 1989). For instance, compared to older cultivars, modern stay-green cultivars typically show higher post-silking DM accumulation, with much less DM reallocation from vegetative parts to grains (Liu et al., 2017; Zhang et al., 2022). In addition, at high N availability leaf senescence is typically postponed allowing plants to maintain high LAI longer, and thus to increase post-silking canopy photosynthesis and post-silking DM accumulation (Olmedo Pico and Vyn, 2021; Zhang et al., 2010; Zhao et al., 2019).

Grain N in turn is derived from either post-silking root N uptake or from reallocation from vegetative organs (Molla et al., 2019; Wang et al., 2018). For a given N content, there is a trade-off between leaf N reallocation and leaf photosynthesis rate during post-silking stages, because as more N is reallocated from leaves to grains, less N remains in leaves to support photosynthesis (Brown et al., 2019; Liu et al., 2022; Ning et al., 2017). Although higher N reallocation is important to support grain growth, it may concomitantly result in lower leaf N content which in turn speeds up leaf senescence, leading to reduced leaf N contents, green leaf area and canopy photosynthesis (Evans and Clarke, 2019; Hikosaka, 2016). This trade-off is mediated by the amount of N

absorbed from the soil and hence by N fertilizer application. Higher N availability entails that more N needed for grain growth can be taken up from the soil reducing the need for N reallocation such that more N remains in leaves and leaf longevity is prolonged (Boomsma et al., 2009; Li et al., 2022).

Previous studies mostly focused on N distribution, and reallocation during the grain-filling stage at the whole plant level (Chen et al., 2019; Liu et al., 2022). However, it is well known that leaf N distribution is uneven among leaves; the upper leaves generally have higher N contents and specific leaf N than the lower leaves (Archontoulis et al., 2011; Bertheloot et al., 2008; Hirose and Werger, 1987). This entails that more N could be reallocated from upper leaves during the grain-filling stage. However, the upper leaves receiving more light generally have higher photosynthetic rates and nitrogen-use efficiency and thus contribute more to canopy photosynthesis than do lower shaded leaves (Anten et al., 1995; Hikosaka, 2016; Li et al., 2019). Therefore, plants tend to reallocate relatively more N from lower leaves partly through shade-induced senescence (Archontoulis et al., 2011; Liu et al., 2022). The vertical pattern of N reallocation and its effects on post-silking canopy photosynthesis is also affected by N availability from the soil, due to the fact that N availability affects leaf area and thus canopy shading, leaf N content as well as the amount of grain N that can directly be derived from the soil (Li et al., 2022; Perez et al., 2019).

The accumulation, partitioning, and reallocation of dry matter (DM) and nitrogen (N) affect the metabolic processes of the crop and are intricately linked to plant physiological events (Fournier and Andrieu, 1999; Zhao et al., 2019). Although aboveground DM or N accumulation, partitioning, and reallocation have been well documented in maize plants (Liu et al., 2022; Molla et al., 2019), little information is available about the vertical distribution and reallocation patterns of DM or N across leaf ranks and the extent to which this distribution is modified by changes in N availability. In this study the objectives were to: (1) quantify the DM and N accumulation, partitioning, and reallocation at the whole plant and organ scales during the reproductive phase, and (2) improve our understanding of the dynamic responses of vertical DM and N reallocation within the maize canopy to different N availabilities. These questions were addressed in a long-term field N fertilizer trial with maize.

2.2 Materials and methods

2.2.1 Experimental Site

A long-term field experiment with maize grown under different nitrogen (urea) fertilizer levels was started in 2009 at the Gongzhuling Experimental Station of the Chinese Academy of Agricultural Sciences (43°53N, 124°81E) in Jilin province, Northeast China. The meteorological data for 2015 and 2016 at the experimental site were recorded daily during the maize growing season (from 1 May to 30 September). The average daily temperature varied from -1.5°C to 28.0°C in 2015 and from 1.1°C to 28.6°C in 2016 respectively (Fig. 2.1). The total precipitation was 433.6 mm in 2015 and 652.0 mm in 2016.

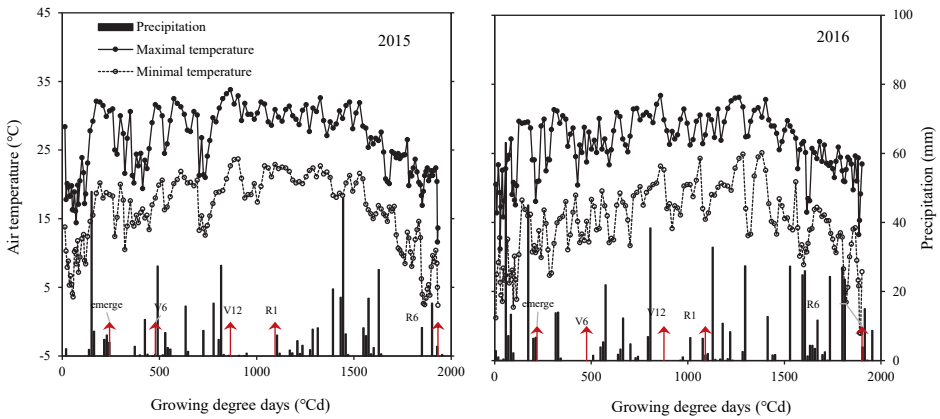


Fig. 2.1. The mean daily maximum and minimum temperature, and daily precipitation during the experimental years. Red arrows indicate key growth stages in maize.

2.2.2 Experimental design

The data in this study was collected in 2015 and 2016, which were the year seven and eight in this long-term trial. Soil samples from the 0 to 20 cm soil layers were taken before fertilizer application during each growing season at the beginning of the field experiments (Table 2.1). The total N amount and application stage of the three N fertilizer management practices were as follows: no N fertilizer was applied throughout the whole growth period (N0), 150 kg N ha⁻¹ was all applied as base fertilizer before sowing (N1), and 150 kg N ha⁻¹ was applied as described for the N1 treatment, and an additional 150 kg N ha⁻¹ splits equally at V6 and silking stages (N2). The chemical fertilizer phosphorus (superphosphate) and potassium (potassium chloride) were applied before sowing at a rate of 42.5 kg P₂O₅ ha/year and 42.5 kg K₂O ha/year to all treatments. As the N treatments had already been maintained for

6-7 years before that start of the experiment, soil N at the start of the experimental years was already different and these differences were increased by current-year N fertilizer application (Table 2.1).

Table 2.1. The soil N content (g/kg) before sowing, the N fertilizer applications, and the different N availabilities in this study

| Year | Treatments | Soil N content (g/kg) | N fertilizer application (kg/ha) | N availability (g/kg) |
|------|------------|-----------------------|----------------------------------|-----------------------|
| 2015 | N0 | 1.09 c | 0 | 1.09 c |
| | N1 | 1.25 b | 150 | 1.29 b |
| | N2 | 1.40 a | 300 | 1.49 a |
| 2016 | N0 | 1.08 c | 0 | 1.08 c |
| | N1 | 1.29 b | 150 | 1.33 b |
| | N2 | 1.46 a | 300 | 1.55 a |

Note: Different letters in the same column indicate significant differences at $P < 0.05$ ($n=10$). The soil bulk density was $1.68/\text{cm}^3$ in this study, and the nutrient availability of P and K were applied the same for all N management treatments.

The experiments used a random block design with three replications each. Individual plots were 45.5m^2 , and each contained seven rows of maize, 10 m long with 0.65 m distance between rows. The seeds of widely grown maize hybrid XY335 were sown by hand at 6.75 plants/ m^2 on 1 May in 2015 and 29 April in the year 2016. All weeds, diseases, and pests were controlled.

2.2.3 Plant sampling

Adjacent plants from the same inner row were tagged by applying a dot of red paint to their leaves at the V3 stage. Leaf rank was counted from the bottom (leaf 1) to the top, and tags were placed on leaves 4, 8, and 12 to avoid confusion as lower leaves senesced (Fan et al., 2020). Maize plants were randomly selected among tagged plants, and the whole plant shoots were cut down from the soil surface at silking (R1) and at physiological maturity (R6). Plant samples were separated into individual leaves (leaf blades), stem (including leaf sheath), grain, cob, bracts, and tassels. The dry weight of each sample was determined after drying for 72 h at 85°C in a forced-air drying oven. The samples were then weighed, ground into a fine powder, and analyzed the N concentration based on the Kjeldahl method (Bremner, 1960). The post-silking DM accumulation and N uptake were calculated at the whole-plant level, and the DM and N allocation to different organs was quantified. The absolute reallocated amounts

and the fractions of the reallocated leaf DM and leaf N, and the contributions of both to grain DM and grain N were calculated at organ scale (leaf or stem sections) and individual leaf scale (individual leaf rank), respectively.

2.2.4 Measurements of accumulation, partitioning, and reallocation processes of dry matter and nitrogen

2.2.4.1 Whole-plant dry matter and N accumulation during post-silking stage

The post-silking DM accumulation Eq. (1) and N uptake Eq. (2) were calculated as:

$$\text{Post-silking DM (g/plant)} = \text{whole plant DM}_{R6} - \text{whole plant DM}_{R1} \quad (1)$$

$$\text{Post-silking N uptake (g/plant)} = \text{whole plant N}_{R6} - \text{whole plant N}_{R1} \quad (2)$$

where DM_{R1} and DM_{R6} represent the whole-plant DM in R1 (silking) and R6 (maturity) stages, and N_{R1} and N_{R6} represent the whole-plant N content in the R1 and R6 stages.

2.2.4.2 Harvest index

Harvest index (HI) and N harvest index (NHI) were calculated as follows:

$$\text{HI} = \text{Grain DM} / \text{whole-plant DM}_{R6} \quad (3)$$

$$\text{NHI} = \text{Grain N} / \text{whole-plant N}_{R6} \quad (4)$$

where Grain DM and Grain N are the grain dry matter (g plant^{-1}) and grain N (g plant^{-1}) content at the R6 stage, respectively.

2.2.4.3 DM and N partitioning

$$\text{DM partitioning (\%)} = \text{DM of specific vegetative organ} / \text{whole-plant DM} \times 100\% \quad (5)$$

$$\text{N partitioning (\%)} = \text{N of specific vegetative organ} / \text{whole-plant N} \times 100\% \quad (6)$$

DM partitioning and N partitioning were defined as the fractions of DM or N of each organ to whole-plant DM or N. Where the specific vegetative organs represent of leaf, stem, cob, brace, tassel, and grain, respectively.

2.2.4.4 *The reallocated amount and reallocation fraction of DM and N*

$$\text{Reallocated DM amount (g)} = \text{DM}_{\text{R1}} - \text{DM}_{\text{R6}} \quad (7)$$

$$\text{Reallocated N amount (g)} = \text{N}_{\text{R1}} - \text{N}_{\text{R6}} \quad (8)$$

$$\text{DM reallocation fraction (\%)} = \text{Reallocated DM amount} / \text{DM}_{\text{R1}} \quad (9)$$

$$\text{N reallocation fraction (\%)} = \text{Reallocated N amount} / \text{N}_{\text{R1}} \quad (10)$$

We calculated the reallocated amount and fraction of DM and N at organ level (leaf or stem) and leaf level (individual leaf rank) in this study, respectively. When calculating at the organ level, the DM_{R1} , N_{R1} , DM_{R6} , and N_{R6} represent the dry matter or N contents of the leaf or stem fractions of the plant at the R1 and R6 stages, respectively. When calculated at leaf level for individual leaves, the DM_{R1} , N_{R1} , DM_{R6} , and N_{R6} represent the dry matter or N contents of individual leaves at each leaf rank at the R1 and R6 stages, respectively.

2.2.4.5 *Contribution of DM and N reallocation to grain*

$$\begin{aligned} &\text{Contribution of reallocated DM to grain DM (\%)} = \\ &\text{Reallocated DM amount} / \text{Grain DM} \end{aligned} \quad (11)$$

$$\begin{aligned} &\text{Contribution of reallocated N to grain N (\%)} = \\ &\text{Reallocated N amount} / \text{Grain N} \end{aligned} \quad (12)$$

Where DM_{R1} and DM_{R6} represent the dry matter of each leaf rank at the R1 and R6 stages. Grain DM represents the dry matter of grain at the R6 stage. N_{R1} and N_{R6} represent the N content of each leaf rank at the R1 and R6 stages, and Grain N represents the N content of grain at the R6 stage.

2.2.5 *Statistical analysis*

Statistical analysis was performed using R software (version 3.6.1). One-way analysis (ANOVA) was used to evaluate the effect of N availability on the accumulation, partitioning, and reallocation of dry matter and N content. Differences were compared using the least significant difference test at a 0.05 level of probability. Due to substantial year-by-treatment interactions for most of the assessed variables, the treatment effect was evaluated separately for each year.

2.3 Results

2.3.1 Whole-plant dry matter and N content accumulation

The whole-plant DM and N increased with N availability in both years regardless of developmental stage, and the effect of N availability on whole-plant N content was stronger than the effects on whole-plant DM (Table 2.2). The post-silking DM increase was slightly larger than the grain DM, in all cases including the N0 treatment. This indicates that net assimilation during the reproductive phase was consistently more than sufficient to meet DM demand for grain filling. However, the N uptake from the soil during the post-silking stage was not sufficient for the amount of N in grains, as indicated by post N/grain N values being < 100%. This fraction increased with the level of fertilizer. This indicates that part of the N in grains must have been made available through reallocation from vegetative organs to grain and that this reallocation requirement was larger at low N availability (Table 2.2).

At the R1 stage, the largest fraction of DM was in the stem (60%) followed by leaves (30%) and the remaining organs (cob, bract, and tassel) together accounting for about 10%. This DM distribution pattern was not significantly affected by N fertilizer treatments. At the R6 stage, maize plants tended to allocate less DM to grains and more to vegetative parts at low than at high N availability. For instance, the proportions of grain DM to whole-plant DM decreased from 0.49 in the N2 treatment to 0.42 in the N0 treatment (Fig. 2.2a). The distribution pattern of N was different. At the R1 stage, around 50% of N was in leaves and 40% in the stem, without a significant effect of N availabilities. At the R6 stage, about two-thirds of the total N was in the grains, with a nitrogen harvest index (NHI) of 0.61-0.67. Increased N availability decreased N allocation to the grains, but caused a considerable increase in the N allocation to other organs (Fig. 2.2b). Overall, the soil N availability did not affect partitioning of DM and N among organs at the R1 stage. However, at the R6 stage, more DM was allocated to leaf and stem, and more N was allocated to grain in response to low N availability.

2.3.3 Reallocation of DM and N in leaf versus stem organs

Overall plants reallocated relatively more N than DM from leaves to grains and grain N hence also depended relatively more on this reallocation than grain DM (Table 2.3). More specifically, leaves reallocated up to 60% of their N, but only 15% of their DM. The reallocated N from both leaf and stem contributed 10.5-35.6% to grain N, but the reallocated DM only account for 1.6-10.9% to grain DM. Furthermore, the amount of N reallocation from leaves was larger than that from stem, and this was consistent

Table 2.2. Whole-plant dry matter (DM) and N content (N) at silking (R1) and maturity (R6), harvest index (HI), nitrogen harvest index (NHI), dry matter accumulation during post-silking (Post DM), and N accumulation during post-silking (Post N) in 2015 and 2016

| Year | N availability | Dry matter per plant (DM) | | | HI | Post DM (g) | Grain DM (g) | Post DM/grain DM (%) | N content per plant (N) | | | Post N/grain N (%) | NHI |
|------|----------------|---------------------------|----------------------|--|---------|-------------|--------------|----------------------|-------------------------|---------------------|--------------------------------------|--------------------|---------|
| | | DM _{R1} (g) | DM _{R6} (g) | DM _{R6} /DM _{R1} (%) | | | | | N _{R1} (g) | N _{R6} (g) | N _{R6} /N _{R1} (%) | | |
| 2015 | N0 | 117.0 c | 230.1 c | 113.1 c | 98.3 c | 0.43 b | 114.9 a | 0.97 c | 1.77 c | 0.80 c | 1.18 c | 0.67 a | 68.1 c |
| | N1 | 151.1 b | 322.9 b | 171.8 b | 159.6 b | 0.49 a | 107.7 b | 2.17 b | 4.43 b | 2.26 b | 2.91 b | 0.66 a | 77.7 b |
| | N2 | 166.6 a | 381.0 a | 214.3 a | 183.6 a | 0.48 a | 116.7 a | 2.65 a | 5.91 a | 3.26 a | 3.60 a | 0.61 b | 90.6 a |
| 2016 | N0 | 119.4 c | 217.3 c | 97.9 c | 90.8 c | 0.42 b | 107.8 a | 0.83 c | 1.56 c | 0.73 c | 1.04 c | 0.67 a | 70.6 b |
| | N1 | 161.4 b | 341.9 b | 180.5 b | 164.5 b | 0.48 a | 109.7 a | 2.12 b | 4.29 b | 2.16 b | 2.77 b | 0.65 b | 78.1 ab |
| | N2 | 175.2 a | 384.7 a | 209.5 a | 187.0 a | 0.49 a | 112.0 a | 2.68 a | 6.10 a | 3.42 a | 3.73 a | 0.61 c | 91.7 a |

Table 2.3. The reallocation amount of DM and N in leaf and stem, reallocation fraction (%) of leaf and stem is the percentage of reallocated DM or N amount to total DM or N at the R1 stage, and the contribution of DM and N reallocation in leaf and stem to grain DM and grain N in 2015 and 2016

| Year | N availability | DM reallocation in leaf versus stem | | | | | | N reallocation in leaf versus stem | | | | | | | | | | | |
|------|----------------|-------------------------------------|-------|--|--------------|-------|--|------------------------------------|--------|--|------------|--------|--|--------------|--------|--|-----------------------------|--------|--|
| | | Amount (g) | | | Fraction (%) | | | Contribution to grain DM (%) | | | Amount (g) | | | Fraction (%) | | | Contribution to grain N (%) | | |
| | | Leaf | Stem | | Leaf | Stem | | Leaf | Stem | | Leaf | Stem | | Leaf | Stem | | Leaf | Stem | |
| 2015 | N0 | 5.5b | 5.2 a | | 15.0 a | 7.2 a | | 5.6 a | 5.3 a | | 0.3 b | 0.1 b | | 59.1 a | 38.1 a | | 24.5 a | 11.1 a | |
| | N1 | 8.8a | 5.3 a | | 18.7 a | 6.0 a | | 5.5 a | 3.3 ab | | 0.5 a | 0.3 a | | 43.7 b | 33.4 a | | 16.5 b | 8.8 a | |
| | N2 | 0.8 c | 2.0 b | | 1.7 b | 2.0 b | | 0.5 b | 1.1 b | | 0.3 b | 0.2 ab | | 22.1 c | 15.0 b | | 7.6 c | 4.4 b | |
| 2016 | N0 | 5.2 ab | 4.0 a | | 14.8 a | 5.4 a | | 5.8 a | 4.4 a | | 0.2 b | 0.1 b | | 57.5 a | 40.6 a | | 20.9 a | 11.6 a | |
| | N1 | 6.1 a | 4.3 a | | 12.7 a | 4.5 a | | 3.7 b | 2.6 a | | 0.4 a | 0.3 a | | 44.6 a | 38.2 a | | 16.0 ab | 11.6 a | |
| | N2 | 2.7 b | 3.2 a | | 5.0 b | 3.2 a | | 1.4 c | 1.7 a | | 0.2 b | 0.2 b | | 17.4 b | 17.1 b | | 5.8 b | 4.7 b | |

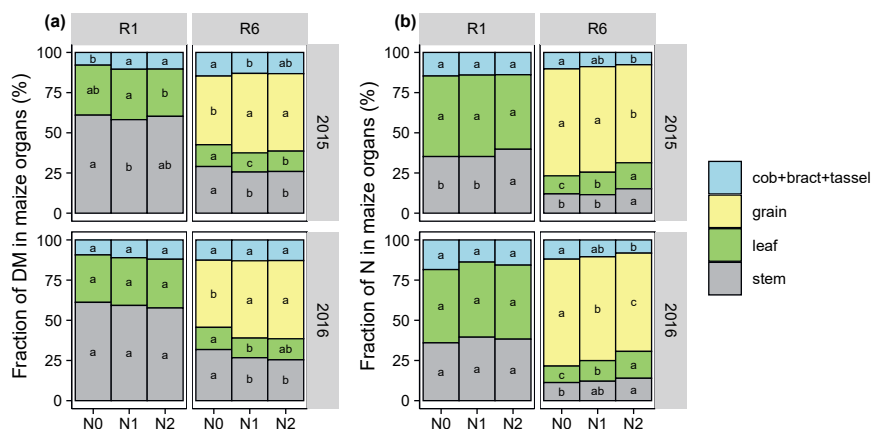


Fig. 2.2. The proportion of DM (a) and N (b) in different plant organs to total DM and total N of the whole plant under different N availabilities at the R1 and R6 stages in 2015 and 2016. Different letters indicate significant ($P < 0.05$) difference between N treatments.

across all treatments. N availability significantly influenced DM and N reallocation, reallocation fractions, as well as the contribution of reallocation to grain DM and N (Table 2.3). Notably, in absolute terms, the highest DM and N reallocation amounts were obtained under the intermediate N1 treatment. The reallocation fractions of reallocated DM or N and their contributions to grain DM or grain N however were greater as N availability became less. This indicates that in terms of relative contributions, the accumulation of DM and N in grains becomes increasingly dependent on DM and N reallocation from vegetative parts when external N availability goes down.

2.3.4 Distribution and reallocation of DM and N from individual leaves

Leaf DM and leaf N content (i.e., the total amounts of DM or N in a leaf) of individual leaves increased with leaf rank up to the ear leaf (rank 14) and then decreased towards the top leaf (Fig. 2.3). The maximum leaf DM was around 6 g/leaf at the R1 stage, whereas the maximum leaf N content was 0.15g N/leaf. N deficiency significantly reduced DM and N content particularly for the leaves closer to the ear. The reduction in leaf N content with decreasing N availability was much greater than the reduction in leaf DM (Fig. 2.3). Furthermore, the differences of leaf N among N availabilities became greater at the R6 stage.

The amount of reallocated DM from individual leaves increased with leaf rank up to the ear leaf and then decreased towards the top leaf (Fig. 2.4a). The negative value of the reallocated DM in the upper leaves indicates there was net DM accumulation rather than reallocation between the R1 and R6 stages in these leaves (Fig. 2.4a).

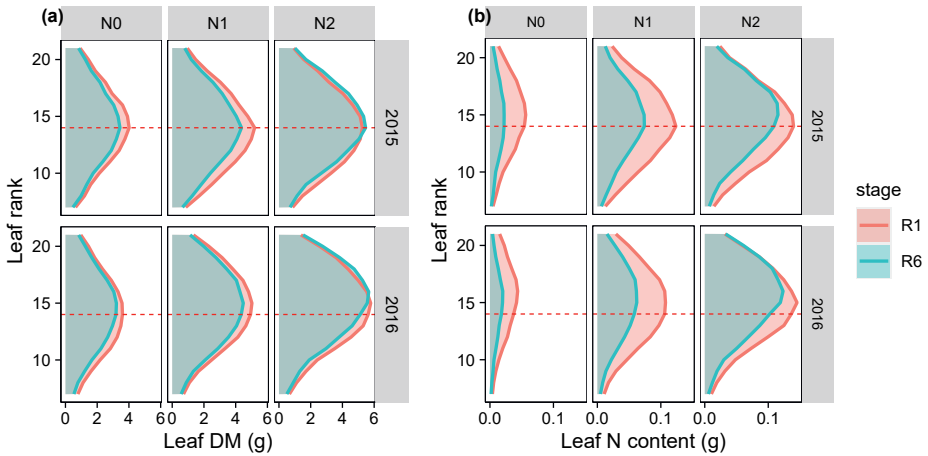


Fig. 2.3. The vertical distribution patterns of leaf DM (a) and leaf N content (b) at each leaf rank under different N availabilities during the R1 and R6 stages in 2015 and 2016. The dashed red line represents the rank of the ear leaf within the maize canopy.

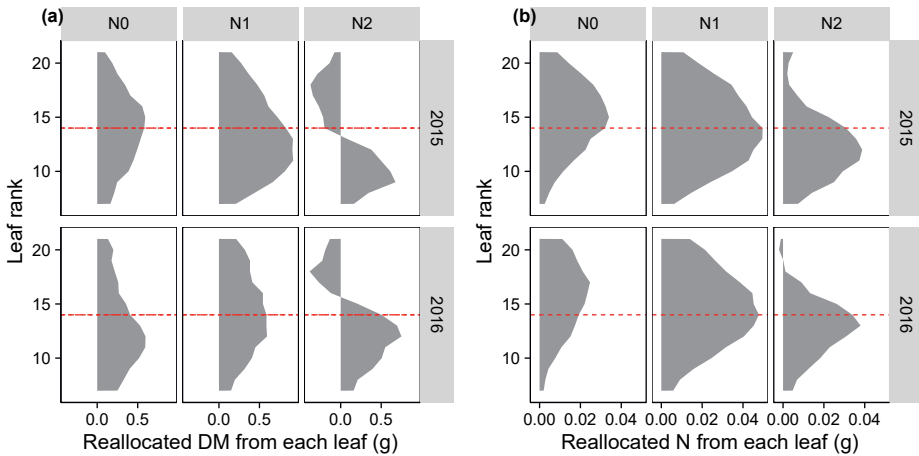


Fig. 2.4. The amount of leaf DM (a) and leaf N (b) reallocated from individual leaves under different N availabilities. The dashed red line represents the rank of the ear leaf within the maize canopy. Negative values (< 0) indicate that on-balance leaves had accumulated DM or N between the R1 and R6 stages.

By contrast, the reallocation of leaf N content was largely from middle and upper leaves within the canopy, especially near the ear leaf. This indicates that the leaf N reallocation was largely from living leaves via re-translocation of N reserves and less from senescing leaves (Fig. 2.4b). The reallocation of DM and N was affected by N availability, with the highest amount of DM and N reallocation occurring in the N1 treatment. The maximum reallocation values of leaf DM and leaf N content were 0.9 g and 0.05 g, respectively (Fig. 2.4). The general pattern of N accumulation

across leaf ranks was an initial increase with leaf rank at low leaf ranks and a decline at higher ones, with the highest values being at somewhat intermediate leaf ranks. But this maximum shifted upwards with decreasing N availability, indicating that as plants are more N limited, N reallocation occurs progressively more from upper leaves (Fig. 2.4b).

The lower the N availability, the higher the reallocation fraction (the ratio of reallocated DM or N per leaf to whole leaf DM or leaf N) of leaf DM or leaf N. For instance, the average value of reallocated leaf DM fractions during the two experimental years were 5%, 17%, and 16% (N2, N1, and N0), and the reallocated leaf N fractions were 23%, 46%, and 53% (Fig. 2.5). Individual leaves reallocated up to 60% of their own N but only up to 30% of their DM. The reallocation fractions of leaf DM and leaf N differed among leaf ranks and these distribution patterns were affected by N availability (Fig. 2.5). The lowest-ranked leaves at the bottom of the canopy exhibited the highest reallocated leaf DM fraction, and the decline in fractions with leaf rank was strongest in the N2 treatment (i.e., where values became negative at the top) (Fig. 2.5a). The reallocated leaf N fraction under N0 and N1 treatments was much bigger than that in N2 treatments, but the range difference among leaf ranks within the maize canopy was much smaller compared with N2 treatments (Fig. 2.5b).

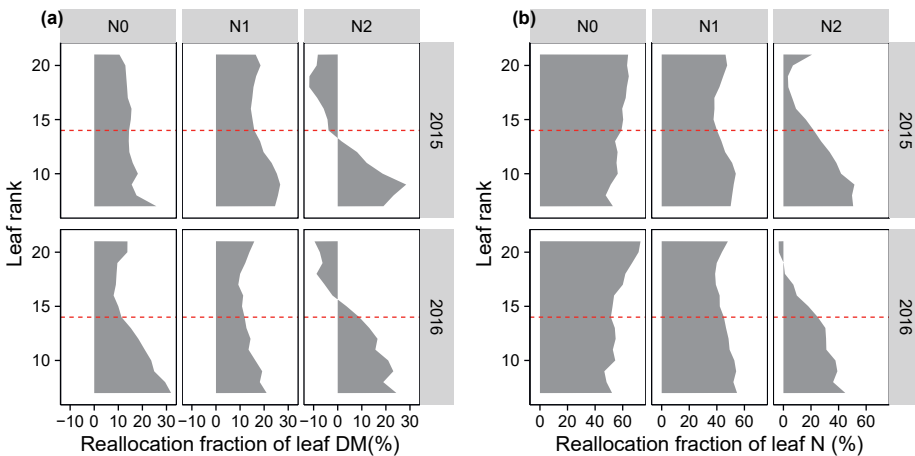


Fig. 2.5. The reallocation fraction of leaf DM (%) and leaf N content (%) (i.e., the net percentage of DM and N in leaves that were retranslated) at individual leaves under different N availabilities in 2015 and 2016. The dot red line represents the rank of the ear leaf within the maize canopy.

2.3.5 The contribution of DM and N reallocation from individual leaves to grain DM and N

The DM and N reallocated from individual leaves under lower N availabilities contributed more to grain DM and grain N than that under high N availability (Fig. 2.6). For instance, the average contributions of total leaf DM reallocation during the two experimental years under N0, N1, and N2 treatments were 5.7%, 4.6%, and 0.9%, respectively (Fig. 2.6a). Grain N depended more on the reallocation from vegetative organs compared with grain DM. The leaf N reallocations in 2015 and 2016 under N0, N1, and N2 treatments averaged to account for 22.7%, 16.2%, and 6.7%, respectively (Fig. 2.6b). Furthermore, the vertical distribution of individual leaf contributions of DM and N was affected by N availability, shifting from it coming more from bottom leaves at N2 treatment via middle leaves at N1 to top leaves at N0 (Fig. 2.6).

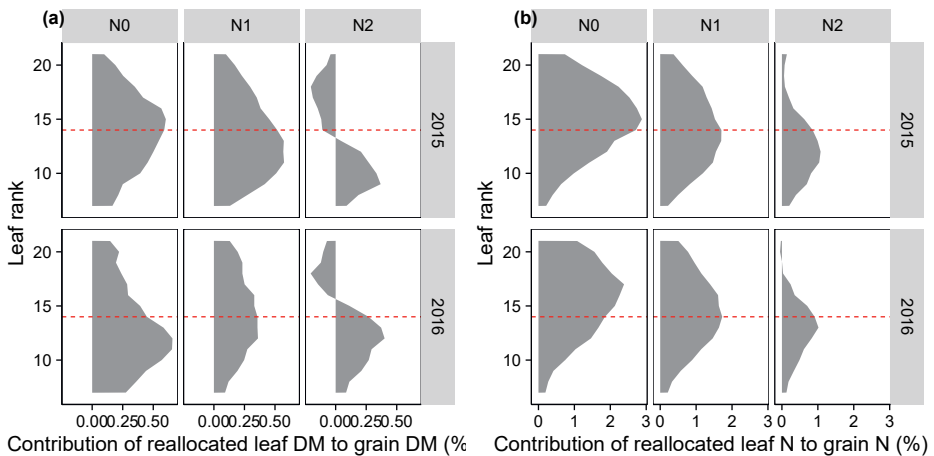


Fig. 2.6. The contribution (%) of reallocated leaf DM and leaf N to grain DM or grain N at individual leaves under different N availabilities in 2015 and 2016. The dashed red line represents the rank of the ear leaf within the maize canopy.

2.4 Discussion

2.4.1 Grain N relies on reallocation more than grain DM.

A high post-silking DM accumulation is vital for achieving high maize grain yields, since the majority of the grain DM was found to come from the photosynthates produced during the post-silking stage (Liu et al., 2023; Ning et al., 2013; Parco et al., 2022). In this study, the post-silking DM accounted for 107.7-116.7% of the grain DM regardless of the N availability (Table 2.2), which was probably because the

accumulated post-silking DM derived by canopy photosynthesis provides sufficient assimilates for grain DM (Koutroubas et al., 2012; Liu et al., 2023). The situation was different for nitrogen, where 8.3-38.8% of grain N was reallocated from vegetative organs during the post-silking stage, and this dependency on N reallocation increased with decreasing N availability (Table 2.2). Since N uptake depends on the continuous carbohydrate supply from shoot to roots, longer duration of post-silking photosynthesis is beneficial for post-silking N uptake of the maize plants, which in turn increases canopy photosynthesis duration and final grain DM (Gallais et al., 2007; Liu et al., 2023; Winterhalter et al., 2012). The delayed leaf senescence, larger leaf area, and longer photosynthesis duration under high N availability contribute to improving canopy photosynthesis capacity and ultimately, greater post-silking N uptake (Liu et al., 2022; Riha et al., 2013). However, as we found, increasing N availability may simultaneously decrease the N reallocation fraction of the N accumulated at the pre-silking stage (Table 2.3), and therefore increase the amount of residual N in vegetative organs, since less reallocated N is needed to meet grain N requirements with increasing N availability (Chen et al., 2015; Ning et al., 2013).

2.4.2 Trade-off between post-silking photosynthesis and N reallocation in leaves in response to N availability

Leaf and stem are the main organs from which DM or N is reallocated to grains, when the accumulated post-silking DM or post-silking N uptake cannot meet the grain requirements (Chen et al., 2015; Liu et al., 2021). The largest amount of DM and N reallocation was at intermediate N availability (Table 2.3), indicating both N deficiency and very high N availability can restrain the DM and N reallocation from vegetative organs to grain (Wei et al., 2019). On the other hand, the relative contribution of DM or N reallocation from stems and leaves to the total accumulation of DM and N in grains increased with decreasing N availability (Liu et al., 2022; Molla et al., 2019). The amount of N reallocated from leaves was larger than the amount reallocated from stems consistent across all three treatments (Table 2.3). Since the reallocated leaf N mainly comes from the breakdown of proteins and chlorophyll (Evans and Clarke, 2019; Ma et al., 2022), there is a trade-off between N reallocation and leaf photosynthesis rate. The more N is reallocated from leaf to grain during post-silking stage, the less N will be left to support photosynthesis (Li et al., 2020; Onoda et al., 2017). This trade-off was mediated by N availability (Chen et al., 2015; Ning et al., 2013). A high N availability entails that more N needed for grain growth can be taken up from the soil reducing the need for N reallocation such that more N remains in leaves and leaf longevity is prolonged (Boomsma et al., 2009; Li et al., 2022).

2.4.3 N availability determines the vertical N reallocation patterns within maize canopy

Increasing N availability does not necessarily result in a proportional increase in the distribution and reallocation of DM and N from individual leaves (Fig. 2.3 and 2.4). It is well-known that the N distribution and photosynthetic rate N are uneven among leaves at different positions in the canopy (Anten et al., 1995; Archontoulis et al., 2011). Leaf DM and leaf N content of individual leaves increased with leaf rank up to the ear leaf and then decreased towards the top leaf, roughly following a bell-shaped function of leaf rank (Fig. 2.3), which was consistent with previous findings (Archontoulis et al., 2011; Ciganda et al., 2008; Winterhalter et al., 2012). The largest amount of reallocated leaf DM and leaf N came from the middle part of the canopy (Fig. 2.4). This is mainly because the middle leaves have higher N concentration and bigger leaf area, which entails more DM and N could be reallocated (Fan et al., 2022; Li et al., 2022). However, the bottom leaves reallocated the largest fraction of their DM and N to grains (Fig. 2.5). Furthermore, the vertical N reallocation pattern across leaves within the canopy was affected by N availability. On the one hand, N reallocation from lower leaves has the smallest opportunity costs in terms of photosynthesis as these leaves are shaded. On the other hand, leaf N contents in upper leaves are higher, hence there being more N available for reallocation. When N availability is limited, maize plants tend to reallocate N to the upper leaves to maximize light interception and photosynthesis, while reducing investment in lower leaves (Fig. 2.4b). The upper leaves have a higher N concentration and photosynthetic rates than the lower leaves, and this distribution pattern positively affects canopy photosynthesis (Ellsworth and Reich, 1993; Yao et al., 2016). However, when N availability is high, the plants also reallocate more N to lower leaves to increase N uptake and maximize DM production (Fig. 2.4b). As N availability from the soil declines, N uptake during the reproductive stage supports a smaller fraction of grain N accumulation and thus plants progressively needed to reallocate from upper leaves even if that entailed a relatively large opportunity cost in terms of photosynthesis.

2.4.4 Implications for N fertilizer management practice and crop models

Increasing grain yield and grain quality, especially protein content, are important for meeting food demand, improving human nutrition, and ensuring a sustainable food system (Duvick, 2005; Ray et al., 2019; Tokatlidis and Koutroubas, 2004). Proper N management can help to ensure that crops receive adequate N for optimal growth and yield, maintain high grain protein content (Hou et al., 2012; Van Oosterom et al., 2001), while preventing losses of N to the environment. In practice, farmers often

apply N at excessive rates to ensure a good maize yield (Li et al., 2022; Ning et al., 2013). An N supply in excess of that required for the highest grain DM may increase grain N to an extent (Table 2.2), but also lead to high levels of residual N in straw at maturity, since less reallocated N from vegetative organs to grain (Table 2.3). Previous studies also demonstrated that postponed N fertilizer topdressing could enhance yields, nitrogen-use efficiency and effectively avoid the environmental problems caused by excessive fertilization during field production (Xu et al., 2023; Zhai et al., 2022). However, we suggest to pay attention to the soil N availability, and pre-silking N fertilizer application, since a relatively high pre-silking DM accumulation provides a strong base for post-silking growth, which in our study contributed to lower DM reallocation and high grain yield. Moreover, the pre-silking N accumulation in vegetative organs contributed to grain N, which has a positive effect to grain quality.

The current crop models, while rather good at simulating yield amounts (Van Ittersum et al., 2003; Wang et al., 2019) are much less capable of simulating yield quality. This is in part because the physiological processes underlying yield quality are not so well understood and quantified. For instance, as pointed out in this paper, the balance between post-silking N reallocation to support grain N demand and canopy photosynthesis to support assimilate supply is mediated by variation in soil N availability, which is not well described yet in crop models. (Van Ittersum et al., 2003; Vos et al., 2010). In this paper, we focused on DM and N reallocation which is an important process driving the balance between post-silking canopy photosynthesis (supporting yield quantity) and N reallocation (mostly, grain protein content). We showed how the balance between N uptake and N reallocation is mediated by N availability, and how this dynamic plays off across different leaves in the canopy providing important data to develop plant models (Barillot et al., 2016; Faverjon et al., 2019). Such models could be used to explore the extent to which vertical DM and N reallocation patterns in maize plant canopies can be optimized in terms of both high grain DM and grain N.

2.5 Conclusion

Adequate soil N availability is essential to ensure higher DM and N accumulation and higher yield in maize. While post-silking leaf photosynthesis was capable of providing sufficient assimilates for grain DM, 8.3-38.8% of grain N were reallocated from vegetative organs accumulated during pre-silking stage. Moreover, the lower the N availability, the higher the contribution of reallocated N to grain N. The vertical patterns of reallocated leaf DM and leaf N within the maize canopy was mediated by

N availability. With decreasing N availability, N reallocation comes increasingly from upper leaves in the canopy and hence comes at a relatively greater cost in terms of photosynthesis. This is important information to improve N management in precision farming and to provide data support for the plant and crop models N treatment effect assessments.

Acknowledgments

This study was supported by grants from the China Agriculture Research System of MOF and MARA (CARS-02) and the Agricultural Science and Technology Innovation Program (CAAS-ZDRW202004). P.F held a scholarship from China Scholarship Council (CSC202003250113). SL and RX conceived the project and provided funding; PF and BM designed the research; PF and YL performed the experiments; PF analyzed the data and wrote the draft; NA and JBE edited and revised the manuscript.

References

- Anten, N. P. R., Schieving, F., & Werger, M. J. A. (1995). Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C 3 and C 4 mono- and dicotyledonous species. *Oecologia*, 101, 504-513.
- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N. G., & Struik, P. C. (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Barillot, R., Chambon, C., & Andrieu, B. (2016). CN-Wheat, a functional-structural model of carbon and nitrogen metabolism in wheat culms after anthesis. I. Model description. *Annals of botany*, 118(5), 997-1013.
- Bertheloot, J., Martre, P., & Andrieu, B. (2008). Dynamics of light and nitrogen distribution during grain filling within wheat canopy. *Plant physiology*, 148(3), 1707-1720.
- Boomsma, C. R., Santini, J. B., Tollenaar, M., & Vyn, T. J. (2009). Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. *Agronomy Journal*, 101(6), 1426-1452.
- Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science*, 55(1), 11-33.
- Brown, H. E., Huth, N. I., Holzworth, D. P., Teixeira, E. I., Wang, E., Zyskowski, R. F., & Zheng, B. (2019). A generic approach to modelling, allocation and redistribution of biomass to and from plant organs. *in silico Plants*, 1(1), diy004.
- Chen, K., Kumudini, S. V., Tollenaar, M., & Vyn, T. J. (2015). Plant biomass and nitrogen partitioning changes between silking and maturity in newer versus older maize hybrids. *Field Crops Research*, 183, 315-328.
- Chen, W., Wang, L., Siddique, K. H., Deng, X., & Chen, Y. (2019). Nitrogen vertical distribution differed in foliar and nonfoliar organs of dryland wheat during grain filling. *Agronomy Journal*, 111(3), 1218-1228.
- Ciganda, V., Gitelson, A., & Schepers, J. (2008). Vertical profile and temporal variation of chlorophyll in maize canopy: Quantitative “crop vigor” indicator by means of reflectance-based techniques. *Agronomy Journal*, 100(5), 1409-1417.
- Cliquet, J. B., Deleens, E., & Mariotti, A. (1990). C and N mobilization from stalk and leaves during kernel filling by ¹³C and ¹⁵N tracing in *Zea mays* L. *Plant Physiology*, 94(4), 1547-1553.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy*, 86, 83-145.
- Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, 78(1), 9-19.
- Evans, J. R., & Clarke, V. C. (2019). The nitrogen cost of photosynthesis. *Journal of Experimental Botany*, 70(1), 7-15.
- Fan, P., Ming, B., Anten, N. P., Evers, J. B., Li, Y., Li, S., & Xie, R. (2022). Plastic response of leaf traits to N deficiency in field-grown maize. *AoB Plants*, 14(6), plac053.
- Fan, P. P., Li, Y. Y., Evers, J. B., Ming, B., Wang, C. X., Li, S. K., & Xie, R. Z. (2020). A new empirical equation to describe the vertical leaf distribution profile of maize. *The Journal of Agricultural Science*, 158(8-9), 676-686.
- Faverjon, L., Escobar-Gutiérrez, A., Litrico, I., Julier, B., & Louarn, G. (2019). A generic individual-based model can predict yield, nitrogen content, and species abundance in experimental grassland communities. *Journal of Experimental Botany*, 70(9), 2491-2504.

- Fournier, C., & Andrieu, B. (1999). ADEL-maize: an L-system based model for the integration of growth processes from the organ to the canopy. Application to regulation of morphogenesis by light availability. *Agronomie*, 19(3-4), 313-327.
- Gallais, A., Coque, M., Le Gouis, J., Prioul, J. L., Hirel, B., & Quillere, I. (2007). Estimating the Proportion of Nitrogen Remobilization and of Postsilking Nitrogen Uptake Allocated to Maize Kernels by Nitrogen-15 Labeling. *Crop science*, 47(2), 685-691.
- Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., & Riha, S. (2013). Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant and soil*, 365, 239-254.
- Hikosaka, K. (2016). Optimality of nitrogen distribution among leaves in plant canopies. *Journal of Plant Research*, 129, 299-311.
- Hirose, T., & Werger, M. J. A. (1987). Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia*, 72, 520-526.
- Hou, P., Gao, Q., Xie, R., Li, S., Meng, Q., Kirkby, E. A., ... & Chen, X. (2012). Grain yields in relation to N requirement: Optimizing nitrogen management for spring maize grown in China. *Field Crops Research*, 129, 1-6.
- Koutroubas, S. D., Fotiadis, S., & Damalas, C. A. (2012). Biomass and nitrogen accumulation and translocation in spelt (*Triticum spelta*) grown in a Mediterranean area. *Field Crops Research*, 127, 1-8.
- Kumar, R., Bishop, E., Bridges, W. C., Tharayil, N., & Sekhon, R. S. (2019). Sugar partitioning and source-sink interaction are key determinants of leaf senescence in maize. *Plant, cell & environment*, 42(9), 2597-2611.
- Li, G., Wang, L., Li, L., Lu, D., & Lu, W. (2020). Effects of fertilizer management strategies on maize yield and nitrogen use efficiencies under different densities. *Agronomy Journal*, 112(1), 368-381.
- Li, Y., Ming, B., Fan, P., Liu, Y., Wang, K., Hou, P., ... & Xie, R. (2022). Quantifying contributions of leaf area and longevity to leaf area duration under increased planting density and nitrogen input regimens during maize yield improvement. *Field Crops Research*, 283, 108551.
- Li, Y., Song, H., Zhou, L., Xu, Z., & Zhou, G. (2019). Vertical distributions of chlorophyll and nitrogen and their associations with photosynthesis under drought and rewatering regimes in a maize field. *Agricultural and Forest Meteorology*, 272, 40-54.
- Liu, G., Hou, P., Xie, R., Ming, B., Wang, K., Xu, W., ... & Li, S. (2017). Canopy characteristics of high-yield maize with yield potential of 22.5 Mg ha⁻¹. *Field Crops Research*, 213, 221-230.
- Liu, G., Yang, Y., Guo, X., Liu, W., Xie, R., Ming, B., ... & Hou, P. (2023). A global analysis of dry matter accumulation and allocation for maize yield breakthrough from 1.0 to 25.0 Mg ha⁻¹. *Resources, Conservation and Recycling*, 188, 106656.
- Liu, Z., Hu, C., Wang, Y., Sha, Y., Hao, Z., Chen, F., ... & Mi, G. (2021). Nitrogen allocation and remobilization contributing to low-nitrogen tolerance in stay-green maize. *Field Crops Research*, 263, 108078.
- Liu, Z., Sha, Y., Huang, Y., Hao, Z., Guo, W., Ke, L., ... & Mi, G. (2022). Efficient nitrogen allocation and reallocation into the ear in relation to the superior vascular system in low-nitrogen tolerant maize hybrid. *Field Crops Research*, 284, 108580.
- Ma, L., Shi, L., Wang, S., Wang, K., Zheng, W., Li, Z., & Zhai, B. (2022). ¹⁵N labelling of cattle manure reveals the distribution of organic fertiliser nitrogen in a winter wheat system. *Field Crops Research*, 283, 108529.

- Molla, M. S. H., Nakasathien, S., Ali, M. A., Khan, A. S. M. M. R., Alam, M. R., Hossain, A., ... & El Sabagh, A. (2019). Influence of nitrogen application on dry biomass allocation and translocation in two maize varieties under short pre-anthesis and prolonged bracketing flowering periods of drought. *Archives of Agronomy and Soil Science*, 65(7), 928-944.
- Ning, P., Fritschi, F. B., & Li, C. (2017). Temporal dynamics of post-silking nitrogen fluxes and their effects on grain yield in maize under low to high nitrogen inputs. *Field crops research*, 204, 249-259.
- Ning, P., Li, S., Yu, P., Zhang, Y., & Li, C. (2013). Post-silking accumulation and partitioning of dry matter, nitrogen, phosphorus and potassium in maize varieties differing in leaf longevity. *Field Crops Research*, 144, 19-27.
- Olmedo Pico, L. B., & Vyn, T. J. (2021). Dry matter gains in maize kernels are dependent on their nitrogen accumulation rates and duration during grain filling. *Plants*, 10(6), 1222.
- Onoda, Y., Wright, I. J., Evans, J. R., Hikosaka, K., Kitajima, K., Niinemets, Ü., ... & Westoby, M. (2017). Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist*, 214(4), 1447-1463.
- Parco, M., D'Andrea, K. E., & Maddonni, G. A. (2022). Maize prolificacy under contrasting plant densities and N supplies: I. Plant growth, biomass allocation and development of apical and sub-apical ears from floral induction to silking. *Field Crops Research*, 284, 108553.
- Perez, R. P., Fournier, C., Cabrera-Bosquet, L., Artzet, S., Pradal, C., Bricchet, N., ... & Tardieu, F. (2019). Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection. *Plant, cell & environment*, 42(7), 2105-2119.
- Ray, K., Banerjee, H., Dutta, S., Hazra, A. K., & Majumdar, K. (2019). Macronutrients influence yield and oil quality of hybrid maize (*Zea mays* L.). *PloS one*, 14(5), e0216939.
- Tokatlidis, I. S., & Koutroubas, S. D. (2004). A review of maize hybrids' dependence on high plant populations and its implications for crop yield stability. *Field Crops Research*, 88(2-3), 103-114.
- van Ittersum, M. K., Leffelaar, P. A., Van Keulen, H., Kropff, M. J., Bastiaans, L., & Goudriaan, J. (2003). On approaches and applications of the Wageningen crop models. *European journal of agronomy*, 18(3-4), 201-234.
- Van Oosterom, E. J., Carberry, P. S., & Muchow, R. C. (2001). Critical and minimum N contents for development and growth of grain sorghum. *Field Crops Research*, 70(1), 55-73.
- Vos, J., Evers, J. B., Buck-Sorlin, G. H., Andrieu, B., Chelle, M., & De Visser, P. H. (2010). Functional–structural plant modelling: a new versatile tool in crop science. *Journal of experimental Botany*, 61(8), 2101-2115.
- Wang, E., Brown, H. E., Rebetzke, G. J., Zhao, Z., Zheng, B., & Chapman, S. C. (2019). Improving process-based crop models to better capture genotype× environment× management interactions. *Journal of experimental botany*, 70(9), 2389-2401.
- Wang, X., Wang, N., Xing, Y., & Ben El Caid, M. (2018). Synergetic effects of plastic mulching and nitrogen application rates on grain yield, nitrogen uptake and translocation of maize planted in the Loess Plateau of China. *Scientific reports*, 8(1), 14319.
- Wei, S., Wang, X., Li, G., Jiang, D., & Dong, S. (2019). Maize canopy apparent photosynthesis and ¹³C-photosynthate reallocation in response to different density and N rate combinations. *Frontiers in Plant Science*, 10, 1113.
- Winterhalter, L., Mistele, B., & Schmidhalter, U. (2012). Assessing the vertical footprint of reflectance measurements to characterize nitrogen uptake and biomass distribution in maize canopies. *Field Crops Research*, 129, 14-20.

- Xu, K., Chai, Q., Hu, F., Yin, W., & Fan, Z. (2023). Postponed nitrogen fertilizer topdressing enhances nitrogen use efficiency in pea/maize intercropping. *Plant and Soil*, 1-17.
- Yang, Y., Guo, X., Liu, G., Liu, W., Xue, J., Ming, B., ... & Li, S. (2021). Solar radiation effects on dry matter accumulations and transfer in maize. *Frontiers in Plant Science*, 12, 727134.
- Zhai, J., Zhang, Y., Zhang, G., Tian, M., Xie, R., Ming, B., ... & Li, S. (2022). Effects of nitrogen fertilizer management on stalk lodging resistance traits in summer maize. *Agriculture*, 12(2), 162.
- Zhang, J.H., Liu, J.L., Zhang, J.B., Zhao, F.T., Cheng, Y.N., Wang, W.P., & Wang, W.P., (2010). Effects of nitrogen application rates on translocation of dry matter and nitrogen utilization in rice and wheat. *Acta Agronomica Sinica*, 36(10), 1736-1742.
- Zhang, W., Li, H., Zhang, J., Shen, J., Brown, H., & Wang, E. (2022). Contrasting patterns of accumulation, partitioning, and remobilization of biomass and phosphorus in a maize cultivar. *The Crop Journal*, 10(1), 254-261.
- Zhao, Y., Fan, Z., Hu, F., Yin, W., Zhao, C., Yu, A., & Chai, Q. (2019). Source-to-sink translocation of carbon and nitrogen is regulated by fertilization and plant population in maize-pea intercropping. *Frontiers in Plant Science*, 10, 891.



CHAPTER 3

Plastic response of leaf traits to N deficiency in field-grown maize

Panpan Fan^{1,2}, Bo Ming¹, Niels P.R. Anten², Jochem B Evers²,
Yaoyao Li¹, Shaokun Li¹, Ruizhi Xie¹

¹Institute of Crop Science, Chinese Academy of Agricultural Sciences
Key Laboratory of Crop Physiology and Ecology Ministry of Agriculture
Beijing 100081, China

²Center for Crop Systems Analysis (CSA), Wageningen University &
Research, 6708PB Wageningen, The Netherlands

This chapter has been published in *AoB Plants*, 14(6), plac053

Abstract

Nitrogen (N) utilization for crop production under N deficiency conditions is subject to a trade-off between maintaining specific leaf N content (SLN) important for radiation-use efficiency (RUE) versus maintaining leaf area (LA) development, important for light capture. This paper aims to explore how maize deals with this trade-off through responses in SLN, LA, and their underlying traits during the vegetative and reproductive growth stages. In a ten-year N fertilization trial in Jilin province, Northeast China, three N fertilizer levels have been maintained: N-deficiency (N0), low N supply (N1), and high N supply (N2). We analyzed data from years 8 and 10 of this experiment for two common hybrids. Under N deficiency, maize plants maintained LA and decreased SLN during vegetative stages, while both LA and SLN decreased comparably during reproductive stages. Canopy SLA (specific leaf area, cm²/g) decreased sharply during vegetative stages and slightly during reproductive stages, mainly because senesced leaves in the lower canopy had a higher SLA. In the vegetative stage, maize maintained leaf area at low N by maintaining leaf biomass (albeit hence having N content/mass) and slightly increasing SLA. These responses to N deficiency were stronger in maize hybrid XY335 than in ZD958. We conclude that the main strategy of maize to cope with low N is to maintain leaf area, mainly by increasing SLA throughout the plant but only during the vegetative growth phase.

Keywords: Leaf area; leaf N content per unit leaf area; maize strategy; N deficiency; N management practices; specific leaf area

3.1 Introduction

Nitrogen (N) deficiency is one of the most important abiotic factors reducing plant growth and crop yield because N plays a vital role in photosynthesis and crop productivity (Tollenaar and Lee, 2002; Zhang et al., 2020). In agriculture, the amount and efficiency of use of N fertilizer in crops is an issue of intense scientific and societal debate. When the supply of N does not meet the potential demand of the crop, plants exhibit responses in leaf traits such as changes in leaf area production and senescence, specific leaf area (leaf area/mass SLA) and N reallocation, which in turn influence crop growth and yield (Ellsworth and Reich, 1993; Riha et al., 2013). Analyzing these responses is important to elucidate the drivers of crop nitrogen-use efficiency.

Canopy-level response to reduced N uptake and implications for canopy photosynthesis can be viewed by considering that canopy photosynthesis is the product of light absorption and radiation-use efficiency (RUE, canopy photosynthesis/light capture) (Bonelli and Andrade, 2020; Haxeltine and Prentice, 1996). Plants can thus respond to low N uptake through maintenance in leaf area (LA) but reducing specific leaf N content per unit area (SLN). This results in light capture being maintained but a reduction in RUE, given the positive relationship between SLN and leaf photosynthesis (Drouet and Bonhomme, 2004; Field and Mooney, 1986). Conversely, SLN and RUE can be maintained, if LA and consequently in light capture are reduced (Bonelli and Andrade, 2020; Grindlay, 1997). An optimal balance in these two responses helps plants maximize photosynthesis for a given amount of canopy nitrogen (Anten et al., 1995). The strategies followed by plants to cope with N deficiency may differ both among and within plant species (Lemaire et al., 2008; Massignam et al., 2009). For instance, wheat, potato, and canola have predominantly been found to reduce LA and associated light capture, thereby maintaining SLN and RUE (Lemaire et al., 2008; Vos and Van Der Putten, 1998). By contrast, maize and tall fescue respond to N deficiency by a reduction in SLN (Massignam et al., 2011; Vos et al., 2005). Sunflower and sorghum have an intermediate response strategy, showing an almost equal decline in both LA and SLN (Lemaire et al., 2008; Oosterom et al., 2010). Although broad trade-offs among LA and SLN have been demonstrated, both LA and SLN typically decrease with crop development since leaf senescences and N reallocation to grain for protein synthesis during reproductive stages (Bertheloot et al., 2008; Li et al., 2022). We do not know how these responses are mediated by underlying plant traits and the extent to which they are dependent on the developmental stage of the plants. The extent of intraspecific variation in these responses is also not known.

Plants can alter their intraspecific morphological and physiological traits to acquire and use limited available resources through phenotypic plasticity in response to external environment change (Legner et al., 2014; Poorter et al., 2009; Zhu, 2015). An important leaf trait in this respect is specific leaf area (SLA, cm^2/g), as it reflects the trade-off between light capture (leaf area) and mass (and hence photosynthetic compounds) per unit area (Liu et al., 2017; Wilson et al., 1999). For instance, plants typically respond to drought conditions by decreasing SLA, keeping smaller but thicker leaves to decrease water loss (Pérez-Ramos et al., 2013). The acclimation to shade is characterized by increased SLA because higher SLA provides more leaf area for light harvesting (Evans and Poorter, 2001). The interception and spatial distribution of light within the canopy thus induce SLA adjustments, which in turn change the light environment within plant canopies (Archontoulis et al., 2011; Liu et al., 2017; Yao et al., 2016). Furthermore, SLA influences the amount of nitrogen per unit area (specific leaf nitrogen content, SLN) and hence the SLN profile in the canopy and it affects the N allocation between leaf structural components and leaf photosynthesis (Mu et al., 2016; Wilson et al., 1999). Therefore, the plasticity in SLA is crucial in determining canopy photosynthetic capacity and the photosynthetic utilization efficiency of light and N (Reich et al., 1998; Yao et al., 2016). The dynamics in canopy SLA are also affected by leaf age and plant size (Evans and Poorter, 2001; Poorter et al., 2009). In most herbaceous species, canopy SLA increases during leaf expansion, after which there is a decline presumably because of a build-up of cell wall material and chloroplasts (Milla and Reich, 2007; Simioni et al., 2004). Hence, capturing temporal and spatial distribution in leaf SLA has been a long-standing goal of ecological research and is also a crucial part of advancing crop models (Archontoulis et al., 2011; Serbin et al., 2019).

Maize (*Zea mays*. L) is one of the most extensively cultivated cereal crops worldwide and plays an important role in ensuring food security. However, maize is very sensitive to N deficiency under field conditions (Bonelli and Andrade, 2020; Massignam et al., 2009). Therefore, identifying how maize plants respond and adapt to N-limited conditions in terms of leaf traits is critical to enhancing crop productivity and resource-use efficiency. Plants respond to N fertilizer only when soils have low N availability. Long-term field experiments, in which fertilizer treatments are maintained for 5-10 years are therefore most suitable to test the effect of N fertilization. The general objective of this study was to quantify maize response to N deficient conditions at the leaf- and canopy level and in relation to plant ontogeny. Specifically, we address the following questions: (i) what is the trade-off strategy between LA and SLN to adapt to N deficiency in the maize canopy, (ii) does this strategy differ during vegetative and reproductive stages? (iii) How does specific leaf area (SLA) respond to N deficiency

in leaf- and canopy scale? To this end, our study focused on the comparative analysis of leaf traits at the plant and canopy levels for vegetative and reproductive stages in a long-term N fertilized maize field.

3.2 Materials and methods

3.2.1 Site and experimental design

This study was conducted in a long-term N fertilized (urea) field, in which the N fertilizer management practices had been maintained since 2009. Our data were collected in years 8 (2016) and 10 (2018) of the experiment. The experiment was carried out at Gongzhuling Experimental Station of the Chinese Academy of Agricultural Sciences (43°53N, 124°81E), which is located in Jilin province, Northeast China. Two of the most widely-grown maize hybrids in China, XY335 and ZD958, were used in this study. Individual plots were 45.5 m² in size and comprised seven rows of 10 m in length separated by 0.65 m distance. The plant population density was 6.75 plants/m². The plots were arranged in a randomized block design with three replications each. The seeds were sown on 29th April in both years. Weeds were controlled with herbicides. The experimental site received 602.3 and 635.2 mm of precipitation during the maize growing season (from 1th May to 30th September) in 2016 and 2018 and had a mean daily air temperature of 19.9 and 20.4°C, respectively (Table 3.1).

Table 3.1. Mean temperature and precipitation during the growing season in the years 2016 and 2018 at the experimental station

| Month | Year 2016 | | Year 2018 | |
|-----------|------------------|--------------------|------------------|--------------------|
| | Temperature (°C) | Precipitation (mm) | Temperature (°C) | Precipitation (mm) |
| May | 16.3±4.3 | 173.0±13.8 | 17.1±3.5 | 87.1±7.5 |
| June | 20.8±2.7 | 102.2±8.1 | 22.3±2.7 | 121.9±8.3 |
| July | 23.8±2.1 | 62.5±6.2 | 25.5±2.5 | 128.1±10.2 |
| August | 22.5±3.9 | 132.5±9.1 | 21.6±2.5 | 246.7±17.6 |
| September | 16.4±3.4 | 132.1±7.9 | 15.5±3.7 | 51.4±3.1 |

3.2.2 N fertilizer treatments

The total N amount and application stage of the three N fertilizer management practices on the long-term N fertilized field were as follows: no N fertilizer was applied throughout the whole growth period (N0), 150 kg N /ha was all applied as

base fertilizer before sowing (N1), and 150 kg N /ha was applied as described for the N1 treatment, and an additional 150 kg N /ha split equally at V6 and silking stages (N2). P₂O₅ (super phosphate) 42.5 kg/ha and K₂O (potassium sulfate) 42.5 kg/ha were applied as the base fertilizer for all treatments. The fertilizer was applied in the middle of two rows through traditional broadcasting. The topsoil (0-20cm) at the experimental site was classified as chernozem, and the fundamental soil fertility of each growing season was measured before sowing (Table 3.2). Total nitrogen content was determined using an automatic meter (Kjeltec 8400, FOSS, Denmark) according to the Kjeldahl method (Bremner, 1960).

Table 3.2. Total soil N content in the upper 0-20 cm before sowing

| Total N content | Year | N0 | N1 | N2 |
|-----------------------|------|--------|--------|--------|
| | 2016 | 1.08 c | 1.29 b | 1.46 a |
| (g kg ⁻¹) | 2018 | 0.82 c | 1.11 b | 1.24 a |

Note: Different letters in the same row indicate significant differences at $P < 0.05$ ($n=10$). Nutrient availability of P and K were applied the same for all N management treatments.

3.2.3 Plant sampling and measurement

Plants in the three central rows (considering the border effect) were tagged. Leaf rank was counted from the bottom to the top, and tags were placed on leaves 4, 8, and 12 to avoid confusion as lower leaves were senesced (Fan et al., 2020). Focal plants were randomly selected among tagged plants and the whole plants were cut down at soil-surface level at the six-leaf stage (V6), twelve-leaf stage (V12), silking stage (R1), milking stage (R3), and at physiological maturity (R6) (Broeske, 2017). After cutting the fully-expanded leaves from the tagged plants, the maximum length (L , cm) and maximum width (W , cm) of each leaf was measured with a ruler. The leaf area of every single fully-expanded blade was calculated by Eq.(1) with a coefficient of 0.75 (Fan et al., 2020; Zhen et al., 2018), and the leaf area at the plant level was calculated as the sum of every single fully-expanded green leaf area.

$$LA_i = 0.75 \times L \times W \quad (1)$$

Individual leaves were dried at 85°C to a stable weight, weighed, and ground to a fine powder. N concentration of each sample was determined using the Kjeldahl method (Bremner, 1960).

3.2.4 Calculations and statistical analysis

Canopy SLA was defined to be the ratio between green leaf area to the dry weight of leaf biomass Eq.(2), and canopy SLN was calculated as the amount of leaf N per unit of green leaf area Eq.(3).

$$SLA = \sum_{i=1}^n LA_i / \sum_{i=1}^n LB_i \quad (2)$$

$$SLN = \sum_{i=1}^n TLN_i / \sum_{i=1}^n LA_i \quad (3)$$

$$TLN_i = LNC_i \times LB_i \quad (4)$$

where LA is green leaf area (cm^2), LB is leaf biomass (g), LNC is the leaf N concentration (mg/g), TLN is the total leaf N amount (g), and in Eq.(2) and Eq.(3), n is the total number of green leaves of the maize plant and the suffix i indicates an individual leaf value (e.g. LA_i , LB_i is the leaf area and leaf biomass of the leaf rank i , while LA and LB are the leaf area and leaf biomass of the whole plant).

Specific leaf area (SLA, cm^2/g , the leaf area per unit leaf dry biomass) is an essential indicator for estimating plant strategies in response to environmental changes (Legner et al., 2014; Zhou et al., 2020). Hence, the relationship between leaf area (LA) and leaf biomass (LB) at the whole plant level was examined by a power function in this study, as it shows how SLA scales with canopy size (Milla and Reich, 2007).

A power function $LB = \alpha * LA^\beta$ (by ln-transforming form)

$$\ln(LB) = \ln(\alpha) + \beta \ln(LA) \quad (5)$$

$\beta > 1$ indicates that LB increases disproportionately with increasing LA and SLA decreases as LA increase; $\beta < 1$ means the opposite, and $\beta = 1$ represents that SLA is unaffected by LA (Milla and Reich, 2007; Zhou et al., 2020).

A three-way ANOVA test was used to evaluate the effect of N application (at levels N0, N1, and N2), hybrids (ZD958 and XY335), and growth stages (V6, V12, R1, R3, and R6) on leaf traits. A two-way ANCOVA test was used in Table 3.3 to analyze the effects of hybrids and N application on the relationship between leaf area and leaf biomass. The least significant difference (LSD) was used to determine treatment differences at a $P < 0.05$ level of probability (R software, version 3.6.1). The “ggplot2” package of R programming language (Wickham, 2009) was used to produce figures.

3.1 Results

3.1.1 Leaf area and canopy SLN variations under the N deficiency regime

Green leaf area per plant (LA) and canopy average leaf N content per unit area (SLN) differed between the N application rate, stages, and between both hybrids. In most cases, the effects of the three factors depended on each other [see Supporting Information-Table S3.1]. LA reached a maximum value at the R1 stage and then decreased as leaves senesced. There was no difference in LA among N treatments during vegetative stages. However, from the R1 stage LA values in N0 were lower than in N1 and N2, and in N1 were lower than in N2 from the R3 stage (Figs. 3.1A; Fig. 3.2). Compared with the N2 treatment, the LA difference increased with growth stages in N0 and N1 treatments. For N0 this difference increased to 60-78% at the R6 stage, and for the N1 this difference became 19%-26% (Fig. 3.2). Canopy SLN decreased gradually during growth stages in N0. However, at the other two higher N levels, SLN increased up to the V12 stage and then decreased during reproductive stages (Fig. 3.1B). Thus, SLN at the N0 level was consistently lower than SLN at the other two N levels, and the difference between N0 and N2 increased from 20-30% at the V6 stage to 60-70% at the R6 stage. In N1 this difference increased from 12-15% at the V6 stage to 19-23% at the R6 stage (Fig. 3.2). These differences in overall responses to N treatments were associated with differences in initial soil N (Table 3.2). Therefore, in response to low N, the maize plants tended to maintain or sometimes

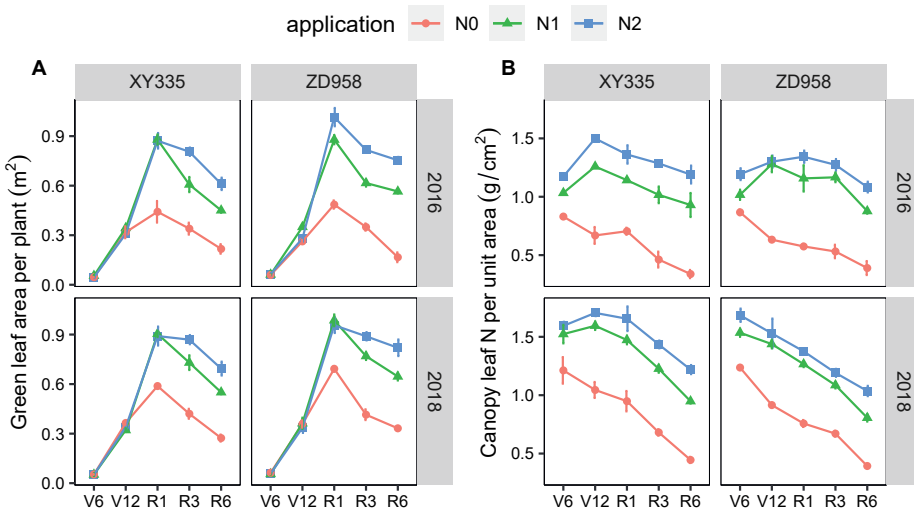


Fig. 3.1. Green leaf area per plant (A) and the canopy leaf N per unit area SLN (B) at five growth stages among three N application rates of XY335 and ZD958 during the 2016 and 2018 growing seasons.

even slightly increase LA and decrease the SLN during vegetative stages to adapt to N deficiency. However, both LA and SLN decreased at reproductive stages, and the proportional reduction was similar for both traits (Fig. 3.2).

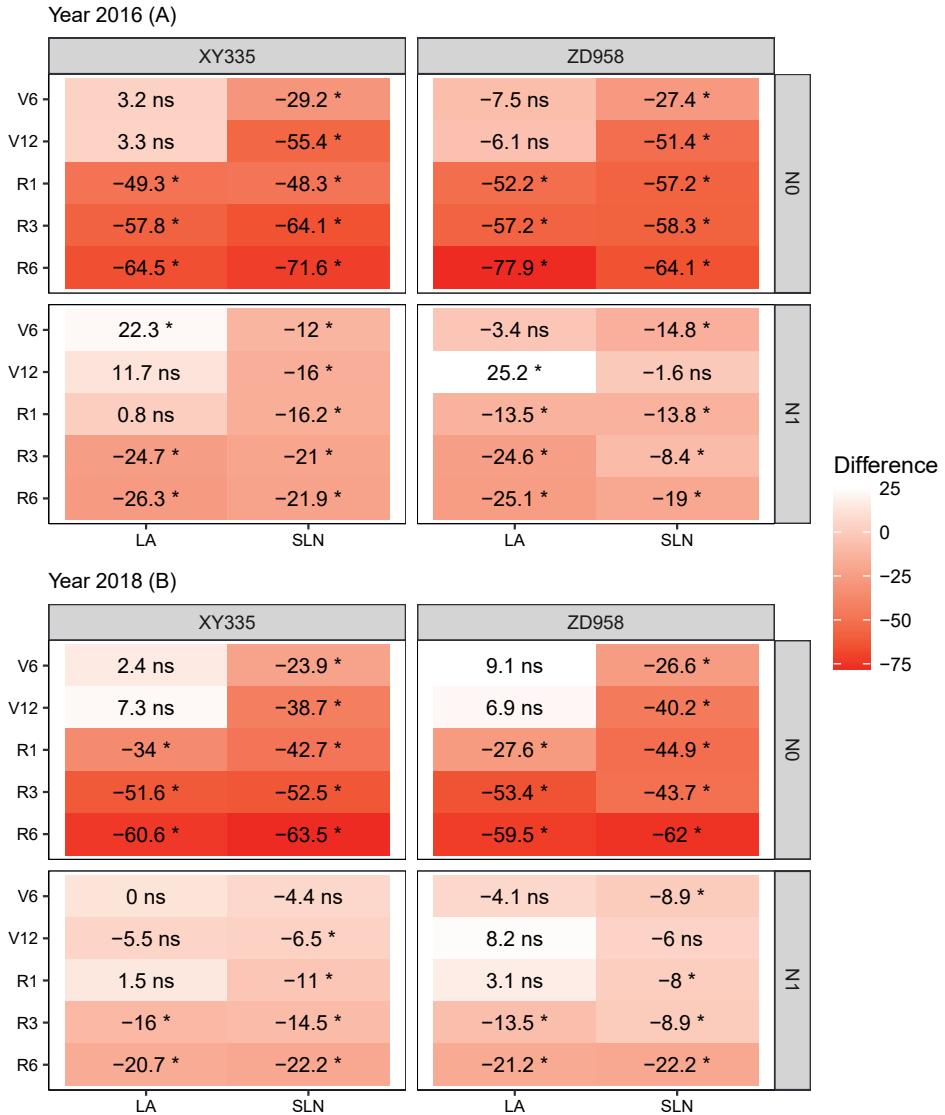


Fig. 3.2. Heatmap of the differences between N applications N0 and N1 compared with N2 application for XY335 and ZD958. Canopy SLN (g/m²); leaf area per plant (LA, m²); * $P < 0.05$, ns = non-significant.

3.3.2 Leaf biomass and total leaf N content variation

At different N levels, maize plants had similar leaf biomass (LB) during vegetative stages, except for the V12 stage of ZD958 in 2016 (Fig. 3.3A). The leaf area in N1 was higher than the other two N levels leading to this exception (Fig. 3.1A). During the reproductive stages, there was an increase in LB with increasing levels of N supply. As of the R1 stage, leaves in N0 had significantly lower biomass than N1 and N2, and the LB difference between N1 and N2 mainly started from the R3 stage (Fig. 3.3A). The total leaf N content per plant (TLN) increased from V6 to R1 stage and decreased thereafter. However, the TLN difference between N0 and other N treatments was significant throughout the whole growth cycle, and the TLN difference increased further with the growth stages (Fig. 3.3B). The TLN difference between N1 and N2 started at the R3 stage, except for ZD958 in 2016.

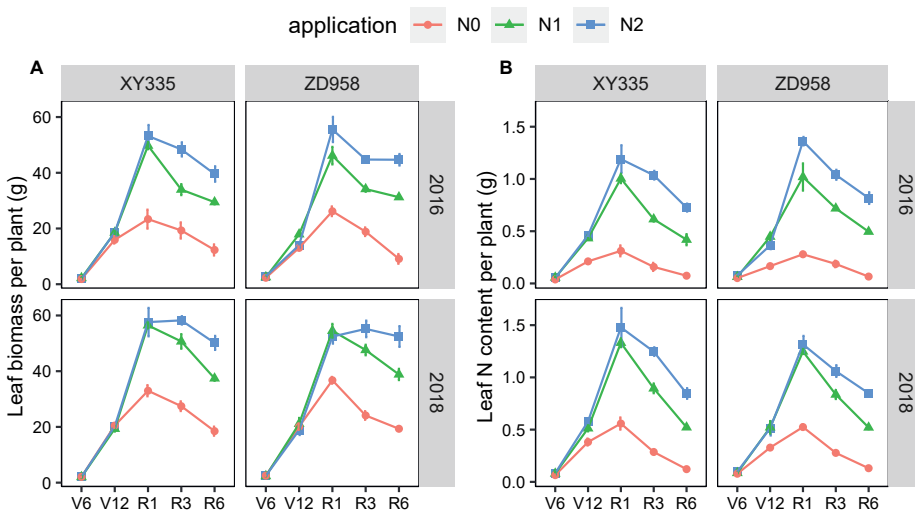


Fig. 3.3. Leaf biomass per plant (A) and the leaf N content per plant (B) at five growth stages among three N application rates of XY335 and ZD958 during the 2016 and 2018 growing seasons.

3.3.3 The scaling relationship between LA and LB

For both ZD958 and XY335, β values in Eq.(5) were greater than 1 in both years, which suggested that LB tended to increase disproportionately faster than LA (Fig. 3.4). Consequently, canopy SLA decreased with increasing leaf size at the plant level, because SLA was defined as the ratio between LA and LB. The β mean values of XY335 were higher than that of ZD958 in 2016 (1.13 vs 1.11) and 2018 (1.19 vs 1.13), which implied that in XY335 SLA declined more strongly with LA than in ZD958, suggesting XY335 invested more photosynthetic products into leaf structure

building (Fig. 3.4). When correcting for differences in plant size, the N application rate did not affect the scaling relationship between LA and LB, given the whole growth stages (Table 3.3).

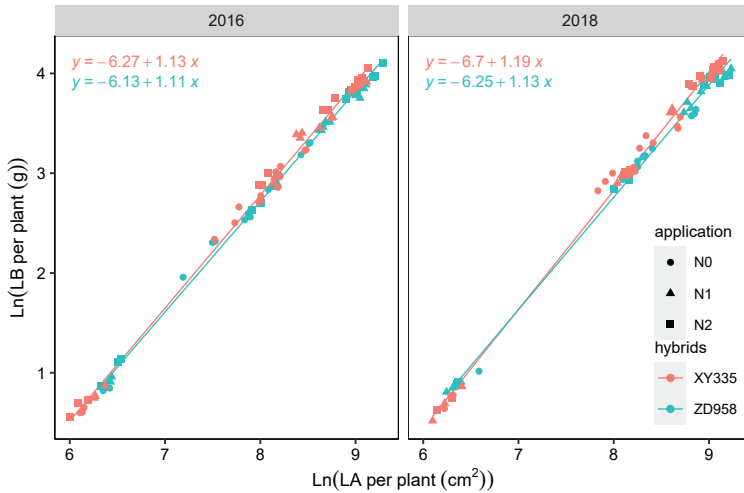


Fig. 3.4. Relationship of leaf biomass (LB, g) and leaf area (LA, cm²) per plant of XY335 and ZD958 under three N treatments in 2016 and 2018. LA and LB were ln-transformed. Statistical fits of the power function $\ln(LB) = \ln(\alpha) + \beta \ln(LA)$. α and β are fitted parameters.

Table 3.3. Two-way ANCOVA (Analysis of Covariance) results in variance

| Effect | Year 2016 | | | | Year 2018 | | | |
|-------------------|-----------|-----|----------|------------|-----------|-----|----------|-------------|
| | DFn | DFd | F value | P value | DFn | DFd | F value | P value |
| LA | 1 | 83 | 217766.8 | 3.14e-102* | 1 | 83 | 14537.95 | 5.46e-95* |
| Hybrids (H) | 1 | 83 | 20.88 | 1.69e-05* | 1 | 83 | 16.73 | 9.93e-05* |
| N application (N) | 2 | 83 | 2.29 | 1.08e-01ns | 2 | 83 | 0.03 | 9.72e-01 ns |
| H × N | 2 | 83 | 3.08 | 5.10e-02ns | 2 | 83 | 0.005 | 9.95e-01 ns |

For both ZD958 and XY335, β values in Eq.(5) were greater than 1 in both years, which suggested that LB tended to increase disproportionately faster than LA (Fig. 3.4). Consequently, canopy SLA decreased with increasing leaf size at the plant level.

3.3.4 The dynamic characteristics of SLA within maize canopy

SLA of individual leaves within maize canopy changed with leaf rank, growth stages, N application, and hybrids [see Supporting Information-Table S3.2]. SLA decreased with increasing leaf rank during vegetative stages, indicating an increase in SLA from the top canopy towards the bottom of the canopy (note that leaf ranks were contented from the bottom canopy to the top canopy). During reproductive stages, SLA decreased with leaf rank and then slightly increased in the top canopy (Fig. 3.5).

3.3.5 Canopy SLA response to N deficiency

Hybrids, N application rate, growth stages, and their interactions had a significant effect on canopy SLA [see Supporting Information-Table S3.2]. Canopy SLA sharply declined during vegetative stages, and declined further but less steeply during the reproductive stage (Fig. 3.6), and this was associated with the vertical SLA distribution within the maize canopy (Fig. 3.5). Leaves with lower ranks in the part of the lower canopy had higher SLA but senesced faster, leading to the sharp decline of canopy SLA during the vegetative stage as it were the lower-canopy higher-SLA leaves that were dropped. The SLA of the middle and upper leaves in the canopy were relatively stable, hence, the canopy SLA decreased slightly over time during reproductive stages [Fig. 3.6; see Supporting Information-Fig. S3.2].

Among N applications, both individual leaf area and leaf lifespan were significantly smaller under N0 treatment than in the other two N treatments in both hybrids [see Supporting Information-Figs. S3.1 and S3.2]. The two hybrids tended to increase SLA in response to N deficiency regardless of the growth stages. In 2016 for XY335, SLA in the N0 treatment was higher than in the other treatments but this was not the case in ZD958 as indicated by the significant difference between hybrids and N application interaction in 2016. In 2018 there was a trend towards higher SLA values in both hybrids. In 2018 the decline in SLA with growth stage was larger in XY335 than in ZD958 as indicated by the interaction effect between hybrids and stage in that year [see Supporting Information Table S3.2].

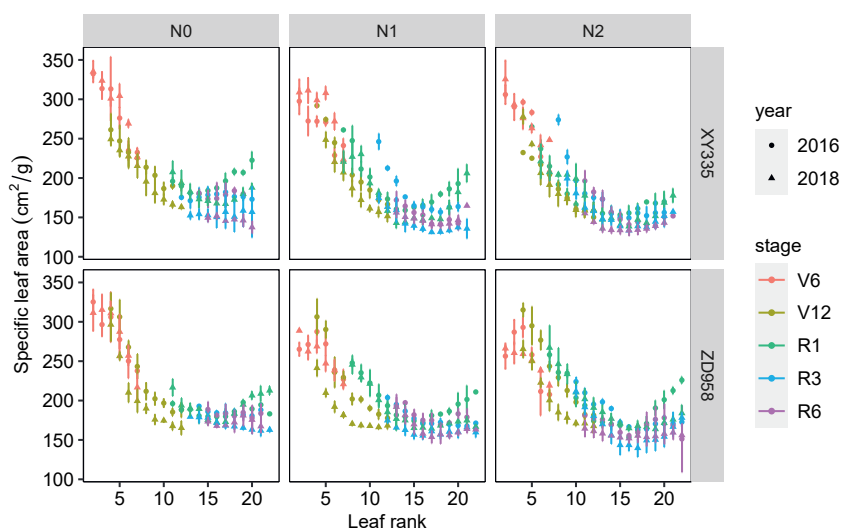


Fig. 3.5. Relationship between specific leaf area (SLA cm^2/g) and leaf rank among three N application rates and five stages of XY335 and ZD958 in 2016 and 2018.

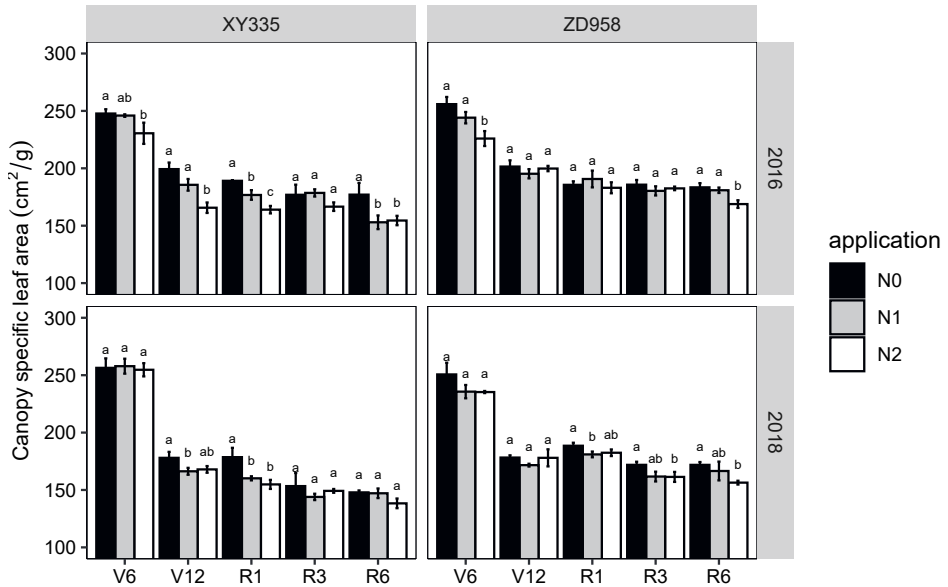


Fig. 3.6. Canopy SLA (specific leaf area, cm²/g) at five growth stages among three N application rates of XY335 and ZD958 during the 2016 and 2018 growing seasons. Different lowercase letters indicate significant differences between treatments at $P < 0.05$. The vertical dot lines indicate the shift from vegetative to reproductive growth.

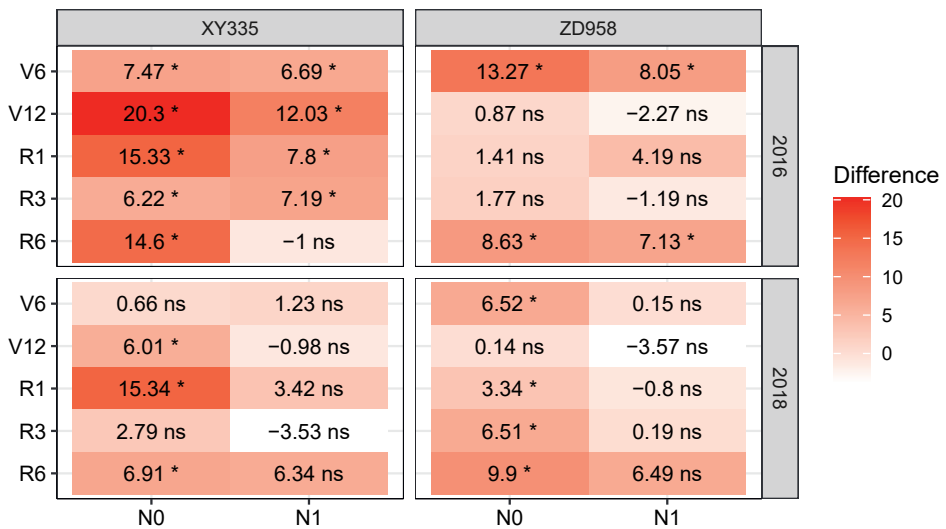


Fig. 3.7. Heatmap of the differences in canopy-specific leaf area (SLA, cm²/g) between N applications compared with N2 application of XY335 and ZD958 in 2016 and 2018. Significance level: * $P < 0.05$, ns = non-significant.

3.4 Discussion

3.4.1 Trade-off between LA and SLN in response to N deficiency

At the canopy level, crops can respond to N deficiency through a reduction in either leaf area (reducing light capture), specific leaf N content (reducing photosynthetic radiation use efficiency, RUE), or both (Hammer et al., 2011; Lemaire et al., 2008). Different response patterns to N deficiency have been associated with different crop species (Lemaire et al., 2008), whereby maize was denoted as a species that tends to maintain its leaf area at the expense of SLN under N deficiency (Vos et al., 2005). Previous research mainly focused on the vegetative growth stages (Lemaire et al., 2008; Massignam et al., 2011; Oosterom et al., 2010). Our findings confirm that maize responds to N stress by the maintenance of LA at the expense of SLN during vegetative stages, but extend this by showing that maize reduced both LA and SLN about equally during the reproductive stages (Fig. 3.2). This suggests that part of the photosynthetic assimilates and N stored in the vegetative organs were translocated and used for grain filling during reproductive stage (Liu et al., 2022; Wei et al., 2019). In general, increasing N supply to the crop might improve LA, prolong leaf lifespan, and boost photosynthesis (Antonietta et al., 2019; Li et al., 2022), however, in our study, there was no remarkable difference in LA across N treatments throughout the vegetative stages (Fig. 3.1). This was primarily caused by the specific leaf area (SLA) of the N0 treatment being higher than that of other N-fertilized treatments (Figs. 3.1 and Fig. 3.2). Probably the higher SLA leaves could capture more light by spreading a given amount of leaf N over a greater area and allocating a greater fraction to photosynthesis, than by overlapping it in a given area (Knops and Reinhart, 2000). As a result, maize plants tended to increase SLA to maintain LA when N was scarce.

3.4.2 Leaf morphological plasticity in response to N deficiency

Individual leaf area and leaf lifespan within plant canopies determine the temporal and spatial distribution of green leaf area and the ability of the crop to intercept light at the canopy level (Perez et al., 2019; Simioni et al., 2004). N supply affects both leaf area development and leaf senescence [as shown in Supporting Information-Fig. S3.1 and S3.2], and consequently the leaf area distribution in the canopy (Bonelli and Andrade, 2020; Vos et al., 2005). However, the negative effect of N deficiency on individual leaf areas was apparent only in the middle to upper leaf ranks, i.e. upwards of leaf rank 10-12 [see Supporting Information-Fig. S3.1]. This explains why the canopy leaf area was not affected by N deficiency at the V6 and V12 stages, and the

results agree with previous studies under field conditions (Dwyer et al., 1992; Fan et al., 2020). Smaller upper leaves allow more light to penetrate the maize canopy, which improves photosynthesis of middle and lower leaves and enhances RUE, as photosynthesis of leaves lower in the canopy is often strongly light limited (Boomsma et al., 2009; Kant et al., 2011). Such an increase in RUE could thus be a positive effect of the upper leaf size reduction under low N found here. Additionally, the onset of leaf senescence begins earlier when N uptake and allocation to leaves is insufficient, and senescence of leaves below the ear leaf (leaf rank 9-12) was most sensitive to N availability [see Supporting Information-Fig. S3.2]. Accelerating the senescence of lower leaves allows more resources (e.g. nitrogen and carbohydrates) to be re-invested in the production of younger leaves and later on in reproduction, and such remobilization and translocation become more important at low N (Massignam et al., 2011).

3.4.3 The relationship between LA and LB

Specific leaf area (SLA, cm²/g) is an essential functional trait since it indicates the amount of light-capturing surface area that leaves have invested per unit of dry mass (Liu et al., 2017; Milla and Reich, 2007; Pérez-Ramos et al., 2013). LB scaled disproportionately faster than LA, as indicated by the scaling exponents $\beta > 1$ in Eq.(5) in XY335 and ZD958 (Fig. 3.4). However, the scaling exponent β of XY335 was somewhat larger than that of ZD958, indicating that for a given canopy size, XY335 tended to have lower SLA and that LB typically increased faster with increasing LA in XY335 than in ZD958 (Fig. 3.4). These findings suggest that the biomass costs (associated with developing and maintaining leaf structure) of deploying light-absorbing leaf area are greater for XY335 than ZD958 (Chen et al., 2014; Li et al., 2019). Lower SLA (thicker leaves) is typically correlated with longer or more palisade cells and larger bundle-sheath cells. This provides space for more or larger chloroplasts and hence a greater amount of photosynthetic enzymes, increasing photosynthetic capacity per unit of leaf area (Evans and Poorter, 2001; Mu et al., 2016; Yao et al., 2016). This is supported by the finding that XY335 has a greater net photosynthetic rate and higher photosynthetic N-use efficiency (PNUE) compared to ZD958 (Chen et al., 2014, 2016). ZD958 displayed stay-green characteristics throughout the grain-filling stage, due to higher leaf number and lower leaf N remobilization efficiency in comparison to XY335 (Chen et al., 2014; Liu et al., 2021). Intriguingly, there appears to be no difference in yield potential between these two hybrids, suggesting that positive effects of the stay-green behaviour of ZD958 compensated for its lower photosynthetic capacity (Li et al., 2022). The connection between LA and LB at the plant level was not substantially different between N treatments after accounting for

the impact of canopy size (Table 3.3). This is most likely because N treatments had minimal impact on LA during vegetative phases due to the maize adaptation strategy, and the LB was driven by LA build-up (Oosterom et al., 2010; Vos et al., 2005).

3.4.4 Spatial and temporal distribution of SLA within maize canopy

Capturing spatial and temporal variability in SLA is crucial in crop growth simulation models. However, many modeling studies use a fixed SLA value even though SLA varies as a function of leaf size, leaf age, growth rate, and climate conditions (Liu et al., 2017; Serbin et al., 2019; Yao et al., 2016; Zhou et al., 2020). SLA within the maize canopy varied with leaf rank (Fig. 3.5), leaves in the lower canopy had larger SLA, while the middle and upper leaves' SLA stayed the same or even increased somewhat with leaf rank, indicating that they may be thicker than lower leaves (Knops and Reinhart, 2000; Yao et al., 2016). This higher SLA of lower leaves allows more light to be captured in the lower part of the canopy, and as leaves there are light limited, possible reductions in SLN and associated photosynthetic capacity will not have a large negative effect (Knops and Reinhart, 2000). We observed that canopy SLA significantly decreased during the vegetative stages and slowly decreased during the reproductive stages across all N treatments (Fig. 3.6). However, the N0 treatment had a slightly higher canopy SLA during the vegetative stage (Fig. 3.7). This probably because leaves in the lower part of the canopy have a shorter leaf lifespan [see Supporting Information-Fig. S3.2] and higher SLA (Serbin et al., 2019; Zhou et al., 2020). Faster leaf senescence in N0 plants should negatively affect canopy SLA as it is the lower canopy leaves that were dropped [see Supporting Information-Fig. S3.2], which generally had relatively high SLA values (Fig. 3.5). These two contrasting leaf sizes and senescence effects may explain why canopy SLA in the reproductive phase no longer differed between N treatments.

3.5 Conclusion

The current study confirmed and extended the trade-off in maize plants' response to N deficiency in terms of leaf area (LA) and specific leaf N content (SLN). At low N availability, maize tended to maintain LA and decrease SLN during vegetative stages, while both LA and SLN declined comparably during the reproductive stage. Individual leaf size, lifespan, and SLA within maize canopy contribute to shaping canopy SLA. Canopy SLA decreased with time mainly because leaves that senesce are lower canopy leaves that have higher SLA than upper leaves that remain. Maize

plants tend to increase SLA to adapt to N deficient conditions, and this could partly offset the negative effects of N deficiency.

Acknowledgments

This study was supported by grants from the National Key Research and Development Program of China (2017YFD0300302). P.F held a scholarship from China Scholarship Council (CSC202003250113). SL and RX conceived the project and provided funding; PF and BM designed the research; PF and YL performed the experiments; PF analyzed the data and wrote the draft; NA and JBE edited and revised the manuscript.

References

- Anten, N. P.R., Schieving, F., Medina, E., Werger, M. J. A., & Schuffelen, P. (1995). Optimal leaf area indices in C3 and C4 mono- and dicotyledonous species at low and high nitrogen availability. *Physiologia plantarum*, 95(4), 541-550.
- Antonietta, M., Girón, P., Costa, M. L., & Guiamét, J. J. (2019). Leaf protein allocation across the canopy and during senescence in earlier and later senescing maize hybrids, and implications for the use of chlorophyll as a proxy of leaf N. *Acta Physiologiae Plantarum*, 41, 1-10.
- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N. G., & Struik, P. C. (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Bertheloot, J., Martre, P., & Andrieu, B. (2008). Dynamics of light and nitrogen distribution during grain filling within wheat canopy. *Plant physiology*, 148(3), 1707-1720.
- Bonelli, L. E., & Andrade, F. H. (2020). Maize radiation use-efficiency response to optimally distributed foliar-nitrogen-content depends on canopy leaf-area index. *Field Crops Research*, 247, 107557.
- Boomsma, C. R., Santini, J. B., Tollenaar, M., & Vyn, T. J. (2009). Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review. *Agronomy Journal*, 101(6), 1426-1452.
- Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science*, 55(1), 11-33.
- Broeske, M., (2017). *Visual Guide To Corn Development*. University of Wisconsin-Madison 1, 72.
- Chen, Y., Xiao, C., Chen, X., Li, Q., Zhang, J., Chen, F., ... & Mi, G. (2014). Characterization of the plant traits contributed to high grain yield and high grain nitrogen concentration in maize. *Field Crops Research*, 159, 1-9.
- Chen, Y., Wu, D., Mu, X., Xiao, C., Chen, F., Yuan, L., & Mi, G. (2016). Vertical Distribution of Photosynthetic Nitrogen Use Efficiency and Its Response to Nitrogen in Field-Grown Maize. *Crop Science*, 56(1), 397-407.
- Drouet, J. L., & Bonhomme, R. (2004). Effect of 3D nitrogen, dry mass per area and local irradiance on canopy photosynthesis within leaves of contrasted heterogeneous maize crops. *Annals of Botany*, 93(6), 699-710.
- Dwyer, L. M., Stewart, D. W., Hamilton, R. I., & Houwing, L. (1992). Ear position and vertical distribution of leaf area in corn. *Agronomy Journal*, 84(3), 430-438.
- Ellsworth, D. S., & Reich, P. B. (1993). Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia*, 96, 169-178.
- Evans, J., & Poorter, H. J. P. C. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, cell & environment*, 24(8), 755-767.
- Fan, P. P., Li, Y. Y., Evers, J. B., Ming, B., Wang, C. X., Li, S. K., & Xie, R. Z. (2020). A new empirical equation to describe the vertical leaf distribution profile of maize. *The Journal of Agricultural Science*, 158(8-9), 676-686.
- Field, C. H. (1986). *The photosynthesis-nitrogen relationship in wild plants. On the economy of plant form and function*, Cambridge, pp25-55.
- Grindlay, D. J. C. (1997). REVIEW Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. *The Journal of Agricultural Science*, 128(4), 377-396.

- Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C., & Riha, S. (2013). Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant and soil*, 365, 239-254.
- Hammer, G. L., Van Oosterom, E. J., Chapman, S. C., & McLean, G. (2001). The economic theory of water and nitrogen dynamics and management in field crops. In *Proceedings Fourth Australian Sorghum Conference*, Kooralbyn, Queensland (pp. 5-8).
- Haxeltine, A., & Prentice, I. C. (1996). A general model for the light-use efficiency of primary production. *Functional Ecology*, 551-561.
- Kant, S., Bi, Y. M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of experimental Botany*, 62(4), 1499-1509.
- Knops, J. M., & Reinhart, K. (2000). Specific leaf area along a nitrogen fertilization gradient. *The American Midland Naturalist*, 144(2), 265-272.
- Legner, N., Fleck, S., & Leuschner, C. (2014). Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance. *Trees*, 28, 263-280.
- Lemaire, G., van Oosterom, E., Jeuffroy, M. H., Gastal, F., & Massignam, A. (2008). Crop species present different qualitative types of response to N deficiency during their vegetative growth. *Field Crops Research*, 105(3), 253-265.
- Li, Y., Ming, B., Fan, P., Liu, Y., Wang, K., Hou, P., ... & Xie, R. (2022). Quantifying contributions of leaf area and longevity to leaf area duration under increased planting density and nitrogen input regimens during maize yield improvement. *Field Crops Research*, 283, 108551.
- Li, Y., Song, H., Zhou, L., Xu, Z., & Zhou, G. (2019). Vertical distributions of chlorophyll and nitrogen and their associations with photosynthesis under drought and rewatering regimes in a maize field. *Agricultural and Forest Meteorology*, 272, 40-54.
- Yu, L., Huang, Y., Zhang, W., Li, T., & Sun, W. (2017). Methane uptake in global forest and grassland soils from 1981 to 2010. *Science of the Total Environment*, 607, 1163-1172.
- Liu, Z., Hu, C., Wang, Y., Sha, Y., Hao, Z., Chen, F., ... & Mi, G. (2021). Nitrogen allocation and remobilization contributing to low-nitrogen tolerance in stay-green maize. *Field Crops Research*, 263, 108078.
- Liu, Z., Sha, Y., Huang, Y., Hao, Z., Guo, W., Ke, L., ... & Mi, G. (2022). Efficient nitrogen allocation and reallocation into the ear in relation to the superior vascular system in low-nitrogen tolerant maize hybrid. *Field Crops Research*, 284, 108580.
- Massignam, A. M., Chapman, S. C., Hammer, G. L., & Fukai, S. (2012). Effects of nitrogen supply on canopy development of maize and sunflower. *Crop and Pasture Science*, 62(12), 1045-1055.
- Massignam, A. M., Chapman, S. C., Hammer, G. L., & Fukai, S. (2009). Physiological determinants of maize and sunflower grain yield as affected by nitrogen supply. *Field crops research*, 113(3), 256-267.
- Milla, R., & Reich, P. B. (2007). The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proceedings of the Royal Society B: Biological Sciences*, 274(1622), 2109-2115.
- Mu, X., Chen, Q., Chen, F., Yuan, L., & Mi, G. (2016). Within-leaf nitrogen allocation in adaptation to low nitrogen supply in maize during grain-filling stage. *Frontiers in Plant Science*, 7, 699.

- Van Oosterom, E. J., Borrell, A. K., Chapman, S. C., Broad, I. J., & Hammer, G. L. (2010). Functional dynamics of the nitrogen balance of sorghum: I. N demand of vegetative plant parts. *Field Crops Research*, 115(1), 19-28.
- Perez, R. P., Fournier, C., Cabrera-Bosquet, L., Artzet, S., Pradal, C., Bricchet, N., ... & Tardieu, F. (2019). Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection. *Plant, cell & environment*, 42(7), 2105-2119.
- Pérez-Ramos, I. M., Volaire, F., Fattet, M., Blanchard, A., & Roumet, C. (2013). Tradeoffs between functional strategies for resource-use and drought-survival in Mediterranean rangeland species. *Environmental and Experimental Botany*, 87, 126-136.
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New phytologist*, 182(3), 565-588.
- Reich, P. B., Ellsworth, D. S., & Walters, M. B. (1998). Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology*, 12(6), 948-958.
- Serbin, S. P., Wu, J., Ely, K. S., Kruger, E. L., Townsend, P. A., Meng, R., ... & Rogers, A. (2019). From the Arctic to the tropics: multibiome prediction of leaf mass per area using leaf reflectance. *New Phytologist*, 224(4), 1557-1568.
- Simioni, G., Gignoux, J., Le Roux, X., Appé, R., & Benest, D. (2004). Spatial and temporal variations in leaf area index, specific leaf area and leaf nitrogen of two co-occurring savanna tree species. *Tree Physiology*, 24(2), 205-216.
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field crops research*, 75(2-3), 161-169.
- Vos, J. P. E. L., Van Der Putten, P. E. L., & Birch, C. J. (2005). Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Research*, 93(1), 64-73.
- Vos, J., & Van der Putten, P. E. L. (1998). Effect of nitrogen supply on leaf growth, leaf nitrogen economy and photosynthetic capacity in potato. *Field Crops Research*, 59(1), 63-72.
- Wei, S., Wang, X., Li, G., Jiang, D., & Dong, S. (2019). Maize canopy apparent photosynthesis and ¹³C-photosynthate reallocation in response to different density and N rate combinations. *Frontiers in Plant Science*, 10, 1113.
- Wilson, P. J., Thompson, K. E. N., & Hodgson, J. G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *The New Phytologist*, 143(1), 155-162.
- Yao, H., Zhang, Y., Yi, X., Zhang, X., & Zhang, W. (2016). Cotton responds to different plant population densities by adjusting specific leaf area to optimize canopy photosynthetic use efficiency of light and nitrogen. *Field Crops Research*, 188, 10-16.
- Zhang, L., Liang, Z. Y., He, X. M., Meng, Q. F., Hu, Y., Schmidhalter, U., ... & Chen, X. P. (2020). Improving grain yield and protein concentration of maize (*Zea mays* L.) simultaneously by appropriate hybrid selection and nitrogen management. *Field Crops Research*, 249, 107754.
- Zhen, X., Shao, H., Zhang, W., Huo, W., Batchelor, W. D., Hou, P., ... & Zhang, F. (2018). Testing a bell-shaped function for estimation of fully expanded leaf area in modern maize under potential production conditions. *The Crop Journal*, 6(5), 527-537.
- Zhou, H., Zhou, G., He, Q., Zhou, L., Ji, Y., & Zhou, M. (2020). Environmental explanation of maize specific leaf area under varying water stress regimes. *Environmental and Experimental Botany*, 171, 103932.

- Zhu, J. (2015). Plant plasticity in intercropping: mechanisms and consequences. Wageningen University and Research.
- Zhai, J., Zhang, Y., Zhang, G., Tian, M., Xie, R., Ming, B., ... & Li, S. (2022). Effects of nitrogen fertilizer management on stalk lodging resistance traits in summer maize. *Agriculture*, 12(2), 162.
- Zhang, D., Sun, Z., Feng, L., Bai, W., Yang, N., Zhang, Z., ... & Zhang, L. (2020). Maize plant density affects yield, growth and source-sink relationship of crops in maize/peanut intercropping. *Field Crops Research*, 257, 107926.
- Zhang, F., Chen, X., & Vitousek, P. (2013). An experiment for the world. *Nature*, 497(7447), 33-35.
- Zhang, N., Van Westreenen, A., Evers, J. B., Anten, N. P., & Marcelis, L. F. (2020). Quantifying the contribution of bent shoots to plant photosynthesis and biomass production of flower shoots in rose (*Rosa hybrida*) using a functional-structural plant model. *Annals of botany*, 126(4), 587-599.
- Zhang, T. A., Chen, H. Y., & Ruan, H. (2018). Global negative effects of nitrogen deposition on soil microbes. *The ISME journal*, 12(7), 1817-1825.
- Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, 528(7580), 51-59.
- Zhu, J. (2015). Plant plasticity in intercropping: mechanisms and consequences. Wageningen University and Research.

Supporting information

Table S3.1. ANOVA analysis of leaf area per plant (LA) and canopy-averaged SLN of XY335 and ZD958 under different N treatments during the two experimental years

| | Year 2016 | | Year 2018 | |
|-------------------|-----------|-----|-----------|-----|
| | LA | SLN | LA | SLN |
| Hybrids (H) | *** | ns | *** | *** |
| N application (N) | *** | *** | *** | *** |
| Stage (S) | *** | *** | *** | *** |
| H×N | *** | ** | ns | ** |
| H×S | *** | ** | *** | *** |
| N×S | *** | *** | *** | *** |
| H×N×S | *** | ** | ns | * |

LA = leaf area per plant (m²); SLN = canopy-averaged leaf N content per unit leaf area (g/m²). Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non-significant.

Table S3.2. Analysis of variance results for canopy SLA

| Variance | Canopy SLA | |
|-------------------|------------|-----------|
| | Year 2016 | Year 2018 |
| Hybrids (H) | *** | *** |
| N application (N) | *** | *** |
| Stage (S) | *** | *** |
| H×N | *** | ns |
| H×S | ns | *** |
| N×S | *** | ** |
| H×N×S | ** | * |

Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = non-significant.

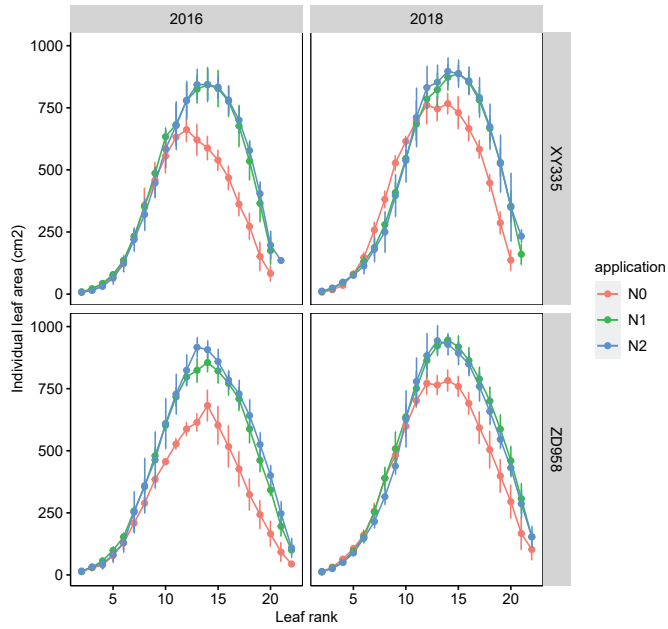


Fig. S3.1. Individual leaf area (cm^2) of fully expanded leaves versus leaf rank among three N application rates of XY335 and ZD958. The dataset of individual leaf areas in 2018 was published and cited by *Li et al.* (2022).

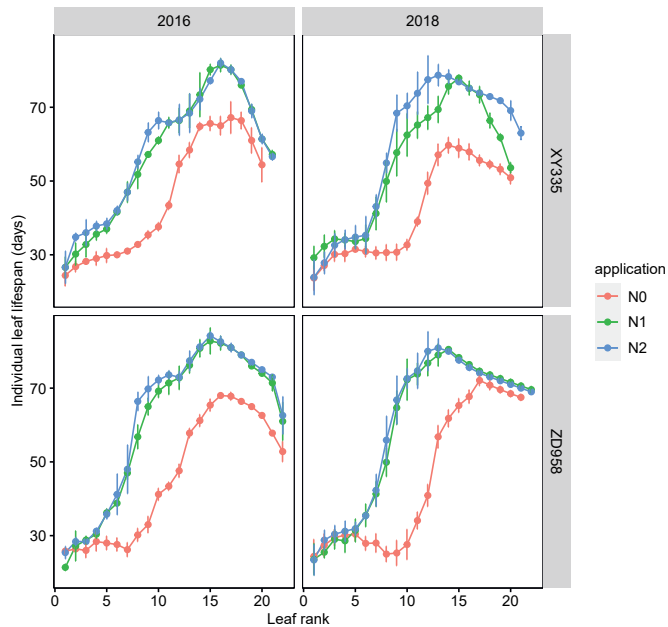


Fig. S3.2. Leaf lifespan (days) of individual leaf rank among three N application rates of XY335 and ZD958. The dataset of individual leaf lifespans in 2018 was published and cited by *Li et al.* (2022).



CHAPTER 4

Changes in the vertical distribution of light and nitrogen within the maize canopy during decades of breeding

Panpan Fan^{1,2}, Niels P.R. Anten², Jochem B Evers²,
Yaoyao Li¹, Shaokun Li¹, Bo Ming¹, Ruizhi Xie¹

¹Key Laboratory of Crop Physiology and Ecology, Institute of Crop
Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

²Center for Crop Systems Analysis, Wageningen University & Research,
P.O. Box 430,6700 AK Wageningen, the Netherlands

This chapter has been submitted

Abstract

Context: Canopy architecture and associated light distribution, nitrogen distribution, and leaf physiological characteristics drive canopy photosynthesis, and ultimately determine grain yields. Breeding has increased those yields during the past decades of breeding history but it is not clear if this also improved efficiency in nitrogen allocation for optimal light use.

Research questions: To what extent has selection for yields affected maize canopy architecture and the vertical distribution of light and nitrogen?

Methods: A 2-year field experiment was conducted in Jilin province, Northeast China, involving six maize cultivars released between 1950 and 2004. The canopy architecture, the vertical distribution patterns of light and nitrogen, and grain yields of these cultivars were quantified and analysed.

Results: The genetic gain in grain yield through breeding was 109 kg ha⁻¹ year⁻¹. Modern cultivars had steeper leaves compared to older cultivars, especially the leaves above the ear leaf, showing a decrease of 0.31° year⁻¹. The changes in leaf area index (LAI) and leaf angle contributed to the improved light distribution within the canopy over generations of selection, as the light extinction coefficient K_L decreased significantly by 0.67% year⁻¹ since 1950. The canopy nitrogen content increased by 0.09 g N m⁻² ground year⁻¹, but the average canopy specific leaf nitrogen (canopy N/LAI) tended to decrease. The relationship between light and nitrogen distribution within the canopy differed among cultivars but did not relate to year of release (YOR), indicating that breeding of maize cultivars from the 1950 to 2004 primarily led to changes in the canopy architecture and did not improve the coordination between light and nitrogen in maize canopy.

Conclusion: Yield gain from older to newer cultivars was strongly associated with changes in canopy architecture, which improved light distribution and increased light interception by higher LAI and steeper leaf angle. In contrast, the coordination of light and nitrogen was not changed much through breeding history from 1950 to 2004. We conclude that the higher yields that are obtained with modern cultivars do not appear to rely on a coordinated light and N distribution within the canopy.

Keywords: Canopy architecture, light distribution, nitrogen distribution, breeding selection.

4.1 Introduction

Maize is cultivated across a wide range of environments and delivering one-third of the total global grain production (Duvick, 2005; Liu et al., 2021). Maize grain yields per unit area have increased gradually over time due to improvements in both genetics and management practices such as higher population densities (Duvick, 2005; Qin et al., 2016). Specifically, the genetic improvements through breeding have contributed to increased maize yields worldwide, including China (Liu et al., 2021; Qin et al., 2016), USA (Tollenaar and Lee, 2002), Europe (Perez et al., 2019; Welcker et al., 2022), and Argentina (Cagnola et al., 2021; Lacasa et al., 2022). This genetic yield improvement has been associated with changes in morphological and physiological traits improving tolerance to high densities, and further exploration of these associations can give insights into the mechanisms of yield improvements.

Several morphological traits have changed in newly released genotypes over the past several decades (Ma et al., 2014; Perez et al., 2019). The leaf area increased with the year of release (YOR), and the modern cultivars tend to show a markedly different vertical leaf area distribution compared with older cultivars (Liu et al., 2023; Perez et al., 2019). For instance, modern cultivars tend to have more erect leaves and relatively more leaf area located at lower relative positions in the canopy (Lacasa et al., 2022; Liu et al., 2021). These morphological traits reduce the photosynthetically active radiation (PAR) absorbed by upper leaves in the canopy and increases the amount of PAR reaching lower leaves (Ma et al., 2014; Qin et al., 2016). The changes in vertical distribution of leaf area over generations of selection allowed optimizing the utilization of light in the different layers and a better interception by the layer containing the leaves that mainly supply carbon to the ear (Cagnola et al., 2021; Welcker et al., 2022). The middle leaves are the main functional leaves for dry matter production, and they capture more solar radiation when the leaves in the upper canopy are more upright (Fan et al., 2023; Li et al., 2019). The more compact plant type of modern maize suits high planting densities better, allowing higher whole-stand light capture and preventing of lodging (Cagnola et al., 2021; G. Liu et al., 2022). Moreover, modern cultivars tend to have delayed leaf senescence and thus longer duration of photosynthesis (Li et al., 2022; Valentinuz and Tollenaar, 2006).

The process of light attenuation within the canopy is responsible for determining the vertical distribution of light and nitrogen (N). The vertical distribution of light capture can be described quantitatively using the extinction coefficient K_L , which relates the fraction of intercepted photosynthetically active radiation (iPAR) to the leaf area index (LAI) (Monsi and Saeki, 2005). At a given plant density, lower values of K_L correspond

to more vertically-inclined leaves and a more uniform vertical distribution of light. This enhances radiation-use efficiency (RUE) (Huang et al., 2017; Liu et al., 2021). The RUE was found to increase by about 31% from the oldest cultivar to modern cultivar from 1980 to 2010 under an optimal environment (Liu et al., 2021). However, a too low K_L value can result in too much light reaching the soil, not contributing to crop productivity. The optimal K_L value also depends on other canopy traits such as nitrogen distribution in the canopy (Anten et al., 1995b; Hikosaka, 2016).

Similar to light capture in the canopy, the N distribution among leaves in both natural vegetations and in crop stands declines with depth, i.e. the upper sunlit leaves have a higher nitrogen content than the lower shaded leaves (Anten et al., 1995b; Hirose and Werger, 1987). This distribution positively affects canopy photosynthesis and nitrogen-use efficiency, compared to a uniform distribution, as the greater illumination of upper leaves allows them to use N more efficiently for photosynthesis (Evans, 1993; Hirose and Werger, 1987). Hence, the canopy N distribution is an important crop trait for canopy photosynthesis (Dreccer et al., 2000; Stewart et al., 2003). Typically, the vertical N distribution within the canopy can be described by an exponential function of the downwards cumulative leaf area index (Chen et al., 2019; Hirose and Werger, 1987; Liu et al., 2020). However, in maize, a more quadratic N distribution pattern has been observed, with specific leaf N (SLN, g N m^{-2}) of middle-canopy leaves being similar or even higher than those of the top leaves (Chen et al., 2016; Ciganda et al., 2008; Li et al., 2019). This difference probably depends on species, cultivar, environmental conditions and field management strategies.

At a given canopy N content, canopy photosynthesis is maximized when the nitrogen distribution exactly follows the light distribution within a canopy (Evans, 1993; Field, 1983). However, the N gradients in the actual canopy have been found to be less steep compared with the optimal gradients, leading to a reduction in canopy photosynthesis by over 20% (Yin and Struik, 2015). Moreover, the gradient of both light and nitrogen show a strong degree of genotypic variation and environmental conditions (Chen et al., 2019; Zhou et al., 2023). The modern cultivars may make it possible to close the gap between light and nitrogen profiles because of the erect canopy architecture and the more uniform light distribution (Lacasa et al., 2022; Ma et al., 2014), but whether this is the case and how breeding may have resulted in changes in terms of the vertical distributions of light and nitrogen is not clear.

Although some studies have assessed performance of a time series of maize cultivars to identify the morphological and physiological traits associated with resource-use efficiency (Liu et al., 2021; Mueller et al., 2019; Welcker et al., 2022), information is also limited

regarding the maize genetic improvement from the viewpoint of vertical distributions of light and nitrogen within the canopy. Thus, the primary objectives of this work were to: (1) evaluate how key morphological and physiological traits have been changed over six decades of maize breeding; and (2) how this in turn may have resulted in canopy architecture changes and the vertical light and nitrogen distributions within the canopy. Studying these changes for maize cultivars released across different breeding eras is relevant for assessing the benefit of future modifications aimed at improving yield gain.

4.2 Materials and methods

4.2.1 Experimental design and management

The field experiments were conducted at the Gongzhuling Experimental Station, Jilin Province, China (43°30 N, 124°50 E) in 2016 and 2018. The soil at the site is classified as black soil. Relevant physical and chemical properties for the top 0-20cm soil layer were as follows: organic matter content 26.3g kg⁻¹; total N, 1.5 g kg⁻¹; available N, 124.9mg kg⁻¹; Olsen P, 28.5mg kg⁻¹; and available K, 184.5 mg kg⁻¹. The cultivars used in this study were Baihe (BH) released in 1950, Jidan 101 (JD101) in 1967, Zhongdan 2 (ZD2) in 1972, Yedan 13 (YD13) in 1998, Zhengdan 958 (ZD958) in 2000, and Xianyu 335 (XY335) in 2004 together spanning a 54-year range in release years (Table 4.1). All of the cultivars are successful, widely grown cultivars, representative of the elite germplasm of the period in which they were released. Individual cultivars were selected because they were known to be well-adapted to the agro-ecological conditions of the study area and seeds of proprietary parental lines were available, which allowed the creation of new F1 hybrid seeds.

Table 4.1. The maize cultivars were used at the experimental sites in this study

| Cultivars | Pedigree | Year of release | Institutions that developed the cultivar |
|-----------|------------------|-----------------|---|
| BH | OPC | 1950 | Gongzhuling farm of Jilin province, China |
| JD101 | Ji63×M14 | 1967 | Maize institute of Jilin AAS, Gongzhuling, China |
| ZD2 | Mo17×Zi330 | 1972 | Chinese AAS, Beijing, China |
| YD13 | Ye478×Dan340 | 1998 | Lanzhou AAS of Shandong province, Lanzhou, China |
| ZD958 | Zheng58×Chang7-2 | 2000 | Luohe AAS of Henna province, Luohe, China |
| XY335 | PH6WC×PH4CV | 2004 | The Tieling Pioneer limited company, Tieling, China |

BH was popularly used in maize production in the 1950s; # AAS, Academy of Agricultural Sciences; OPC: open-pollinated cultivar.

The experiments were arranged in a randomized design with three replications. Individual plots were 45.5 m² in size and comprised seven rows of 10 m in length separated by 0.65 m distance. The plant population density was 6.75 plants per m². Each plot was fertilized with 150 kg ha⁻¹ N (urea), 42.5 kg ha⁻¹ P₂O₅ (superphosphate), and 42.5 kg ha⁻¹ K₂O (potassium sulphate) before planting each year. Additional urea was applied at the jointing (75kg ha⁻¹) and flowering (75kg ha⁻¹) stages. The weeds, diseases, and pests were chemically controlled.

4.2.2 Morphological traits

At emergence, ten successive plants were tagged in the central row of each plot. Plants were tagged and the 4th, 8th, and 12th leaves were marked with red labels to avoid confusion as lower leaves senesced. The leaf rank was counted from the bottom leaf (rank 1) to the top leaf. For each tagged plant, the position of the ear leaf was recorded. Five plants per cultivar treatment in 2016 and 10 plants per cultivar treatment in 2018 were observed as representative plants to measure the morphological traits at silking stage. Leaf angle (i.e., the declination angle between the leaf and stalk), leaf length and leaf width of each leaf blade were recorded when the leaf ligule appeared. Leaf area (cm²) and leaf area index (LAI) were calculated as (Fan et al., 2020):

$$\text{Leaf area} = \text{leaf length} \times \text{maximum width} \times 0.75 \quad (1)$$

$$\text{LAI} = \sum_1^n \text{Leaf area} \times \text{planting density} \quad (2)$$

where n is the leaf rank of green leaves. Progression of senescence of each leaf was recorded twice weekly from emergence to harvest in tagged plants. Senescence was visually estimated in terms of the percentage of leaf yellowing or drying. Leaf lifespan was considered the time between leaf expansion and 50% leaf surface area senescence (Li et al., 2022).

4.2.2.2 Vertical light and N distribution within the canopy

At silking stage, the instantaneous photosynthetically active radiation (PAR) was measured between 12:00 and 15:00 AM local time on clear days, using a 1-m line quantum with 64 sensors (SUNSCAN, Delta, UK) in 2016 and 2018. PAR measurements were taken at each leaf rank from the ground upwards and above the canopy, with three repeated measurements in each leaf position for each plot. The measured datapoint was at the middle height between adjacent leaves on the same side. The light attenuation within the canopy was described following the Beer-Lambert law as:

$$I_i = I_0 e^{-K_L \text{LAI}_i} \quad (3)$$

Where I_i and I_0 are PAR at the leaf rank I and above the canopy, respectively. K_L is the light extinction coefficient; LAI_i (m^2 green leaves m^{-2} ground) is the cumulative surface area of green leaves per unit ground area from the top layer of the canopy (Hirose, 2005; Monsi and Saeki, 2005). Since maize leaves are long and curved, one leaf may actually be present in multiple layers. In our study therefore, LAI_i is the cumulative LAI at the leaf rank i counted from the top of the canopy downwards. Both the absolute and relative values of cumulative LAI were used in this study to eliminate differences in the total LAI among cultivars, and in the relative cumulative LAI, 0 represents the top of the canopy and 1 the bottom.

After the light distribution was measured, three representative plants selected were cut from the shoot base and separated into sections at each leaf rank to assess the green leaf area (LA) and leaf nitrogen per unit area (specific leaf area, SLN, g N m^{-2}). Individual leaves were dried at 85°C to a stable weight, weighed and ground to a fine powder. Nitrogen concentration of each sample was determined using the Kjeldahl method (Bremner, 1960).

4.2.2.3 Grain yield and biomass

At physiological maturity, three rows of each cultivar were hand harvested to measure yield. All plants in the central three rows of each plot, excluding those on the end of each row, were hand-harvested. Ears were harvested, counted, and weighed, then the mean weight of each ear was calculated. Kernel moisture content was determined with a portable moisture meter (PM8188, Kett Electric Laboratory). The grain yield of each subplot was weighted and adjusted to 14% moisture for analysis.

4.2.3 Statistical analyses

Linear regression was used to test the relationship between the year of release (YOR) and grain yield, yield components, biomass before silking, leaf angle, the light extinction coefficient K_L , total canopy N content and canopy SLN. A quadratic equation was used to fit the relationship between YOR and maximum leaf area index (maximum LAI) at silking. Nonlinear fitting was carried out using the “nls” package of R software (version 3.6.1) to estimate K_L in Eq.(3). Comparisons among different cultivars were performed with Duncan’s multiple range test only when the ANOVA indicated significant difference.

4.3 Results

4.3.1 Yield and genetic gain

The grain yield, biomass before silking (biomass from emerge to silking stage), kernel weight, and kernel number per ear significantly increased with year of release (YOR). Among the six cultivars, the cultivar XY335, released in 2004, had the highest yield, with 12.4 and 13.7 t/ha in 2016 and 2018, respectively (Fig. 4.1A). Grain yield increased linearly with YOR at the rate of 109 kg ha⁻¹ year⁻¹, resulting in an 88% increase from 1950 to 2004 (Fig. 4.1A). The increase of grain yield was driven by a combination of both higher kernel number per ear and kernel weight. The significant

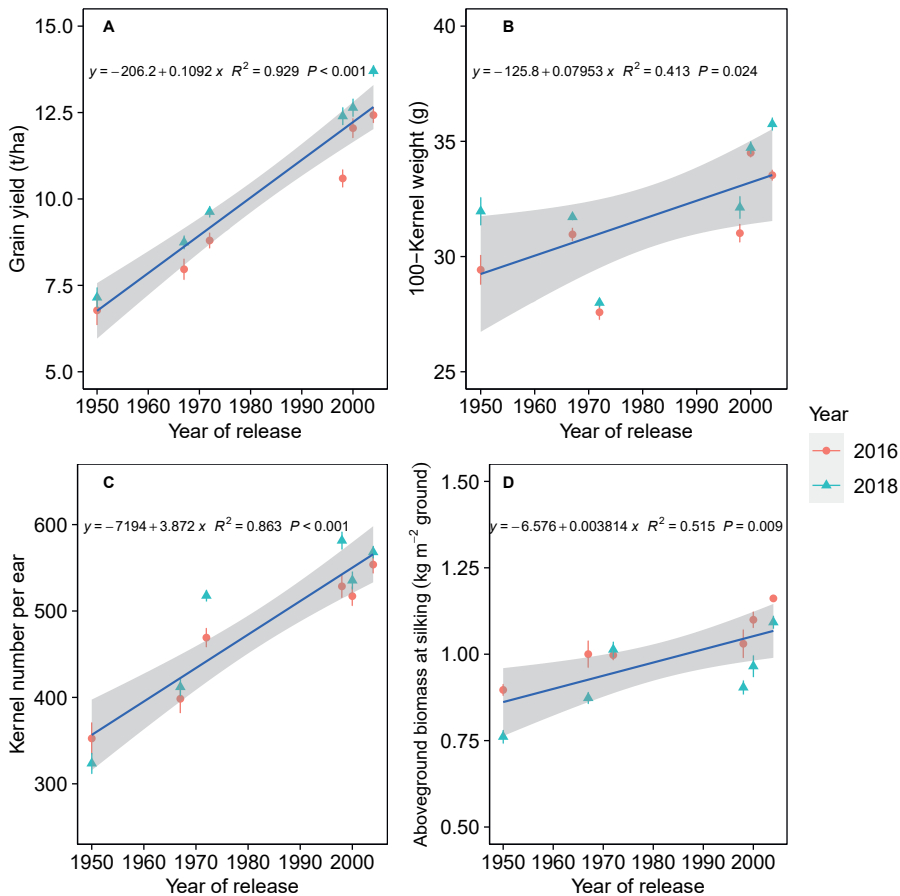


Fig. 4.1. Yield and yield component changes in maize hybrids over time. Variables are grain yield (A), 100-kernel weight (B), Kernel number per ear (C) and canopy biomass before silking (D). canopy biomass before silking is the biomass accumulation from emerge to silking stage. Red and blue symbols represent experimental years 2016 and 2018, respectively. Linear regression lines were for overall data in the two years.

increase in kernel number per ear was greater over time compared with grain kernel weight (Fig. 4.1B and 4.1C). Specifically, the 100-kernel weight and kernel number per ear increased at a rate of 0.08 g year^{-1} ($0.24\% \text{ YOR}^{-1}$) and 3.87 year^{-1} ($1.23\% \text{ YOR}^{-1}$), respectively (Fig. 4.1B and 4.1C). Similar with grain yield, the total canopy biomass of modern cultivars was higher than older cultivars at silking stage, resulting in an overall increase of 29.1% at a rate of $3.8 \text{ g m}^{-2} \text{ ground year}^{-1}$ across the evaluated cultivars in this study (Fig. 4.1D). Additionally, the grain yield and yield components in 2018 were higher, possibly due to higher average temperature and precipitation during June and July compared to 2016 (Table 4.2).

Table 4.2. Monthly accumulated air temperature, and monthly total rainfall during the three growing seasons at the Gongzhuling experimental station

| Month | 2016 | | 2018 | |
|-------|---------------------------|--------------------|---------------------------|--------------------|
| | Averaged Temperature (°C) | Precipitation (mm) | Averaged Temperature (°C) | Precipitation (mm) |
| May | 16.3 | 173.0 | 17.1 | 87.1 |
| June | 20.8 | 102.2 | 22.3 | 121.9 |
| July | 23.8 | 62.5 | 25.5 | 128.1 |
| Aug. | 22.5 | 132.5 | 21.6 | 246.7 |
| Sept. | 16.4 | 132.1 | 15.5 | 51.4 |

4.3.2 Canopy architecture

The maximum LAI at silking versus YOR fit well into a significant quadratic function (Fig. 4.2A). The maximum LAI of modern cultivar XY335 released in 2004 was average 65% higher than that of oldest cultivar BH released in 1950, and the cultivar ZD958 released in 2000 had the highest LAI during the two experimental years (Fig. 4.2A). From 1950 to 2004, breeding had reduced the leaf declination angle (Fig. 4.2B). The leaf angles above and below the ear leaf of modern cultivars were both significantly smaller (i.e., leaves being more vertically inclined) compared with old cultivars, which decreased by $0.31^\circ \text{ year}^{-1}$ and $0.09^\circ \text{ year}^{-1}$, respectively (Fig. 4.2B). The mean leaf angle of above and below the ear leaf changed from 17.8° to 37.5° and from 27.7° to 36.0° in a time series of cultivars (Fig. S4.1). Furthermore, from the bottom to the top leaf, the changes in leaf angle were smaller for the old cultivar than for the modern cultivars, determining by the difference in leaf angle between above and below the ear (Fig. 4.2B). For instance, the difference between the mean leaf angle of leaves above and below the ear leaf for ZD958 and XY335 (modern cultivars) were 10.2° and 11.8° in both experimental years compared to 0.95° and 2.0° for BH and JD101 (older cultivars) (Fig. S4.1).

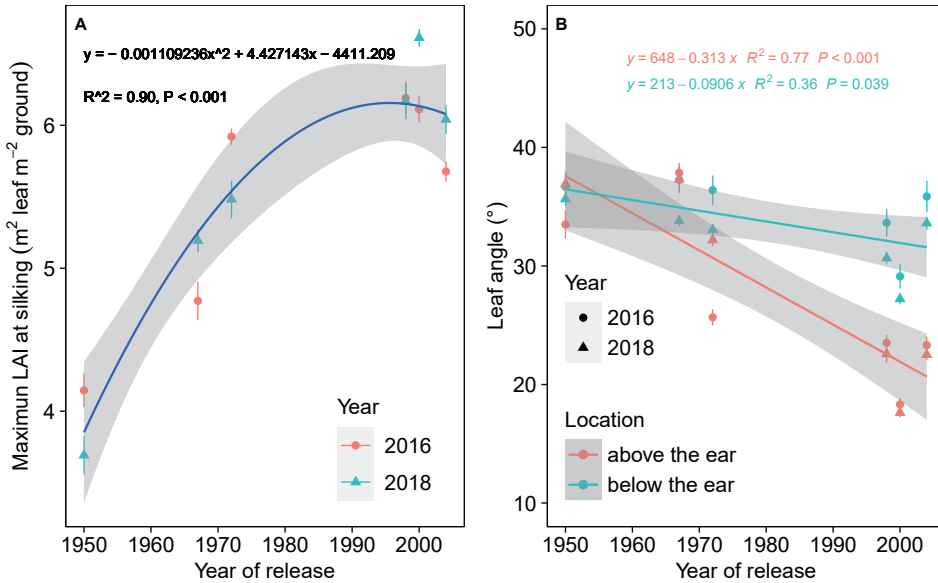


Fig. 4.2. The effect of maize cultivar release of years (YOR) on maximum LAI at silking (A) and leaf angle (B). Leaf angle ($^\circ$) above or below the ear was averaged value from all the leaves above or below the ear leaf. Solid lines indicate that the linear fitted models were significant. Linear regression lines were for overall data in the two years.

4.3.3 The vertical light distribution within the canopy

Among cultivars, the relative photosynthetically active radiation profile (PAR) decreased negative exponentially with increasing cumulative LAI counted from the top of the canopy (Fig. 4.3). At a given cumulative LAI, the modern cultivars ZD958 and XY335 had higher relative PAR values compared with older cultivars (Fig. 4.3). When comparing relative light levels at the same LAI, the two most modern varieties had the highest values while ZD2 and YD13 (released in 1972 and 1998) had the lowest values (Fig. 4.3). Moreover, the variation in K_L among cultivars was from 0.61 to 0.39, leading a decrease at a rate of 0.0044 year^{-1} or $0.67\% \text{ YOR}^{-1}$ from 1950 to 2004 (Fig. 4.4). Lower values of K_L corresponded to more upright leaves. Specifically, relatively more light penetrated into the lower layers of the canopy for modern cultivars due to them having more vertically-inclined leaves, as reflected by an average of 36% lower K_L for modern cultivar XY335 than for the oldest cultivar BH (Fig. 4.4).

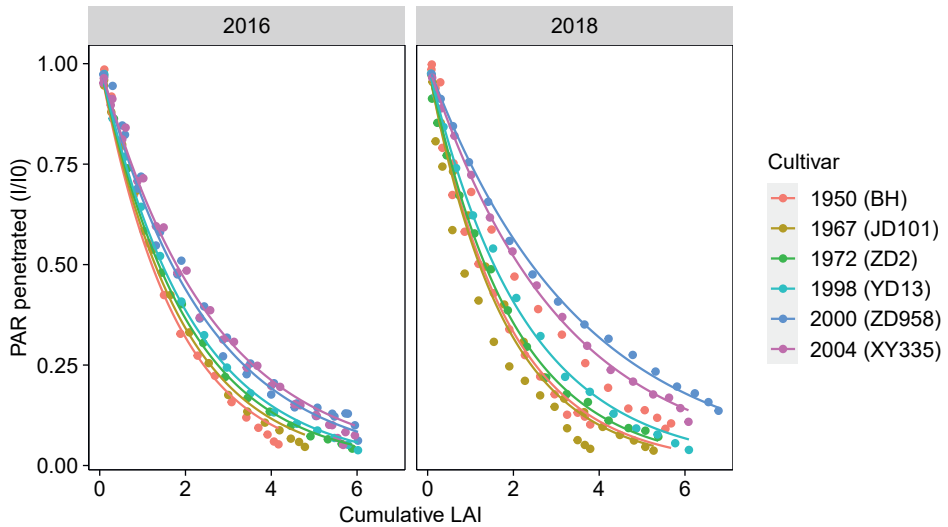


Fig. 4.3. Vertical light distribution in the canopies of maize cultivars in the two experimental years. The canopy relative photosynthetically active radiation profile (iPAR) are plotted against cumulative leaf area index (LAI) counted from the top of the canopy of the six maize cultivars at silking stage. In the relative cumulative LAI, 0 represents the top of the canopy and 1 represents the bottom. Each data point represents the mean of six replicates. Curves for light profile are drawn from Eq. (3) with parameter K_L fitted.

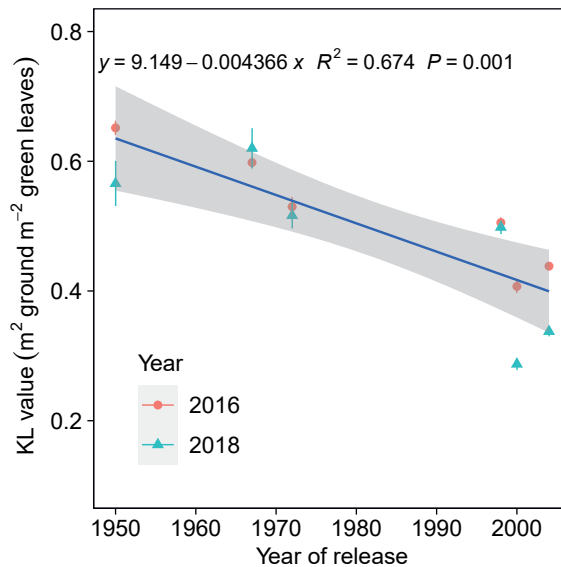


Fig. 4.4. Relationships between canopy light extinction coefficient (K_L) and year of release in the two experimental years. The canopy light extinction coefficient (K_L) was derived from the vertical profile of relative values of photosynthetically-active radiation with Eq. (3). Linear regression lines were for overall data of the two experimental years. Solid lines indicate that the linear fitted models were significant.

4.3.4 Canopy nitrogen content

The total canopy N content increased 34% between 1950 and 2004 at a rate of $0.09 \text{ g N m}^{-2} \text{ ground year}^{-1}$, indicating that modern cultivars allocated more N to the canopy (Fig. 4.5A). However, there was a negative trend between canopy SLN with YOR albeit not significant ($p=0.138$) (Fig. 4.5B), indicating both canopy N content and LAI increased with YOR and the increase in canopy nitrogen content tended to be less than the increase in LAI. For instance, the canopy nitrogen content of the most modern cultivar XY335 was 23% and 46% higher compared with the oldest cultivar BH in the two experimental years respectively, but the differences in LAI were 37% and 64% (Fig. 4.5A and Fig. 4.2A).

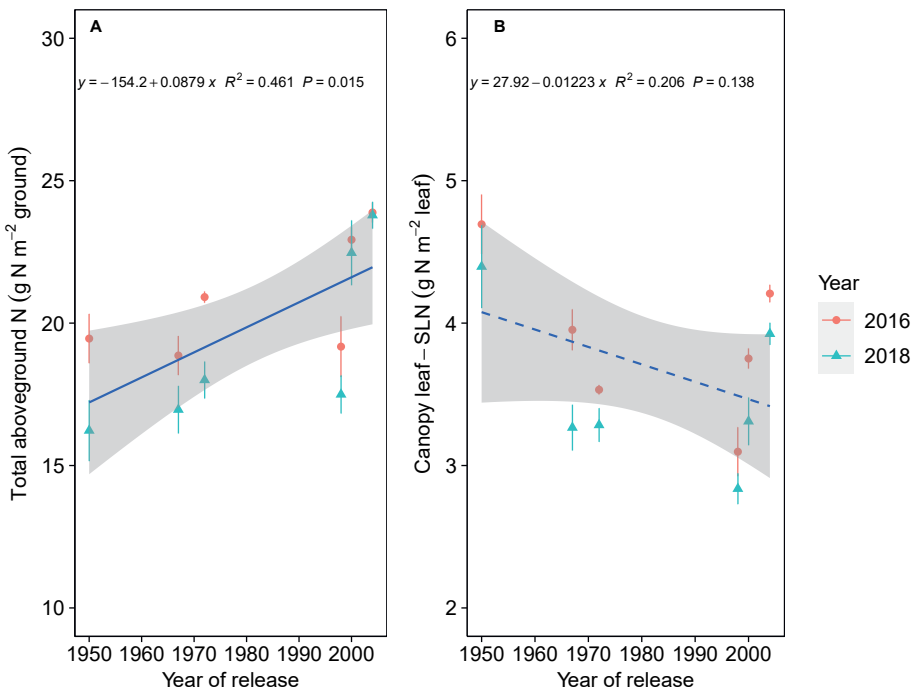


Fig. 4.5. Relationships between total canopy N content ($\text{g N m}^{-2} \text{ ground}$) (A) and canopy specific nitrogen (canopy SLN, $\text{g N m}^{-2} \text{ leaf}$) (B) and year of release in the two experimental years. Linear regression lines were for overall data of the two experimental years. Solid lines indicate that the linear fitted models were significant.

4.3.5 The vertical nitrogen distribution within the canopy

The relationship between specific leaf nitrogen (SLN) and the cumulative LAI from the top to the bottom within the maize canopy was generally unimodal (i.e., hump-shaped). Across cultivars, the upper-middle leaves often had the highest SLN, and

then the SLN decreased sharply towards the bottom of the canopy (Fig. 4.6). At a given cumulative LAI, the modern cultivars ZD958 and XY335 had higher SLN values than other older cultivars. However, the relationship between SLN and relative cumulative LAI differed with cumulative LAI, as the different total LAI and vertical distribution patterns among cultivars (Fig. 4.6). Specifically, the oldest cultivar BH released in 1950 had similar SLN with modern cultivars ZD958 and XY335 during the two experimental years, and all of them were higher than other cultivars released from 1967 to 1998 (Fig. 4.6).

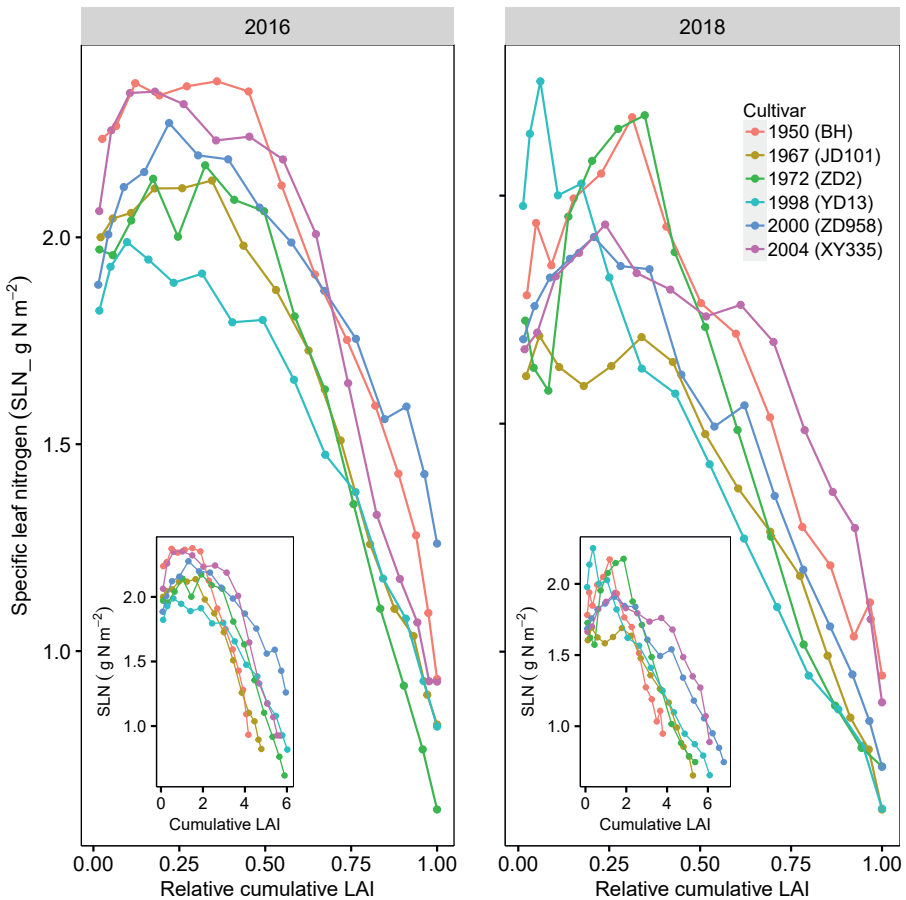


Fig. 4.6. The vertical specific leaf nitrogen (SLN, g N m^{-2} leaf) distribution in the canopies of maize cultivars in 2016 and 2018. The cumulative LAI counted from the top canopy at silking stage. In the relative cumulative LAI, 0 represents the top of the canopy and 1 the bottom. Each data point represents the mean of three replicates.

4.3.6 Relationship between SLN and PAR distributions

Although both light and SLN values generally decreased with cumulative LAI from top to bottom in the canopy, the patterns in which they did so were quite different (Fig. 4.3 and 4.6). Across cultivars, the relative PAR (I/I_0) decreased 51% from the top canopy 0.97 to the middle canopy (the ear leaf) 0.47, but the SLN increased 9% over the same part of the canopy. Both PAR and SLN decreased more strongly in the lower canopy (from the ear leaf to bottom leaf), compared with the top canopy (Fig. 4.7). For instance, the relative PAR decreased 85% from the ear leaf 0.47 to the bottom leaf 0.07, and the average leaf SLN decreased 62% over the same part of the canopy. Additionally, the relationship between PAR and SLN distributions differed significantly between cultivars. The oldest cultivar BH and modern cultivar XY335 tended to have higher leaf SLN at the same relative light intensity in the canopy, compared with other cultivars. Moreover, the cultivar ZD2 released in 1972 tended to have higher SLN than other cultivars in the 2018, especially in the lower canopy (Fig. 4.7).

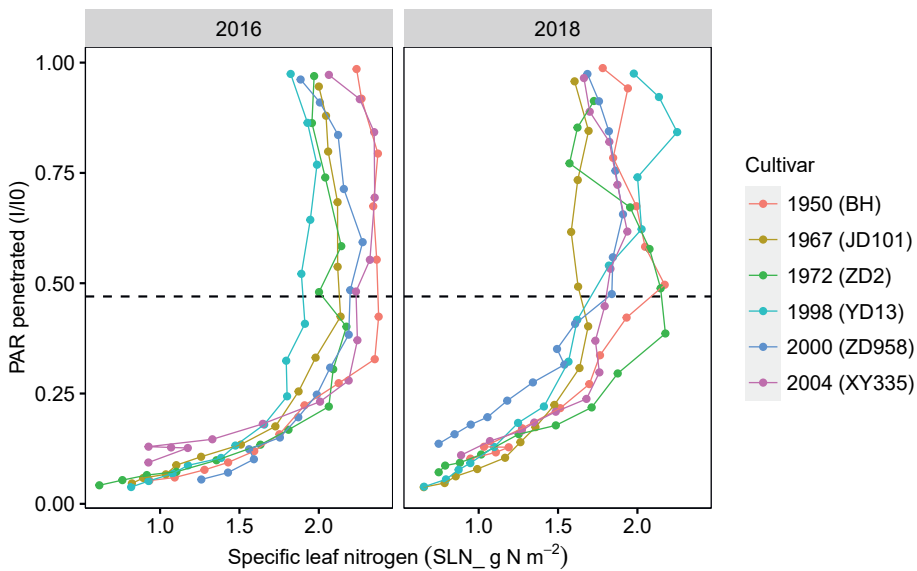


Fig. 4.7. The relationship between light (PAR penetrated, I/I_0) and specific leaf nitrogen (SLN, g N m^{-2} leaf) within the canopy among different cultivars in 2016 and 2018. The black dashed lines represent the average location of the ear leaf within maize canopies.

4.4 Discussion

4.1.1 Grain yield improved linear with year of release

The genetic gain in grain yield (i.e., the slope of the regression between yield and year of release) was about $109 \text{ kg ha}^{-1} \text{ year}^{-1}$ or $1.63\% \text{ YOR}^{-1}$ among maize cultivars released in China from 1950 to 2004 (Fig. 4.1), being similar to reported increase in Europe, i.e., about $100 \text{ kg ha}^{-1} \text{ year}^{-1}$ among 60 maize cultivars, ranging from 1950 to 2015 (Perez et al., 2019; Welcker et al., 2022). Over 50 years of genetic improvement in China represented by the 10 cultivars evaluated, grain yield increased at a rate of $86 \text{ kg ha}^{-1} \text{ year}^{-1}$ from 1950s to 2000s (Ma et al., 2014), which is lower than that in our study. This difference may be due to the optimal planting density, as modern cultivars yield better at higher planting densities than older cultivars, which tend to yield better at lower densities typical of the cultivation practices during their years of release (Liu et al., 2021; Ma et al., 2014; Tollenaar and Lee, 2002). Since the cultivars were cultivated in the same and relatively low plant density in this study, we have likely underestimated the yield gains with year of release as modern varieties tend to do particularly well at high density (Hammer et al., 2009; Ma et al., 2014).

4.4.2 Genetic progress in canopy architecture

Genetic improvements have contributed to increased maize yield, and that mainly results from an increase in plant density tolerance, and from changes in maize morphological traits in response to breeding (G. Liu et al., 2022; Mueller et al., 2019; Perez et al., 2019). The breeding of maize cultivars primarily focused on the leaf area index (LAI) and leaf angle, which influence the light distribution in the canopy (Huang et al., 2017; Liu et al., 2017; Monsi and Saeki, 2005). The indirect crop trait selected for by breeders was the vertical distribution of leaf area that appreciably contributed to light penetration in the canopy, and the largest fraction of leaf area being located at the middle of the canopy, near the ear leaf (Fan et al., 2020; Li et al., 2022). This is in agreement with the possible yield enhancement through optimizing the utilization of light in the different canopy layers, especially a better interception by the middle leaves that mainly supply carbon to the ear (Chen et al., 2016; Li et al., 2019; Perez et al., 2019). Compared with older cultivars, newer cultivars have higher LA per plant (Ci et al., 2012; Ma et al., 2014). The maximum LAI (LAI_{max}) increased by 65% from the oldest cultivar BH to newest cultivar XY335 in this study (Fig. 4.2A). Moreover, modern cultivars tend to be “stay-green” with faster leaf growth and longer lifespan, ultimately contributing to higher yield (Bonelli and Andrade, 2020; Li et al., 2022; Liu et al., 2023).

The leaf angle above the ear leaf decreased significantly by $0.31^{\circ} \text{ year}^{-1}$ from 1950 to 2004, and the leaf angle below the ear also decreased by $0.09^{\circ} \text{ year}^{-1}$ (Fig. 4.2B). Previous studies have shown that high-yielding cultivars should have a more compact plant type with steeper leaves, and that was consistent with our results (G. Liu et al., 2022; Ma et al., 2014). As the plant type changed to a more erect stature for modern cultivars over the time, light distribution in the canopy improved since increased leaf erectness allows lower leaves to receive more light (Ouyang et al., 2021; Perez et al., 2019). This trait is particularly beneficial to improve tolerance of modern cultivars under higher planting density (Maddonni et al., 2001; Sher et al., 2017). The vertical light distribution throughout the canopy was relatively more uniform in modern cultivars (Fig. 4.3) as indicated by a decreased extinction coefficient (K_L) from 0.61 in 1950 to 0.39 in 2004 at a rate of $0.67\% \text{ year}^{-1}$ (Fig. 4.4). Consistently, the variation in K_L across breeding decreased at a rate of $1.1\% \text{ year}^{-1}$ since 1989 in Argentina regardless of plant density (Lacasa et al., 2022). Moreover, there is a positive correlation between K_L and the average leaf angle (Ouyang et al., 2021). Erect architectures in modern cultivars prevent upper leaves from receiving supra-saturating light and allow lower leaves in the canopy to receive more light, therefore, resulting in a more even vertical light distribution and lower K_L (Li et al., 2019; Ouyang et al., 2021). Furthermore, modern hybrids presented improved light penetration and distribution in the canopy, thereby increasing the amount of light absorbed in the canopy layer where the ears are located, hence, maize cultivars with more upright leaves were selected through the yield-driven breeding process (Ci et al., 2011; Lacasa et al., 2022; Liu et al., 2021).

4.4.3 The vertical distributions of light and nitrogen

Total canopy nitrogen content (canopy N) increased with year of release (YOR) (Fig. 4.5A), mainly due to the modern cultivars have higher nitrogen uptake from the soil (Gallais et al., 2007; Yan et al., 2014). However, the canopy-averaged nitrogen per leaf area (canopy SLN) tended to be lower for modern cultivars compared with older cultivars (Fig. 4.5B), as the increase in LAI is proportionally greater than the increase in canopy N (Fig. 4.2A). However, studies using optimality models, predict that canopy photosynthesis and associated nitrogen-use efficiency would increase if crop stands would have relatively lower LAIs for a given canopy N, and hence higher average SLN (canopy N/LAI), and they argued that crop selection should be for higher SLN values (Anten, 2002; Anten et al., 1995a).

The development of vertical nitrogen distribution in the canopy is a strategy to optimize nitrogen utilization with respect to canopy carbon assimilation (Bertheloot

et al., 2008; Fan et al., 2022). The non-uniformity of N within the canopy has been reported for many crops (Anten et al., 1995b; Archontoulis et al., 2011; Ouyang et al., 2021). However, the largest specific leaf nitrogen (SLN, g N m^{-2}) in maize canopies are in the upper-middle leaves (Fig. 4.6), rather than the top leaves as it is in most other herbaceous plants (Anten et al., 1995b; Archontoulis et al., 2011; Hikosaka, 2016). Another study shows the SLN revealed a good fit with a quadratic function rather than an exponential function in maize, also indicating higher N was distributed in the upper canopy (Li et al., 2019). The middle-upper canopy layer had larger leaf photosynthetic capacity and larger leaf area, which play a more important role in deciding the grain yield (Li et al., 2019; N. Liu et al., 2022). Hence, the optimized canopy architecture by breeding selection improved light penetration and distribution in the canopy, this potentially increased the carbon availability to ears, via the amount of light absorbed by the intermediate canopy layer (Li et al., 2022; N. Liu et al., 2022; Ouyang et al., 2021). Although selection for increased yield in maize has improved nitrogen-use efficiency over time (Mueller et al., 2019; Noor, 2017), it does not appear to be associated with a consistent change in N distribution (Fig. 4.7).

Optimization theory studies indicated that canopy carbon gain could be maximized when the vertical nitrogen distribution is matching the light gradient in the canopy (Anten et al., 1995b; Field, 1983; Hirose and Werger, 1987). However, the vertical distribution of nitrogen is more uniform than of light, and far from optimized for the actual canopy (Hirose and Werger, 1987; Ouyang et al., 2021). Previous studies prove there is a positive correlation between the extinction coefficient for light (K_L) and nitrogen (K_N) both across species (Anten et al., 1995b) and e.g. within rice, and their ratio ($K_N:K_L$) as main determinant of canopy photosynthesis and daily growth rate (Ouyang et al., 2021; Zhou et al., 2023). However, the changes in the relationship between nitrogen and light have been related to LAI and nitrogen status, and the gradients of both light and nitrogen are strongly affected by genotypic variation (Zhou et al., 2023). For modern cultivars, more light penetrated into the lower layers of the canopy, as reflected by lower K_L values compared with older cultivars (Fig. 4.4) with as noted a potentially more favorable light distribution for canopy photosynthesis (G. Liu et al., 2022; Ma et al., 2014). However, the light and nitrogen distribution patterns were quite different within the maize canopy (Fig. 4.3 and 4.6). The inconsistency between light and nitrogen distribution was also found within wheat canopies during grain filling stage (Bertheloot et al., 2008). Furthermore, the relationships between light and nitrogen distribution within the canopy differed among cultivars. Specifically, the oldest cultivar BH and modern cultivar XY335 tended to have higher leaf SLN at the same relative light intensity in the canopy, compared with other cultivars.

Moreover, the cultivar ZD2 released in 1972 tended to have higher SLN than other cultivars in the 2018, especially in the lower canopy (Fig. 4.7). The results showed the relationships between light and nitrogen distribution within the canopy was not significantly related to YOR, indicating the coordination between light and nitrogen in maize canopy was not a consequence of maize breeding history from the 1950 to 2004 in Chinese cultivars.

Apparently, the predictions from optimality models regarding the balance between canopy N and LAI and the vertical SLN distribution seem inconsistent with our findings. This suggests that optimization of nitrogen distribution and associated coordination with light distribution may not be critical for yield improvement in maize. However, as far as we know this is the first study that explores the association between breeding history and canopy nitrogen traits and more work is needed in this regard including for instance different nitrogen treatments and crop species other than maize.

4.5 Conclusion

The rate of yield gain increase with YOR was $109 \text{ kg ha}^{-1} \text{ year}^{-1}$ or $1.63\% \text{ YOR}^{-1}$ for the cultivars released from 1950 to 2004 in China. By selecting for yield, breeders indirectly selected maize cultivars with higher leaf area index (LAI) and upright architecture, which were responsible for the variation in light profile among cultivars over generations selection. Conversely, the coordination of light and nitrogen distributions showed no signatures of selection. Together, the current higher yields apparently rely on canopy architecture rather than the coordinated light and N distribution, during the breeding history from 1950 to 2004 in China.

Acknowledgments

This study was supported by grants from the China Agriculture Research System of MOF and MARA (CARS-02) and the Agricultural Science and Technology Innovation Program (CAAS-ZDRW202004). P.F held a scholarship from China Scholarship Council (CSC202003250113). SL and RX conceived the project and provided funding; PF and BM designed the research; PF and YL performed the experiments; PF analysed the data and wrote the draft; NA and JBE edited and revised the manuscript.

References

- Anten, N. P. R. (2002). Evolutionarily stable leaf area production in plant populations. *Journal of Theoretical Biology*, 217(1), 15-32.
- Anten, N. P. R., Schieving, F., Medina, E., Werger, M. J. A., & Schuffelen, P. (1995). Optimal leaf area indices in C3 and C4 mono-and dicotyledonous species at low and high nitrogen availability. *Physiologia plantarum*, 95(4), 541-550.
- Anten, N. P. R., Schieving, F., & Werger, M. J. A. (1995). Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C 3 and C 4 mono-and dicotyledonous species. *Oecologia*, 101, 504-513.
- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N. G., & Struik, P. C. (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Bertheloot, J., Martre, P., & Andrieu, B. (2008). Dynamics of light and nitrogen distribution during grain filling within wheat canopy. *Plant physiology*, 148(3), 1707-1720.
- Bonelli, L. E., & Andrade, F. H. (2020). Maize radiation use-efficiency response to optimally distributed foliar-nitrogen-content depends on canopy leaf-area index. *Field Crops Research*, 247, 107557.
- Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science*, 55(1), 11-33.
- Cagnola, J. I., Parco, M., Rotili, D. H., Ploschuk, E. L., Curin, F., Amas, J. I., ... & Casal, J. J. (2021). Artificial selection for grain yield has increased net CO₂ exchange of the ear leaf in maize crops. *Journal of Experimental Botany*, 72(10), 3902-3913.
- Chen, W., Wang, L., Siddique, K. H., Deng, X., & Chen, Y. (2019). Nitrogen vertical distribution differed in foliar and nonfoliar organs of dryland wheat during grain filling. *Agronomy Journal*, 111(3), 1218-1228.
- Chen, Y., Wu, D., Mu, X., Xiao, C., Chen, F., Yuan, L., & Mi, G. (2016). Vertical Distribution of Photosynthetic Nitrogen Use Efficiency and Its Response to Nitrogen in Field-Grown Maize. *Crop Science*, 56(1), 397-407.
- Ci, X., Li, M., Liang, X., Xie, Z., Zhang, D., Li, X., ... & Zhang, S. (2011). Genetic contribution to advanced yield for maize hybrids released from 1970 to 2000 in China. *Crop science*, 51(1), 13-20.
- Ci, X., Li, M., Xu, J., Lu, Z., Bai, P., Ru, G., ... & Dong, S. (2012). Trends of grain yield and plant traits in Chinese maize cultivars from the 1950s to the 2000s. *Euphytica*, 185, 395-406.
- Ciganda, V., Gitelson, A., & Schepers, J. (2008). Vertical profile and temporal variation of chlorophyll in maize canopy: Quantitative “crop vigor” indicator by means of reflectance-based techniques. *Agronomy Journal*, 100(5), 1409-1417.
- Dreccer, M. F., Van Oijen, M., Schapendonk, A. H. C. M., Pot, C. S., & Rabbinge, R. (2000). Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. *Annals of Botany*, 86(4), 821-831.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy*, 86, 83-145.
- Evans, J. R. (1993). Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. *Functional Plant Biology*, 20(1), 55-67.
- Fan, P., Ming, B., Anten, N. P., Evers, J. B., Li, Y., Li, S., & Xie, R. (2022). Plastic response of leaf traits to N deficiency in field-grown maize. *AoB Plants*, 14(6), plac053.

- Fan, P., Ming, B., Evers, J. B., Li, Y., Li, S., Xie, R., & Anten, N. P. (2023). Nitrogen availability determines the vertical patterns of accumulation, partitioning, and reallocation of dry matter and nitrogen in maize. *Field Crops Research*, 297, 108927.
- Fan, P. P., Li, Y. Y., Evers, J. B., Ming, B., Wang, C. X., Li, S. K., & Xie, R. Z. (2020). A new empirical equation to describe the vertical leaf distribution profile of maize. *The Journal of Agricultural Science*, 158(8-9), 676-686.
- Field, C. (1983). Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia*, 56, 341-347.
- Gallais, A., Coque, M., Le Gouis, J., Prioul, J. L., Hirel, B., & Quillere, I. (2007). Estimating the Proportion of Nitrogen Remobilization and of Postsilking Nitrogen Uptake Allocated to Maize Kernels by Nitrogen-15 Labeling. *Crop science*, 47(2), 685-691.
- Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., ... & Cooper, M. (2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt?. *Crop Science*, 49(1), 299-312.
- Hikosaka, K. (2016). Optimality of nitrogen distribution among leaves in plant canopies. *Journal of Plant Research*, 129, 299-311.
- Hirose, T. (2005). Development of the Monsi-Saeki theory on canopy structure and function. *Annals of botany*, 95(3), 483-494.
- Hirose, T., & Werger, M. J. A. (1987). Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia*, 72, 520-526.
- Huang, S., Gao, Y., Li, Y., Xu, L., Tao, H., & Wang, P. (2017). Influence of plant architecture on maize physiology and yield in the Heilonggang River valley. *The Crop Journal*, 5(1), 52-62.
- Lacasa, J., Ciampitti, I. A., Amas, J. I., Curín, F., Luque, S. F., & Otegui, M. E. (2022). Breeding effects on canopy light attenuation in maize: a retrospective and prospective analysis. *Journal of Experimental Botany*, 73(5), 1301-1311.
- Li, Y., Ming, B., Fan, P., Liu, Y., Wang, K., Hou, P., ... & Xie, R. (2022). Quantifying contributions of leaf area and longevity to leaf area duration under increased planting density and nitrogen input regimens during maize yield improvement. *Field Crops Research*, 283, 108551.
- Li, Y., Song, H., Zhou, L., Xu, Z., & Zhou, G. (2019). Vertical distributions of chlorophyll and nitrogen and their associations with photosynthesis under drought and rewatering regimes in a maize field. *Agricultural and Forest Meteorology*, 272, 40-54.
- Liu, G., Hou, P., Xie, R., Ming, B., Wang, K., Xu, W., ... & Li, S. (2017). Canopy characteristics of high-yield maize with yield potential of 22.5 Mg ha⁻¹. *Field Crops Research*, 213, 221-230.
- Liu, G., Yang, H., Xie, R., Yang, Y., Liu, W., Guo, X., ... & Li, S. (2021). Genetic gains in maize yield and related traits for high-yielding cultivars released during 1980s to 2010s in China. *Field Crops Research*, 270, 108223.
- Liu, G., Yang, Y., Liu, W., Guo, X., Xie, R., Ming, B., ... & Li, S. (2022). Optimized canopy structure improves maize grain yield and resource use efficiency. *Food and Energy Security*, 11(2), e375.
- Liu, N., Li, L., Li, H., Liu, Z., Lu, Y., & Shao, L. (2023). Selecting maize cultivars to regulate canopy structure and light interception for high yield. *Agronomy Journal*, 115(2), 770-780.
- Liu, T., Pan, Y., Lu, Z., Ren, T., & Lu, J. (2020). Canopy light and nitrogen distribution are closely related to nitrogen allocation within leaves in *Brassica napus* L. *Field Crops Research*, 258, 107958.

- Liu, Z., Gao, J., Zhao, S., Sha, Y., Huang, Y., Hao, Z., ... & Mi, G. (2023). Nitrogen responsiveness of leaf growth, radiation use efficiency and grain yield of maize (*Zea mays* L.) in Northeast China. *Field Crops Research*, 291, 108806.
- Ma, D. L., Xie, R. Z., Niu, X. K., Li, S. K., Long, H. L., & Liu, Y. E. (2014). Changes in the morphological traits of maize genotypes in China between the 1950s and 2000s. *European Journal of Agronomy*, 58, 1-10.
- Maddoni, G. A., Otegui, M. E., & Cirilo, A. G. (2001). Plant population density, row spacing and hybrid effects on maize canopy architecture and light attenuation. *Field Crops Research*, 71(3), 183-193.
- Monsi, M., & Saeki, T. (2005). On the factor light in plant communities and its importance for matter production. *Annals of botany*, 95(3), 549-567.
- Mueller, S. M., Messina, C. D., & Vyn, T. J. (2019). Simultaneous gains in grain yield and nitrogen efficiency over 70 years of maize genetic improvement. *Scientific Reports*, 9(1), 9095.
- Noor, M. A. (2017). Nitrogen management and regulation for optimum NUE in maize—A mini review. *Cogent Food & Agriculture*, 3(1), 1348214.
- Ouyang, W., Yin, X., Yang, J., & Struik, P. C. (2021). Roles of canopy architecture and nitrogen distribution in the better performance of an aerobic than a lowland rice cultivar under water deficit. *Field Crops Research*, 271, 108257.
- Perez, R. P., Fournier, C., Cabrera-Bosquet, L., Artzet, S., Pradal, C., Bricchet, N., ... & Tardieu, F. (2019). Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection. *Plant, cell & environment*, 42(7), 2105-2119.
- Qin, X., Feng, F., Li, Y., Xu, S., Siddique, K. H., & Liao, Y. (2016). Maize yield improvements in China: past trends and future directions. *Plant Breeding*, 135(2), 166-176.
- Sher, A., Khan, A., Cai, L. J., Ahmad, M. I., Asharf, U., & Jamoro, S. A. (2017). Response of maize grown under high plant density; performance, issues and management—a critical review. *Adv. Crop Sci. Technol*, 5(3), 1-8.
- Stewart, D. W., Costa, C., Dwyer, L. M., Smith, D. L., Hamilton, R. I., & Ma, B. L. (2003). Canopy structure, light interception, and photosynthesis in maize. *Agronomy Journal*, 95(6), 1465-1474.
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field crops research*, 75(2-3), 161-169.
- Valentinuz, O. R., & Tollenaar, M. (2006). Effect of genotype, nitrogen, plant density, and row spacing on the area-per-leaf profile in maize. *Agronomy Journal*, 98(1), 94-99.
- Welcker, C., Spencer, N. A., Turc, O., Granato, I., Chapuis, R., Madur, D., ... & Tardieu, F. (2022). Physiological adaptive traits are a potential allele reservoir for maize genetic progress under challenging conditions. *Nature Communications*, 13(1), 3225.
- Yan, P., Yue, S., Qiu, M., Chen, X., Cui, Z., & Chen, F. (2014). Using maize hybrids and in-season nitrogen management to improve grain yield and grain nitrogen concentrations. *Field Crops Research*, 166, 38-45.
- Yin, X., & Struik, P. C. (2015). Constraints to the potential efficiency of converting solar radiation into phytoenergy in annual crops: from leaf biochemistry to canopy physiology and crop ecology. *Journal of Experimental Botany*, 66(21), 6535-6549.
- Zhou, Z., Struik, P. C., Gu, J., van der Putten, P. E., Wang, Z., Yin, X., & Yang, J. (2023). Leaf-colour modification affects canopy photosynthesis, dry-matter accumulation and yield traits in rice. *Field Crops Research*, 290, 108746.

Supplementary data

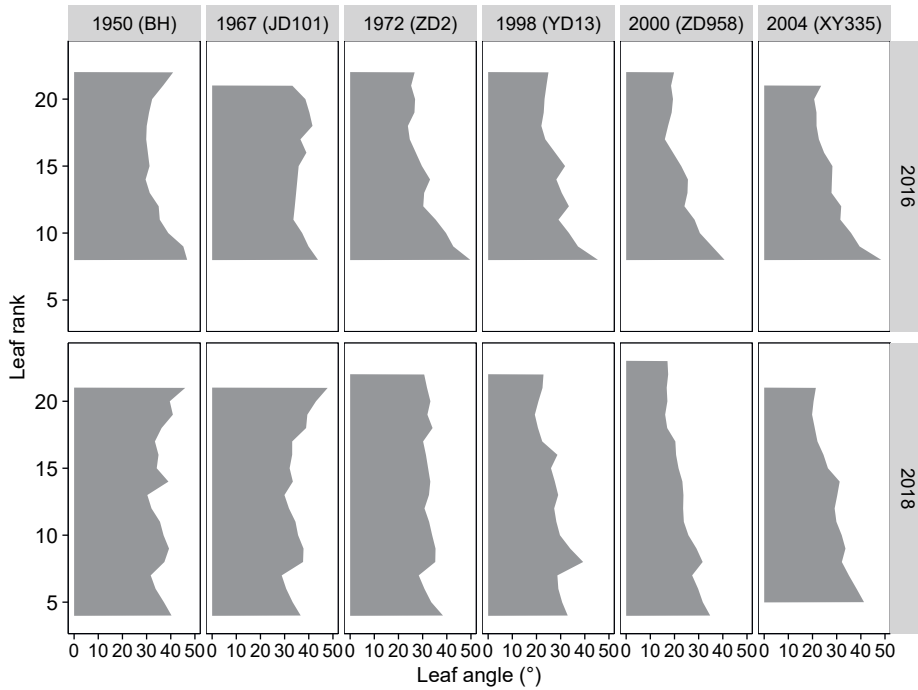


Fig. S4.1. Changes of leaf declination angle at individual leaf ranks within the maize canopy among different cultivars from old to new in both experimental years 2016 and 2018.



CHAPTER 5

A new empirical equation to describe the vertical leaf distribution profile of maize

Panpan Fan^{1,2}, Yaoyao Li¹, Jochem B Evers², Bo Ming¹,
Chunxia Wang¹, Shaokun Li¹, Ruizhi Xie¹

¹Institute of Crop Sciences, Chinese Academy of Agriculture Sciences,
A Key Laboratory of Crop Physiology and Ecology, Ministry of
Agriculture, Beijing 10008, China

²Centre for Crop System Analysis, Wageningen University & Research,
Wageningen 430, 6700AK, The Netherlands

This chapter has been published in *The Journal of Agricultural Science*,
158(8-9), 676-686.

Abstract

The characteristic traits of maize (*Zea mays* L.) leaves affect light interception and photosynthesis. Measurement or estimation of individual leaf area has been described using discontinuous equations or bell-shaped functions. However, new maize hybrids show different canopy architecture, such as leaf angle in modern maize which is more upright and ear leaf and adjacent leaves which are longer than older hybrids. The original equations and their parameters, which have been used for older maize hybrids and grown at low plant densities, will not accurately represent modern hybrids. Therefore, the aim of this paper was to develop a new empirical equation that captures vertical leaf distribution. To characterize the vertical leaf profile, we conducted a field experiment in Jilin province, Northeast China from 2015 to 2018. Our new equation for the vertical distribution of leaf profile describes leaf length, width or leaf area as a function of leaf rank, using parameters for the maximum value for leaf length, width or area, the leaf rank at which the maximum value is obtained, and the width of the curve. It thus involves one parameter less than the previously used equations. By analyzing the characteristics of this new equation, we identified four key leaf ranks (4, 8, 14 and 20) for which leaf parameter values need to be quantified in order to have a good estimation of leaf length, width and area. Together, the method of leaf area estimation proposed here adds versatility for use in modern maize hybrids and simplifies the field measurements by using the four key leaf ranks to estimate vertical leaf distribution in maize canopy instead of all leaf ranks.

Keywords: Leaf length, leaf width, leaf area, leaf rank, new empirical equation

5.1 Introduction

Maize shows great diversity in canopy architecture (Maddonni et al., 2001; Stewart et al., 2003), the arrangements of leaves in space and time affects light distribution and the way in which plants make use of the intercepted light for photosynthesis (Ellsworth and Reich, 1993; Wang et al., 2007). Leaf architecture traits that include leaf size and leaf orientation. Specifically, the number of leaves, leaf shape, leaf area, leaf angle and leaf azimuth in maize canopy (Perez et al., 2019). The vertical distribution of leaf area is an important factor to influence light capture in canopy, which is an essential part of the development of plant and crop models (Fournier and Andrieu, 2000; Vos et al., 2010).

Several approaches have been used to describe the distribution of leaf area of a maize plant. A direct method is to measure individual leaf area by an electronic planimeter (LI-COR, Lincoln, USA) or by calculating leaf area based on leaf length and maximum leaf width (Stewart and Dwyer, 1999; Zhu et al., 2009). However, these direct methods are usually time-consuming, labor-intensive, and may cause canopy damage. Indirect methods are gap-fraction estimation, remote sensing or three-dimensional (3D) point clouds of plants, but they are less precise because indirect methods always use the top of canopy foliar samples to represent the whole plant architecture (Jiang et al., 2018; Tang et al., 2014). Non-destructive and mathematical approaches of modeling present a potential alternative for describing the vertical profile of leaf size, that may avoid these issues.

Previous studies used discontinuous equations to describe the relationship between leaf rank and leaf area, but the results were unsatisfactory because the area of leaves above the 12th leaf is often underestimated (Carberry et al., 1993). Another approach used a continuous equation to predict leaf area by using a skewed, bell-shaped function and its deformation equations (Stewart and Dwyer, 1999; Zhen et al., 2018). The bell-shaped function is more generally applicable because it needs less parameters than the discontinuous equations and gives good predictions of leaf area for a large of hybrids with modified parameters (Dwyer and Stewart, 1986; Valentinuz and Tollenaar, 2006). The equation of the original bell-shaped function is

$$y = y_0 e^{[a(x-x_0)^2 + b(x-x_0)^3]} \quad (1)$$

where y is the fully expanded leaf area of each individual leaf, x is the leaf rank (leaves are numbered from the bottom to the top), y_0 is the maximum individual leaf area, x_0 is the leaf rank that corresponds to the maximum of leaf area, and a and b are

dimensionless empirical constants (Dwyer and Stewart, 1986). Studies focused on parameters of the original bell-shaped function [Eq.(1)]. Specifically, y_0 and x_0 could be simply estimated from total leaf number (Muchow and Carberry, 1989). In addition, nonlinear relationships were also found to exist between total leaf number and the parameters a and b [Eq.(1)] (Keating and Wafula, 1992). However, the regression functions are developed by using plants varying in total leaf number from 12 to 17 with the maize hybrids released before 1990 (Zhen et al., 2018). The average total leaf number of modern high yielding maize hybrids is beyond the scope of the original leaf area model, and is strongly affected by genetic improvement and environmental conditions (Liu et al., 2013; Tsukaya, 2005). Although the coefficients of determination (R^2) were too low to justify the use of total leaf number to estimate the function parameters (Valentinuz and Tollenaar, 2006; Zhen et al., 2018), the original bell-shaped function [Eq.(1)] is still a robust way to predict the fully-expanded leaf area of maize with modified parameters and fitted empirical constants (Zhen et al., 2018).

The common equation of estimating maize fully-expanded leaf area was calculated as length \times maximum width \times coefficient (Bosi and Struikl, 2000; Stewart and Dwyer, 1999). This equation is still widely used, although there is a slight modifications of the constant coefficient (Keating and Wafula, 1992; Zhen et al., 2018). Research of leaf length and width could lead to a better understanding of the distribution of leaf area. In addition, these geometrical variables of leaf length and width determine leaf shape, and leaf shape is of critical important in mathematically characterizing the two-dimensional structure of maize. Modelling the morphology of leaves is helpful for designing optimal plant shape and modelling plant growth (Archontoulis et al., 2011; Fournier and Andrieu, 1999; Zhu et al., 2009).

The first objective was to develop and test a new equation that captures vertical leaf area distribution and describe the morphology of the various leaves of maize based on a series of observations and analyses of the length and width of leaves at individual leaf ranks. The second objective was to find the key leaf ranks based on the constitutive equations, which could simplify the field measurement process.

5.2 Materials and Methods

5.2.1 Experimental site and design

The field experiments were conducted during the growing seasons (from 1 April to 30 September) from 2015 to 2018 at the Gongzhuling Experimental Station, Jilin

province, China (43°30N, 124°50E). This area is typical of the rain-fed spring maize regions in Northeast China. We used maize hybrids ZD958 and XY335 in the same field for this 4 years, and this two hybrids were the most widely cultivated in China at the time of the current study. Maize hybrid ZD958 was developed by the Luohe Academy of Agricultural Science of Henan province, and XY335 was developed by the Tieling pioneer limited company. Normally, the total leaf number of ZD958 is 22 and the rank of ear leaf is 16. The total leaf number of XY335 is 21 and the rank of ear leaf is 14 (Huang et al., 2017; Ma et al., 2014). Maize seeds were sown by hand on 1 May 2015, 29 April 2016, 27 April 2017, and 29 April 2018. Prior to sowing, the plots were fertilized with 150 kg N/ha (urea), 42.5 kg/ha P₂O₅ (super phosphate), and 42.5 kg/ha K₂O (potassium sulfate). Row orientation was east-west, row spacing was 65cm, and population density was 6.75 plants/m². Individual plots measured about 45.5 m², comprised 7 rows and 10 m long. The experiments were arranged in a randomized design with three replications. Plots were kept free of weeds, insects and diseases with chemicals based on standard practices. Monthly meteorological data of mean air temperature and total precipitation during the maize growing seasons in the years from 2015 to 2018 at the experimental site are shown in Table 5.1. Total precipitation during 2015 growing season was significantly lower than in other years, particularly during June and July.

Table 5.1. Monthly mean air temperature and total precipitation during the maize growing seasons in the years from 2015 to 2018 at the Gongzhuling experimental station

| Month | Mean air temperature (°C) | | | | Precipitation (mm) | | | |
|-------|---------------------------|------|------|------|--------------------|-------|-------|-------|
| | 2015 | 2016 | 2017 | 2018 | 2015 | 2016 | 2017 | 2018 |
| Apr | 10.6 | 8.5 | 10.2 | 10.4 | 24.0 | 49.7 | 4.06 | 12.5 |
| May | 16.6 | 16.3 | 16.5 | 17.1 | 77.1 | 173.0 | 53.3 | 87.1 |
| Jun | 21.6 | 20.8 | 20.8 | 22.3 | 87.9 | 102.2 | 62.8 | 121.9 |
| Jul | 24.0 | 23.8 | 24.7 | 25.5 | 42.4 | 62.5 | 190.8 | 128.1 |
| Aug | 22.7 | 22.5 | 21.8 | 21.6 | 149.4 | 132.5 | 239.5 | 246.7 |
| Sep | 15.1 | 16.4 | 16.2 | 15.5 | 52.8 | 132.1 | 37.1 | 51.4 |

At the emergence period, ten successive plants were tagged in the central row of each plot. Red paint spray was applied on leaf 4, 8 and 12, which ensured the identification of leaf ranks (Fig. 5.1a). For each tagged plant, the rank of the ear leaf was noted as soon as it was identified. The appearance of the leaf collar at the base of a leaf signaled the termination of individual leaf area growth, which means the leaf was fully expanded (Fournier and Andrieu, 1998). The length and width of each leaf was

manually measured in tagged plants using a non-destructive method by ruler when a leaf was considered fully expanded. The leaf length was defined as the distance from the base of the ligula to the tip of the leaf, and the leaf width was defined as the widest part of the leaf (Fig. 5.1b). The fully expanded leaf area was calculated as leaf length \times leaf width \times 0.75, and length-width ratio was calculated as length divided by width (Keating and Wafula, 1992; Stewart and Dwyer, 1999).

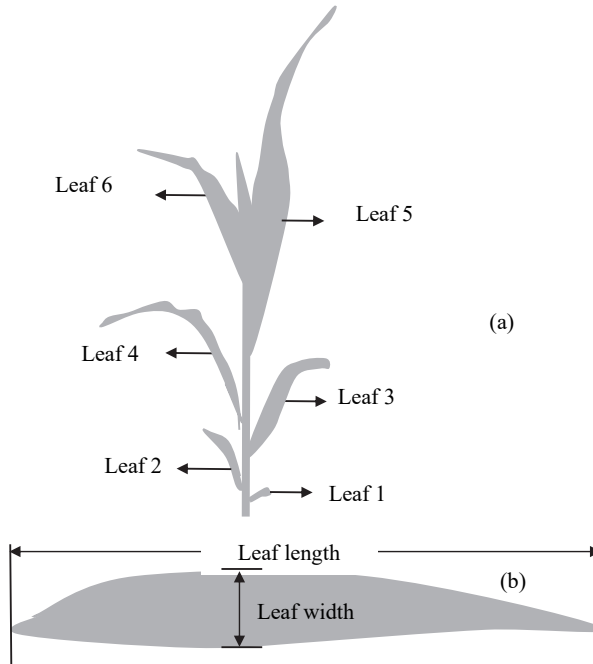


Fig. 5.1. Diagram of leaf rank (a) and leaf morphological traits of leaf length and width (b).

5.2.3 New empirical equation of leaf size distribution

Bell-shaped functions are widely used in mathematical models, for example, in crop growth model (Yin et al., 2003) and plant morphology model (Zhu et al., 2009). The original equation used to describe the vertical distribution of leaf area (Dwyer and Stewart, 1986) is [Eq.(1)]. Factoring out $(x - x_0)^2$ gives

$$y = y_0 e^{(x-x_0)^2[(a+b(x-x_0))]} \quad (2)$$

The parameter b that controls the degree of skewness ranges from -0.007 to 0.001 and varies little with total leaf number (Keating and Wafula, 1992; Zhen et al., 2018). In other words, the polynomial $b(x - x_0)$ is small and has little effect on the output of the equation. Removing the polynomial factor in the exponent results in:

$$y = y_0 e^{a(x-x_0)^2} \quad (3)$$

The parameter a is a dimensionless empirical constant. Then the form of this equation also can be expressed as:

$$y = y_0 e^{a(x-x_0)^2} \quad (4)$$

where y_0 is the maximum value of y , which is reached at leaf rank x_0 . The new empirical equation [Eq.(4)] in this study was thus a simplified form of the original bell-shaped function [Eq.(1)] by omitting the limited effect from parameter b . An advantage of the simplicity of this new equation is that it can be easily evaluated because it only has three parameters, one parameter less than previous bell-shaped function.

The shape of the new empirical equation [Eq.(4)] is similar to the normal distribution which is widely used in plant modelling (Lizaso et al., 2003; Matsunaga et al., 2016), and it has three parameters. The effects of different parameters on the output are shown by varying the parameter values (Fig. 5.2). Parameter y_0 determines the height of the curve and gives the maximum value y_{max} of the curve [Fig. 5.2(a)]. Parameter x_0 is the center of symmetry on the x axis and corresponds to y_{max} [Fig. 5.2(b)]. Parameter a is the width of the curve and low values of a result in a curve that rise sharply and fall sharply [Fig. 5.2(c)].

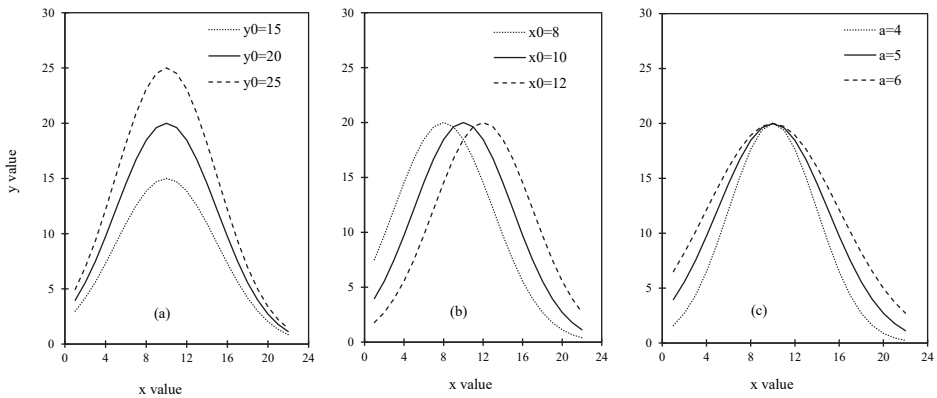


Fig. 5.2. Characteristics of variations in parameters variations of the new empirical equation [Eq. (4)]. Parameters are common to all curves unless stated below: $y_0=20$, $x_0=10$, $a=5$. (a) Effects of varying y_0 : $y_0=15$; 20 ——— ; 25 -----. (b) Effects of varying x_0 : $x_0=8$; 10 ——— ; 12 -----. (c) Effects of varying a : $a=4$; 5 ——— ; 6 ----.

5.2.4 Comparison with existing bell-shaped function

The leaf morphological data (leaf length, width and area) obtained from 2015 to 2017 of all leaf ranks were used to fit the new equation [Eq.(4)] and the original bell-shaped function [Eq.(1)] to determine parameters using the nonlinear least squares algorithm. The independent data from 2018 of all leaf ranks were used to evaluate this two equations.

5.2.5 Derivatives of the new empirical equation

To obtain the key leaf ranks determining the shape of the curve, the first, second and third derivative equations of this new equation were derived (Table 5.2). The key points were obtained when these derivatives were set to zero. The first derivative is the slope of a tangent line through a given point on the curve, and the point where the first derivative is zero is the maximum of the primitive of the new equation [Fig. 5.3(a) and 5.3(b)]. The point where the second derivative is zero is the extreme point of the first derivative, also the inflection point of the primitive new equation [Fig. 5.3(c)]. Finally, the point where the third derivative is zero is the extreme point of the second derivative [Fig. 5.3(d)].

Table 5.2. Derivative equations and key points of the new equation

| | Equations | | Key points |
|-------------------|---|---------------|--|
| New equation | $f(x) = y_0 \times e^{-\frac{(x-x_0)^2}{2a^2}}$ | | |
| First derivative | $f(x)' = \frac{y_0(x_0 - x)}{a^2} \times e^{-\frac{(x-x_0)^2}{2a^2}}$ | $f(x)' = 0$ | $x = x_0$ |
| Second derivative | $f(x)'' = \frac{y_0[(x_0 - x)^2 - a^2]}{a^4} \times e^{-\frac{(x-x_0)^2}{2a^2}}$ | $f(x)'' = 0$ | $x_1 = x_0 - a,$ $x_2 = x_0 + a$ |
| Third derivative | $f(x)''' = \frac{y_0(x_0 - x) \times [(x_0 - x)^2 - 3a^2]}{a^6} \times e^{-\frac{(x-x_0)^2}{2a^2}}$ | $f(x)''' = 0$ | $x_1 = x_0 - \sqrt{3} a,$ $x_2 = x_0$ $x_3 = x_0 + \sqrt{3} a$ |

The first, second and third derivative equations were obtained by taking the derivative of the new equation. The key points were got when the first derivative, the second derivative and the third derivative were equal to zero.

Five key points are obtained in total by the derivatives equations of the new equation, and they are determined by parameters x_0 and parameter a (Table 5.2). When the derivative equations are equal to zero, the number of key point is one, two and three, respectively, but one of the points obtained by the third derivative is the same with the results of first derivative (Fig. 5.3). Therefore, key leaf ranks are selected among these five key points (Table 5.2).

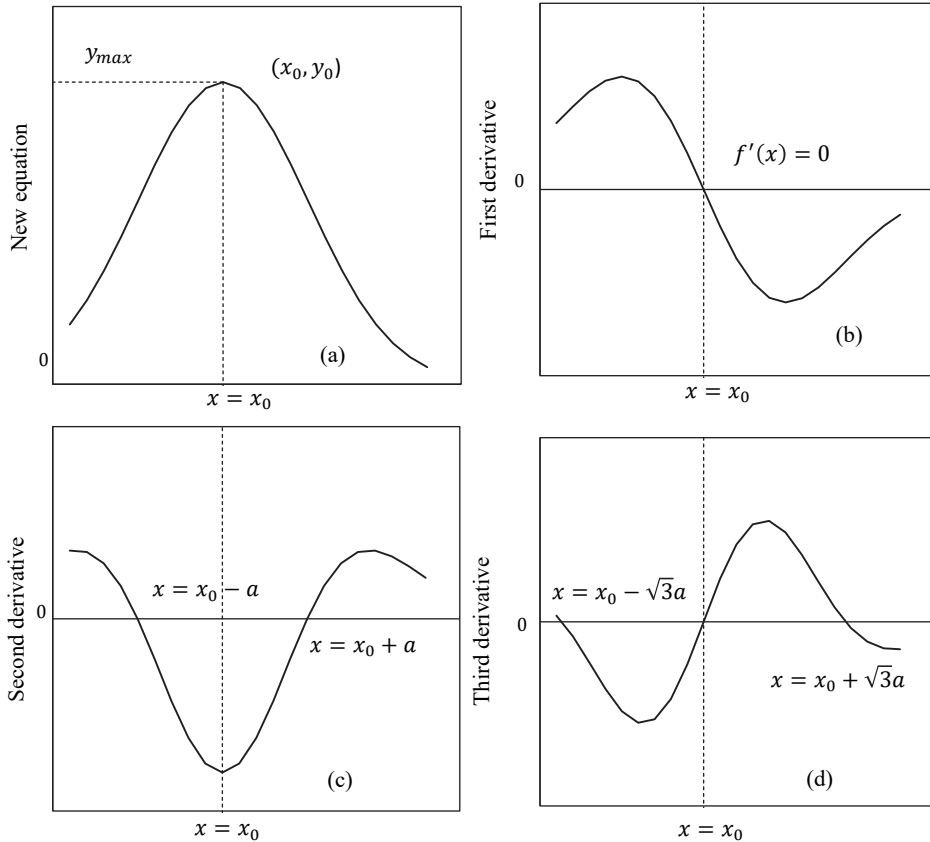


Fig. 5.3. The shape of the new equation (a) and its first (b), second (c) and third (d) derivatives. The key points were obtained when these derivatives were set to zero.

5.2.6 Data analysis

The key leaf ranks were obtained by substituting the value of parameters x_0 and a into the derivative equations of this new equation. The obtained key leaf ranks were rounded value of calculated key points of this new equation because leaf rank is integer number, and the rounded key points were deleted if they beyond the scope of the leaf rank of ZD958 and XY335. Leaf area was calculated by leaf length and

width in this study, then the rounded average values of leaf length and width were assigned as the key leaf ranks of the entire leaf morphological traits distribution. The data from 2015-2017 of the key leaf ranks were used to establish the same equation, and the data from the remaining leaf ranks (except for the key leaf ranks) were used to test the feasibility of the simplified approach.

The root mean square error (RMSE), normalized root mean square error (NRMSE), and coefficient of determination R^2 were used to verify the accuracy of fit between observed values and estimated values (Zhen et al., 2018; Zhu et al., 2009).

5.3 Results

5.3.1 Leaf morphological traits based on individual leaf rank

The relationship between leaf morphology (length, width, and area) and leaf rank can be described quantitatively by the new empirical equation [Eq.(4)] [Fig. 5.4]. The length and width of individual leaves increased with leaf rank up to leaf 14 and then decreased for leaf 15 and above [see Fig. 5.4(a-d)]. The maximum length and width all occurred around leaf 14 both in ZD958 and XY335. The distribution of individual leaf area was similar to that of leaf length and width [Fig. 5.4 (e and f)], whereas the distribution of the length-width ratio differed because the changes from the bottom leaf to the top leaf were small [Fig. 5.4 (g and h)]. The relationships between leaf length, width, area and length-width ratio and leaf rank were the same for maize hybrids ZD958 and XY335 (Fig. 5.4). The results showed a high consistency between estimated and observed values (Fig. 5.5). Overall, the predictability of the new equation [Eq.(4)] in estimating the changes in leaf morphological traits (leaf length, width and area) at different leaf ranks was good.

Each parameter of the new equation [Eq.(4)] can be interpreted in a biologically meaningful way (Fig. 5.2). The parameter y_0 defined as the maximum length, maximum width or maximum area of one plant [Fig. 5.2(a)]. For ZD958, it was 103.2 cm, 11.3cm, or 846.0 cm², respectively, and for of XY335, it was 94.7 cm, 11.9 cm, or 811.6 cm², respectively (Table 5.3). The parameter x_0 defined as the leaf rank corresponding to these maximum leaf morphological traits, and the leaf ranks of both ZD958 and XY335 were all around leaf 14. From the bottom leaf to the top leaf, the changes in leaf length were greater than the changes in leaf width, and the change in leaf area was highest because it had the smallest value for the parameter a [Fig. 5.2(c)].

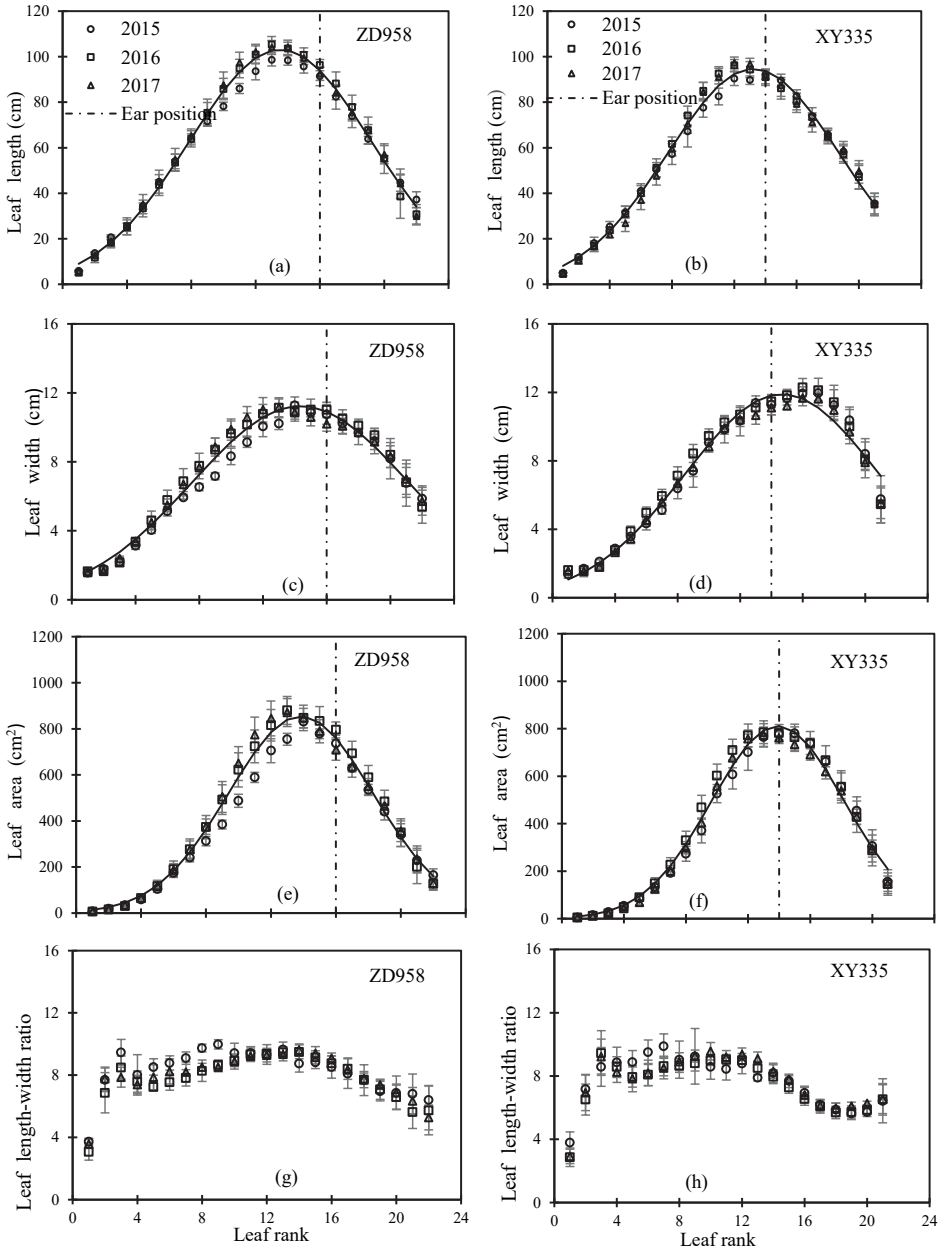


Fig. 5.4. Leaf length (a and b), leaf width (c and d) and leaf area (e and f) of ZD958 and XY335 were simulated by the new empirical equation. The length-width ratio at individual leaf rank of ZD985 (g) and XY335 (h).

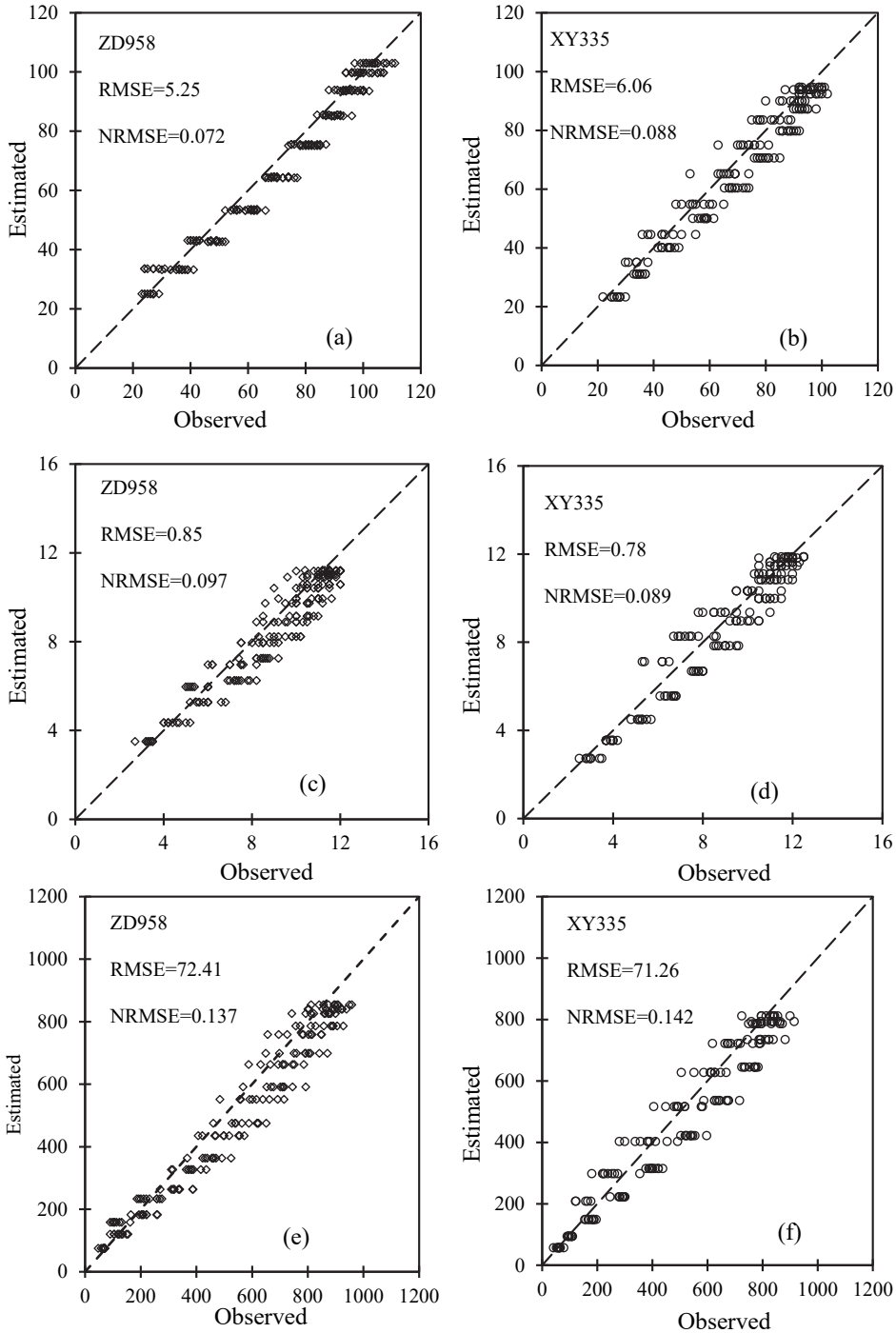


Fig. 5.5. Estimated versus observed leaf length (a and b), leaf width (c and d) and leaf area (e and f) for hybrids ZD958 and XY335 at individual leaf rank. The observed data from 2015 to 2017 were used to fit the new equation [Eq.(4)], and independent data in 2018 were used to test.

5.3.2 Comparison with original bell-shaped function

The new equation [Eq.(4)] was evaluated by comparing with original bell-shaped function [Eq.(1)]. The observed leaf morphological data (leaf length, width and area) from 2015 to 2017 were used to fit the new equation [Eq.(4)] and original bell-shaped function [Eq.(1)] respectively, and the parameter values of ZD958 and XY335 were listed in Table 5.3 and Table 5.4. Eq.(1) and Eq.(4) along with their parameter values were estimated using the independent data from 2018. The R^2 values of both ZD958 and XY335 for leaf length, width and area using Eq.(4) were higher than Eq.(1), especially for leaf width (Table 5.5). The RMSE and NRMSE of ZD958 using Eq.(4) were similar with that using equation Eq.(1), but the RMSE and NRMSE of XY335 using Eq.(1) were much smaller. Therefore, leaf length and leaf area were simulated well by using both Eq.(1) and Eq.(4), but Eq.(4) gave the better estimations for leaf width than Eq.(1) (Table 5.5).

Table 5.3. Values of parameters y_0 , x_0 and a of the new equation

| Hybrids | Morphology traits | y_0 | x_0 | a |
|---------|-------------------|-------|-------|-----|
| ZD958 | Leaf length | 103.2 | 13.5 | 5.7 |
| | Leaf width | 11.3 | 14.3 | 6.8 |
| | Leaf area | 855.0 | 13.8 | 4.5 |
| XY335 | Leaf length | 94.7 | 13.2 | 5.5 |
| | Leaf width | 11.9 | 14.7 | 6.2 |
| | Leaf area | 811.6 | 13.9 | 4.3 |

The observed individual leaf morphology (length, width and leaf area) data from 2015 to 2017 were used to fit the new equation [Eq.(4)] to determine the values of parameters y_0 , x_0 and a of ZD958 and XY335.

Table 5.4. Values of parameters y_0 , x_0 , a and b of original bell-shaped function

| Hybrids | Morphology traits | y_0 | x_0 | a | b |
|---------|-------------------|-------|-------|---------|--------|
| ZD958 | Leaf length | 103.3 | 13.5 | -1.5E-2 | 1.9E-5 |
| | Leaf width | 11.2 | 14.1 | -1.0E-2 | 1.3E-4 |
| | Leaf area | 845.1 | 13.7 | -2.5E-2 | 1.5E-4 |
| XY335 | Leaf length | 94.3 | 13.0 | -1.6E-2 | 2.5E-4 |
| | Leaf width | 12.1 | 15.0 | -1.5E-2 | 2.7E-4 |
| | Leaf area | 810.9 | 13.9 | -2.7E-2 | 1.1E-4 |

The observed individual leaf morphology (length, width and leaf area) data from 2015 to 2017 were used to fit the original bell-shaped function [Eq.(1)] to determine the values of parameters y_0 , x_0 , a and b of ZD958 and XY335.

Table 5.5. Comparison with original bell-shaped function and the new equation

| Hybrids | Morphology traits | RMSE | | NRMSE | | R^2 | |
|---------|-------------------|-------------------------------|--------------|-------------------------------|--------------|-------------------------------|--------------|
| | | Original bell-shaped function | New equation | Original bell-shaped function | New equation | Original bell-shaped function | New equation |
| ZD958 | Leaf length | 5.18 | 5.25 | 0.07 | 0.07 | 0.96 | 0.98 |
| | Leaf width | 0.82 | 0.85 | 0.09 | 0.10 | 0.90 | 0.94 |
| | Leaf area | 71.37 | 72.41 | 0.14 | 0.14 | 0.93 | 0.96 |
| XY335 | Leaf length | 8.13 | 6.06 | 0.12 | 0.09 | 0.94 | 0.93 |
| | Leaf width | 2.85 | 0.78 | 0.32 | 0.09 | 0.72 | 0.92 |
| | Leaf area | 71.71 | 71.27 | 0.04 | 0.14 | 0.93 | 0.93 |

The original bell-shaped function [Eq.(1)] and the new equation [Eq.(4)] along with their parameter values were evaluated using the independent data from 2018.

5.3.3 Key leaf ranks of the new empirical equation for leaf morphological traits

The rounded average values of key leaf ranks of leaf length and width were assigned as the key ranks of the entire leaf morphological traits distribution (Table 5.6). The leaf area was ignored in this study because it was calculated by individual leaf length and width instead of being observed. Finally, the rank of leaf 4, 8, 14 and 20 were defined as the key leaf ranks for the new equation Eq.(4) and the key leaf ranks of ZD958 and XY335 were the same (Table 5.6).

The relationships between leaf morphological traits (i.e. length, width and area) and leaf rank were established based on the four key leaf ranks (leaf 4, 8, 14 and 20) instead of on all leaf ranks (Fig. 5.6). The new equation Eq.(4) were validated by using the data in the remaining leaf ranks except for the four key leaf ranks from the year 2015-2017, and the estimated fit the observed data well (Fig. 5.7). The NRMSE values of ZD958 for leaf length, leaf width and leaf area, were 0.075, 0.092, and 0.137, respectively, and the NRMSE values of XY335 were 0.084, 0.111, and 0.132, respectively (Fig. 5.7). Therefore, the morphological data of the four key ranks (leaf 4, 8, 14 and 20) were used to estimate leaf length, width and area at individual leaf rank were acceptable.

5.4 Discussion

Leaf size together with leaf orientation are important components of leaf architecture, influencing the leaf morphological traits distribution in maize canopy (Fournier and Andrieu, 1999; Huang et al., 2017; Maddonni et al., 2001). However, most previous studies focused only on leaf area of fully-expanded leaves as a function of leaf rank and neglect the more fundamental measurements of leaf length and width (Keating and Wafula, 1992; Muchow and Carberry, 1989; Su et al., 2018). The new empirical equation Eq.(4) describes the distribution of leaf length, width, and area in a quantitative way that remain similar in shape from year to year (Fig. 4). Specifically, the individual leaf length and width of ZD958 and XY335 for the year 2015 were smaller than that for the years 2016 and 2017, mainly because the year 2015 had the least precipitation, particularly during June and July (Table 5.1). Water shortage limited elongation and expansion growth, moisture content and photosynthesis of leaves, which influences on the growth and development of maize plant (Duvick and Cassman, 1999; Zhang et al., 2019).

Table 5.6. Key leaf ranks of the new equation

| Hybrids | Morphology traits | $f(x)' = 0$ | | $f(x)'' = 0$ | | $f(x)''' = 0$ | |
|---------|-------------------|-------------|-----------------|-----------------|-------------------------|---------------|-------------------------|
| | | $x = x_0$ | $x_2 = x_0 - a$ | $x_2 = x_0 + a$ | $x_1 = x_0 - \sqrt{3a}$ | $x_2 = x_0$ | $x_1 = x_0 + \sqrt{3a}$ |
| ZD958 | Leaf length | $x = 13.5$ | $x_1 = 7.8$ | $x_2 = 19.2$ | $x_1 = 3.7$ | $x_2 = 13.5$ | $x_3 = 23.4$ |
| | Leaf width | $x = 14.3$ | $x_1 = 7.5$ | $x_2 = 21.1$ | $x_1 = 2.8$ | $x_2 = 14.3$ | $x_3 = 26.1$ |
| | Leaf area | $x = 13.8$ | $x_1 = 9.4$ | $x_2 = 18.3$ | $x_1 = 6.1$ | $x_2 = 13.8$ | $x_3 = 21.6$ |
| XY335 | Key leaf ranks | $x_1 = 14$ | $x_2 = 8$ | $x_3 = 20$ | $x_4 = 4$ | -- | -- |
| | Leaf length | $x = 13.2$ | $x_1 = 7.7$ | $x_2 = 18.7$ | $x_1 = 3.7$ | $x_2 = 13.2$ | $x_3 = 22.8$ |
| | Leaf width | $x = 14.7$ | $x_1 = 8.5$ | $x_2 = 20.9$ | $x_1 = 3.9$ | $x_2 = 14.7$ | $x_3 = 25.5$ |
| | Leaf area | $x = 13.9$ | $x_1 = 9.6$ | $x_2 = 18.2$ | $x_1 = 6.5$ | $x_2 = 13.9$ | $x_3 = 21.4$ |
| | Key leaf ranks | $x_1 = 14$ | $x_2 = 8$ | $x_3 = 20$ | $x_4 = 4$ | -- | -- |

The data were obtained by substituting the fitted parameter values of Table 5.3 into the first, second and third derivative formulas of key points of Table 5.2, respectively, and they were rounded because they represent leaf ranks (from the bottom to the top). The results beyond the scope of leaf rank were deleted.

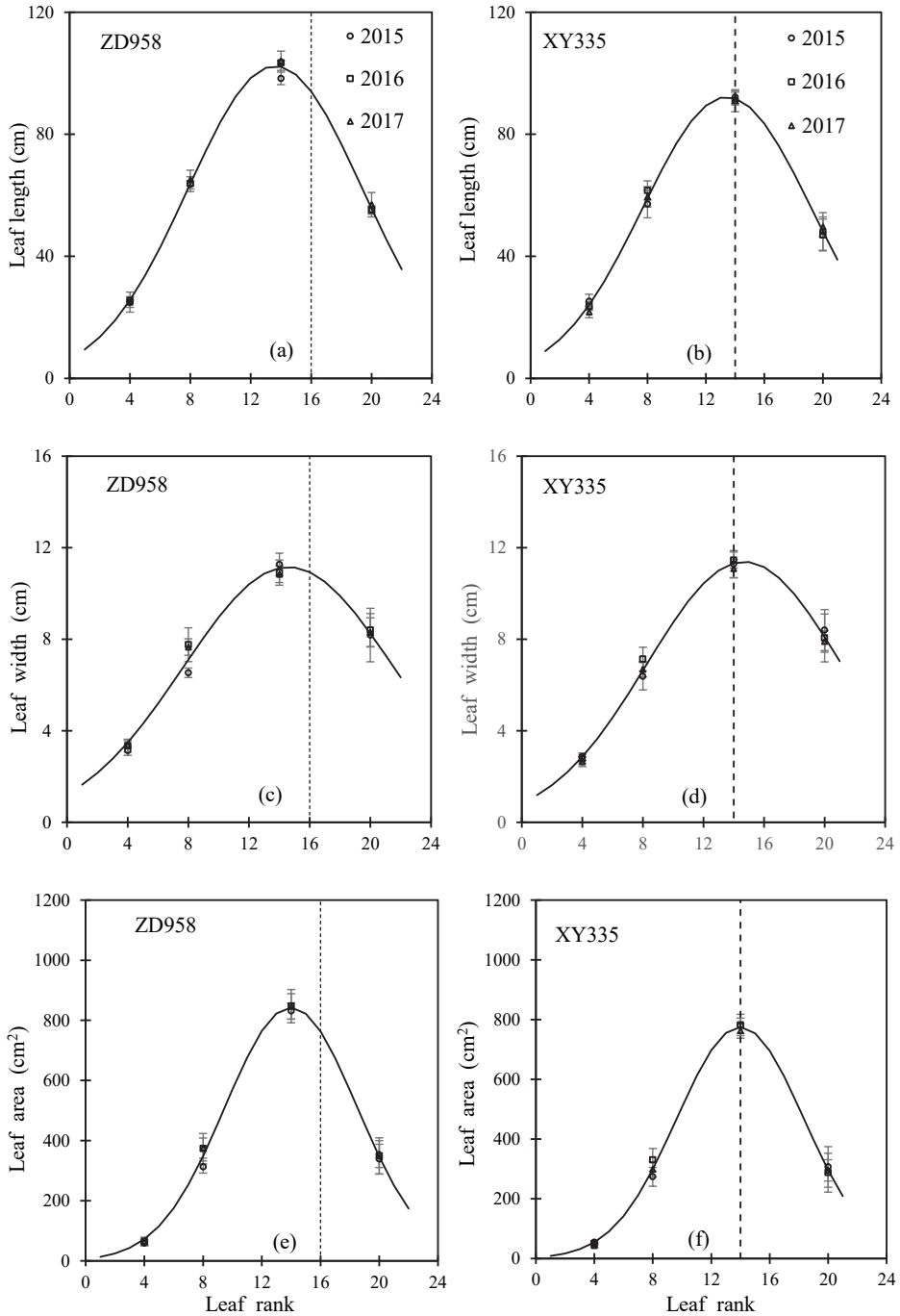


Fig. 5.6. The new empirical equations for leaf length (a and b), leaf width (c and d) and leaf area (e and f) for hybrids ZD958 and XY335. Estimated leaf length, width and area based on four key leaf ranks (leaf 4, 8, 12 and 20) from 2015 to 2017. The vertical dotted line gives the position of the ear leaf and is rounded average over three years.

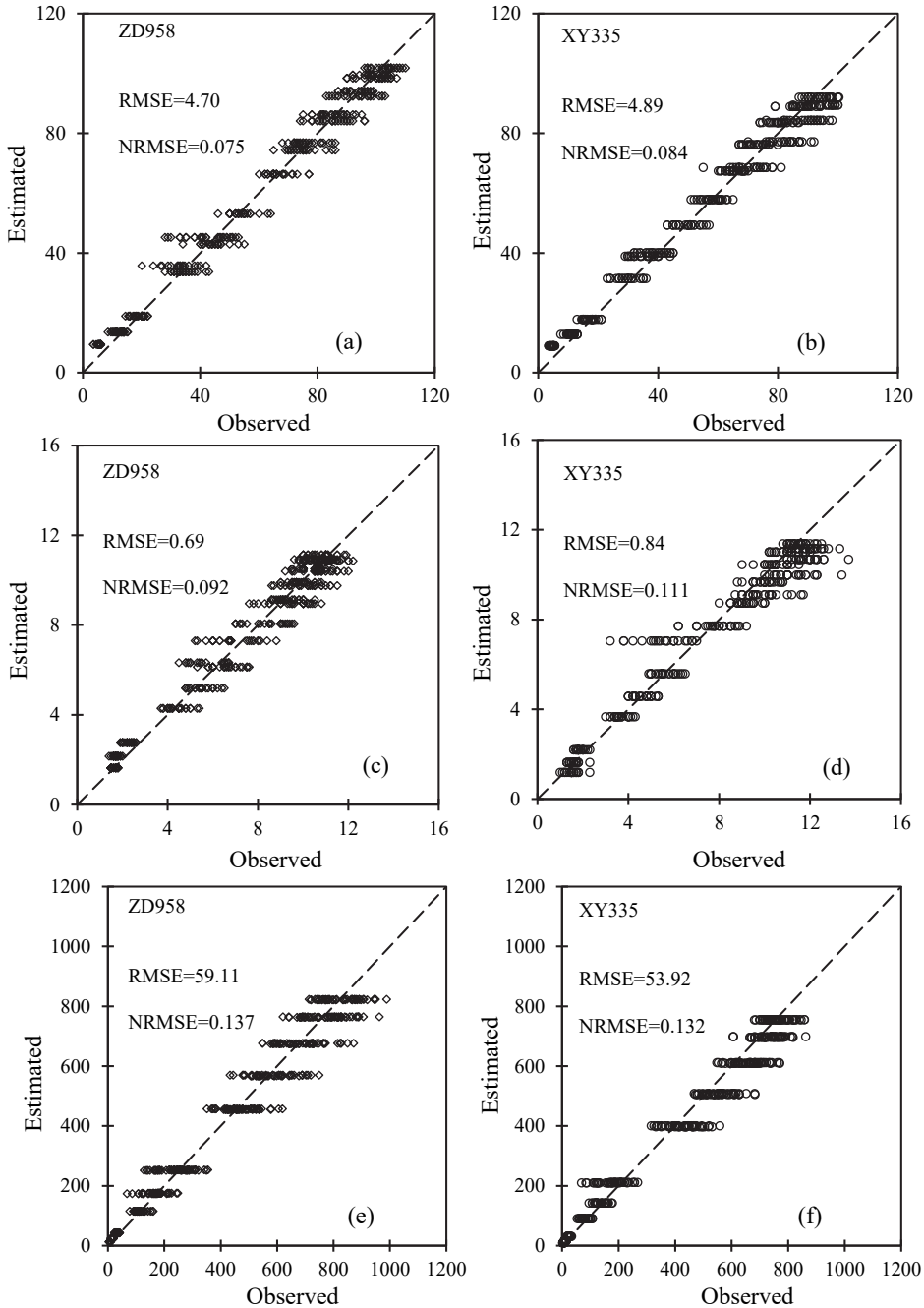


Fig. 5.7. Estimated versus observed leaf length (a and b), leaf width (c and d) and leaf area (e and f) for hybrids ZD958 and XY335 at individual leaf rank. The observed data from key leaf ranks (4, 8, 12 and 20) from 2015 to 2017 were used to fit the new equation [Eq.(4)], and remaining leaf ranks (except key leaf ranks) were used to test.

The morphological changes are observed in maize hybrids released over different generations in American (Duvick and Cassman, 1999), European (Perez et al., 2019) and Chinese (Ma et al., 2014) projects. The number of leaves per plant increased and the leaf area index tend to be higher for modern hybrids than older ones. The leaf orientation became more upright, the length of the middle leaves increase, and modern hybrids have a lower ear and ear leaf position than old hybrids (Ma et al., 2014; Perez et al., 2019). These changes affect the light interception in maize canopy, and also lead to the different architecture of modern maize hybrids, especially for high-yielding modern maize hybrids (Liu et al., 2017). Therefore, the previous mathematical models need to be modified (Zhen et al., 2018).

A great deal of effort has been devoted to measuring and characterizing leaf morphology (Fournier and Andrieu, 1998; Sher et al., 2017). Mathematical approaches with modeling can thus be very convenient and useful for estimating plant growth. The original bell-shaped function [Eq.(1)] is still a robust way to predict the fully-expanded leaf area of maize with modified parameters (Keating and Wafula, 1992; Zhen et al., 2018), and the empirical constants parameters (a and b) were simply estimated from total leaf number (Keating and Wafula, 1992), but the total leaf number of modern hybrids were still beyond the scope of equations calibrated and affected by climate factors, such as thermal time and photoperiod duration of vegetative growth (Muchow and Carberry, 1989). For instance, field experiments are conducted across 34 sites in seven provinces located in China, using maize hybrid ZD958, the total leaf number increased from 18.7 to 23.7 with an average of 21.0 (Liu et al., 2013). The value of parameter b in Eq.(1) were small enough, ranging from 1.9E-5 to 2.7E-4 (Table 5.4) in this study. Previous studies also clarified parameter b had less effects on the output of the function (Keating and Wafula, 1992; Zhen et al., 2018). Then, the Eq.(4) was a simplified form of the Eq.(1) by removing the parameters b [See equation (1)-(4)]. There are some advantages that using the new equation [Eq.(4)] to describe the vertical leaf distribution profile of maize, for example, the prediction accuracy, the number of parameters, the computational complexity and the speed of computer programming. Therefore, the new equation would contribute to more accurate simulation of light capture in relation to phenotype and management, and also lay a foundation for further research into comprehensive simulation systems to produce virtual expressions of leaf growth for maize. Since the morphological characteristics of leaves are complex and difficult to obtain in the field, four key leaf ranks were found by the a method of using derivation equations, because the morphology of any leaf is strongly influenced by the morphology of previously emitted leaves (Fournier and Andrieu, 1999; Stewart and Dwyer, 1993).

This study only focused on the geometrical properties of fully expanded leaf morphology and simplifies field measurements by four key leaf ranks. Further research is needed to test the flexibility of the new equation and the stability of this derivation method with more independent dataset from different hybrids, ecological sites and growing conditions.

5.5 Conclusion

The relationships between leaf length, width, and area and leaf ranks were established by a new empirical equation and the biological meaning of each parameter in this equation is valuable. The use of the new equation reduces the number of parameters required to characterize the leaf morphology, and reduces the workload of computer program. According to the characteristics of this new equation, a method of using derivation formulas to determine key leaf ranks to simplify mathematical equations was proposed in this study. The four key leaf ranks (4th, 8th, 14th, and 20th) were identified using maize hybrids ZD958 and XY335, that could simplify the process of data acquisition in the field.

References

- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N. G., & Struik, P. C. (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Bos, H. J., Tijani-Eniola, H., & Struik, P. C. (2000). Morphological analysis of leaf growth of maize: responses to temperature and light intensity. *NJAS: Wageningen Journal of Life Sciences*, 48(2), 181-198.
- Carberry, P. S., Hammer, G. L., & Muchow, R. C. (1993). Modelling genotypic and environmental control of leaf area dynamics in grain sorghum. III. Senescence and prediction of green leaf area. *Field Crops Research*, 33(3), 329-351.
- Duvick, D. N., & Cassman, K. G. (1999). Post-green revolution trends in yield potential of temperate maize in the North-Central United States. *Crop science*, 39(6), 1622-1630.
- Dwyer, L. M., & Stewart, D. W. (1986). Leaf Area Development in Field-Grown Maize 1. *Agronomy Journal*, 78(2), 334-343.
- Ellsworth, D. S., & Reich, P. B. (1993). Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia*, 96, 169-178.
- Fournier, C., & Andrieu, B. (2000). Dynamics of the elongation of internodes in maize (*Zea mays* L.). Effects of shade treatment on elongation patterns. *Annals of Botany*, 86(6), 1127-1134.
- Fournier, C., & Andrieu, B. (1999). ADEL-maize: an L-system based model for the integration of growth processes from the organ to the canopy. Application to regulation of morphogenesis by light availability. *Agronomie*, 19(3-4), 313-327.
- Fournier, C., & Andrieu, B. (1998). A 3D architectural and process-based model of maize development. *Annals of botany*, 81(2), 233-250.
- Huang, S., Gao, Y., Li, Y., Xu, L., Tao, H., & Wang, P. (2017). Influence of plant architecture on maize physiology and yield in the Heilonggang River valley. *The Crop Journal*, 5(1), 52-62.
- Jiang, B., Wang, P., Zhuang, S., Li, M., Li, Z., & Gong, Z. (2018). Detection of maize drought based on texture and morphological features. *Computers and electronics in agriculture*, 151, 50-60.
- Keating, B. A., & Wafula, B. M. (1992). Modelling the fully expanded area of maize leaves. *Field Crops Research*, 29(2), 163-176.
- Liu, G., Hou, P., Xie, R., Ming, B., Wang, K., Xu, W., ... & Li, S. (2017). Canopy characteristics of high-yield maize with yield potential of 22.5 Mg ha⁻¹. *Field Crops Research*, 213, 221-230.
- Liu, Y., Xie, R., Hou, P., Li, S., Zhang, H., Ming, B., ... & Liang, S. (2013). Phenological responses of maize to changes in environment when grown at different latitudes in China. *Field Crops Research*, 144, 192-199.
- Lizaso, J. I., Batchelor, W. D., & Westgate, M. E. (2003). A leaf area model to simulate cultivar-specific expansion and senescence of maize leaves. *Field crops research*, 80(1), 1-17.
- Ma, D. L., Xie, R. Z., Niu, X. K., Li, S. K., Long, H. L., & Liu, Y. E. (2014). Changes in the morphological traits of maize genotypes in China between the 1950s and 2000s. *European Journal of Agronomy*, 58, 1-10.
- Maddoni, G. A., Otegui, M. E., & Cirilo, A. G. (2001). Plant population density, row spacing and hybrid effects on maize canopy architecture and light attenuation. *Field Crops Research*, 71(3), 183-193.

- Matsunaga, F. T., Tosti, J. B., Androcioli-Filho, A., Brancher, J. D., Costes, E., & Rakocevic, M. (2016). Strategies to reconstruct 3D *Coffea arabica* L. plant structure. *SpringerPlus*, 5(1), 1-15.
- Muchow, R. C., & Carberry, P. S. (1989). Environmental control of phenology and leaf growth in a tropically adapted maize. *Field Crops Research*, 20(3), 221-236.
- Perez, R. P., Fournier, C., Cabrera-Bosquet, L., Artzet, S., Pradal, C., Bricchet, N., ... & Tardieu, F. (2019). Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection. *Plant, cell & environment*, 42(7), 2105-2119.
- Sher, A., Khan, A., Cai, L. J., Ahmad, M. I., Asharf, U., & Jamoro, S. A. (2017). Response of maize grown under high plant density; performance, issues and management-a critical review. *Adv. Crop Sci. Technol*, 5(3), 1-8.
- Stewart, D. W., Costa, C., Dwyer, L. M., Smith, D. L., Hamilton, R. I., & Ma, B. L. (2003). Canopy structure, light interception, and photosynthesis in maize. *Agronomy Journal*, 95(6), 1465-1474.
- Stewart, D. W., & Dwyer, L. M. (1999). Mathematical characterization of leaf shape and area of maize hybrids. *Crop science*, 39(2), 422-427.
- Stewart, D. W., & Dwyer, L. M. (1993). Mathematical characterization of maize canopies. *Agricultural and Forest Meteorology*, 66(3-4), 247-265.
- Su, W., Zhu, D., Huang, J., & Guo, H. (2018). Estimation of the vertical leaf area profile of corn (*Zea mays*) plants using terrestrial laser scanning (TLS). *Computers and Electronics in Agriculture*, 150, 5-13.
- Tang, H., Dubayah, R., Brolly, M., Ganguly, S., & Zhang, G. (2014). Large-scale retrieval of leaf area index and vertical foliage profile from the spaceborne waveform lidar (GLAS/ICESat). *Remote Sensing of Environment*, 154, 8-18.
- Tsukaya, H. (2005). Leaf shape: genetic controls and environmental factors. *The International journal of developmental biology*, 49(5-6), 547-555.
- Valentinuz, O. R., & Tollenaar, M. (2006). Effect of genotype, nitrogen, plant density, and row spacing on the area-per-leaf profile in maize. *Agronomy Journal*, 98(1), 94-99.
- Vos, J., Evers, J. B., Buck-Sorlin, G. H., Andrieu, B., Chelle, M., & De Visser, P. H. (2010). Functional-structural plant modelling: a new versatile tool in crop science. *Journal of experimental Botany*, 61(8), 2101-2115.
- Wang, W. M., Li, Z. L., & Su, H. B. (2007). Comparison of leaf angle distribution functions: Effects on extinction coefficient and fraction of sunlit foliage. *Agricultural and Forest Meteorology*, 143(1-2), 106-122.
- Yin, X., Goudriaan, J. A. N., Lantinga, E. A., Vos, J. A. N., & Spiertz, H. J. (2003). A flexible sigmoid function of determinate growth. *Annals of botany*, 91(3), 361-371.
- Zhang, Y., Wang, R., Wang, S., Ning, F., Wang, H., Wen, P., ... & Li, J. (2019). Effect of planting density on deep soil water and maize yield on the Loess Plateau of China. *Agricultural Water Management*, 223, 105655.
- Zhen, X., Shao, H., Zhang, W., Huo, W., Batchelor, W. D., Hou, P., ... & Zhang, F. (2018). Testing a bell-shaped function for estimation of fully expanded leaf area in modern maize under potential production conditions. *The Crop Journal*, 6(5), 527-537.
- Zhu, Y., Chang, L., Tang, L., Jiang, H., Zhang, W., & Cao, W. (2009). Modelling leaf shape dynamics in rice. *NJAS: Wageningen Journal of Life Sciences*, 57(1), 73-81.



CHAPTER 6

General discussion

General discussion

Maize (*Zea mays* L.) is one of the world's three major cereal crops. As human population and per capita consumption of animal products increase, ensuring a stable and abundant maize supply is essential to meet the demand for food and animal feed is urgent (Huang et al., 2002; Tian et al., 2021). The improvement of crop breeding and management practices play important roles in increasing maize yields (Rizzo et al., 2022). In many growth environments, nitrogen (N) application in the form of either synthetic or organic fertilizer is one of the most effective means to increase yields, since N plays a key role in crop growth (Hou et al., 2012). But this has also down sides. In many parts of the world, farmers apply excessive amounts of N fertilizers, resulting in environmental pollution which in turn results in losses in biodiversity, as well greenhouse gas emissions (Zhang et al., 2015). In order to limit the amount of N applied to crops while maintaining high yields, nitrogen-use efficiency (NUE, defined here as the amount of N harvested per unit nitrogen applied) would have to increased, and higher NUE would be associated with a high canopy photosynthetic nitrogen-use efficiency (Dimkpa et al., 2020; Yan et al., 2022). However, because of the complexity of maize canopy and the plasticity of maize plants, it is still a challenge to clarify how maize plants respond to N availability within the canopy.

The main aim of this thesis was to explore the morphological and physiological trait responses of maize plants to N availability and to explore how these traits may have changed as a result of crop breeding. First, in the General Introduction (Chapter 1), I introduced the general challenge of the need to increase NUE in maize crops and introduced the different ways by which plants can respond to changes in N availability in terms of leaf area and leaf nitrogen dynamics, associated changes in leaf photosynthesis capacity, and yield formation. Then, in Chapter 2, we quantified the responses in the accumulation, partitioning and reallocation patterns of biomass and nitrogen within the canopy in relation to N availability. We investigated how maize plants adapt to N-deficient conditions as plants can respond plastically to the environment (Chapter 3). The vertical distribution patterns of canopy architecture, light, and nitrogen within the maize canopy were also explored among cultivars from old to new (Chapter 4). Moreover, a new empirical equation was established to describe the vertical leaf distribution profile of maize (Chapter 5). The results of this thesis will be important to understand the performance of maize plant in relation to N availability, but can also help guide breeders how to select suitable traits by analysing the genetic gains between old and new cultivars.

In this final chapter, I will place the main findings of this thesis in a broader context and discuss how the findings increase our understanding of the effects of N availability on maize canopy, the vertical distribution patterns of light and nitrogen, biomass and grain formation in section 6.1. Thereafter, the current state and the potential implications of these findings for future research will be discussed in section 6.2 and 6.3, followed by several concluding remarks in section 6.4.

6.1 Overview of the main findings

6.1.1 N availability determines the vertical patterns of N reallocation within the canopy

Typically, plants rely on two primary sources of nitrogen for growth and reproduction. The first is the nitrogen uptake from the soil through their roots, while the second involves the re-translocation of N from aging organs (Ciampitti and Vyn, 2012). If the N uptake rate from the soil is low, sink organs (i.e., organs to which N is transported) may expedite the senescence process in (older) leaves (which are hence N sources) to fulfil their nitrogen requirements (Gallais et al., 2007; Liu et al., 2022). In cereal crops such as maize with a determinate flowering habit (i.e., a complete shift from vegetative to reproductive growth after flowering), developing reproductive organs and their seeds will be the N sink and leaves the main N source during the reproductive growth phase (Molla et al., 2019). Consequently, such plants face a trade-off between reallocating resources to support grain growth or maintaining these resources in the canopy to facilitate additional photosynthesis. This trade-off holds particular significance for N due to its crucial role in both photosynthesis and grain protein content, a vital grain quality trait. Yet, in research on N reallocation in relation to crop photosynthesis this trade-off is rarely considered.

In Chapter 2, we quantified the accumulation, partitioning, and reallocation of N at both the individual leaf and plant scales during the reproductive phase, examining their responses to varying N availabilities resulting from long-term N fertilizer treatments in a field experiment. The results revealed that, regardless of N availability, a portion of grain N comes from reallocation processes from the vegetative organs. Furthermore, it was observed that as N availability decreased, the demand for reallocated N in the grain increased, that is, a relatively larger fraction of seed N was derived from reallocation and a smaller fraction from the soil. However, the absolute quantity of N reallocated was found to be highest under intermediate soil N availability (N1). This suggests that the balance between the demand for reallocated N (which is highest under low N

conditions, N0) and the supply of N (which is highest under high N conditions, N2) tend to co-limit N reallocation at intermediate N levels. When considering the vertical pattern of N reallocation across leaves in the canopy, we noted that a larger fraction of N in leaves was reallocated from lower leaves than from higher ones. But in absolute terms, the largest amounts of N were reallocated from middle-canopy leaves compared to both top and bottom leaves. This suggests that lack of N limits N reallocation from lower leaves while high opportunity costs may have selected for less N reallocation from upper leaves resulting in most N being reallocated from middle leaves.

6.1.2 Maize plants tend to maintain leaf area rather than SLN under N deficient conditions

Crop canopy photosynthesis is the product of radiation interception and radiation-use efficiency (RUE), and both are therefore crucial factors affecting canopy photosynthesis and overall plant productivity (Lemaire et al., 2008). Light interception in turn is strongly determined by the leaf area index (LAI, amount of leaf area per unit soil area) while RUE is strongly determined by specific leaf N contents (SLN, leaf N per unit leaf area) (Massignam et al., 2011). The latter is because of the positive relationship between SLN and leaf photosynthetic capacity (Evans, 1989; Hirose and Werger, 1987). This means that a given amount of nitrogen in the canopy can be used to produce either a large LAI and light capture but with relatively low SLN and associated RUE or the opposite: less light capture but high RUE (Gaudin et al., 2011). When faced with nitrogen deficiency, crop plants are thus faced by a trade-off between maintaining LAI and radiation capture or maintaining SLN and thus RUE (Gaudin et al., 2011; Lemaire et al., 2008).

Chapter 3 aimed to investigate how maize manages this trade-off by examining the responses to different soil N availabilities in terms of specific leaf nitrogen (SLN), leaf area (LA), and their underlying traits during both vegetative and reproductive growth stages. Our findings demonstrate that maize plants employ a strategy of maintaining LA while decreasing SLN during the vegetative stages. But during the reproductive phase, both LA and SLN exhibited comparable reductions. These results characterize maize's response to nitrogen deficiency, giving resource capture takes precedence over resource-use efficiency. The spatial and temporal distribution of specific leaf area (the ratio of leaf area and leaf biomass, SLA, g/m^2) were also explored in Chapter 3. Canopy-SLA (i.e., the canopy leaf area divided by the canopy leaf mass) decreased sharply during vegetative stages and slightly during reproductive stages across all N treatments. This was mainly because leaves in the lower-canopy had a shorter leaf

lifespan and higher SLA. In the vegetative stage, maize maintained LA at low N availability by maintaining leaf biomass (hence having lower N content/mass) and slightly increasing SLA, indicating maize plants tend to increase SLA in order to adapt to nitrogen-deficient conditions while maintaining efficient light capture.

6.1.3 The coordination of light and nitrogen distribution within the maize canopy

In maize, during the last decades breeding has contributed considerably to increased yields, and this genetic yield improvement has been associated with changes in morphological and physiological traits (Welcker et al., 2022). For instance, modern maize hybrids typically exhibit reduced plant height, ear height, and ear-to-stalk ratios, resulting in improved resistance to lodging. Additionally, their more upright leaves, enhance their ability to tolerate high-density planting, and improves light penetration in the canopy which can benefit canopy photosynthesis especially at high plant densities and associated high LAIs (G. Liu et al., 2021). Moreover, the process of light attenuation within the canopy is responsible for determining the vertical distribution of light and nitrogen, which ultimately affects the nitrogen-use efficiency (NUE), as the greater illumination of upper leaves allows them to use N more efficiently for photosynthesis (Hirose and Werger, 1987; Mueller et al., 2019). As noted, this is because A_{max} (leaf-level light saturated leaf photosynthesis) is strongly related to leaf nitrogen content per unit area (SLN, $g\ N/m^2$), and it can be theoretically derived that canopy photosynthesis for a given canopy N (and thus canopy photosynthetic NUE) can be maximized if plants distribute their N such that A_{max} follows the light gradient in the canopy (Anten et al., 1995; Hirose and Werger, 1987). Putting the latter in simple terms, the SLN distribution should be such that if a given leaf receives say 50% of the light that a leaf at the top of the canopy receives its A_{max} should also be half the value of that at the top of the canopy. It is known that the vertical canopy N profile is at least partly driven by the light gradient, but there is still much unexplained variation in N distribution across different growth environments (Archontoulis et al., 2011). Optimizing the relationship between light and nitrogen in the maize canopy could thus be an effective way to improve NUE and plant productivity (Chen et al., 2016). However, to what extent yield-based selection during the last ~70 years of crop breeding indirectly affected canopy architecture, and the vertical distribution of light and nitrogen within the maize canopy has not been reported.

The canopy architecture, the vertical distribution patterns of light and nitrogen, and grain yields of maize cultivars, released between 1950 and 2004, were hence quan-

tified and analysed in Chapter 4. We found that the yield gain from older to newer cultivars was strongly associated with changes in canopy architecture, which increased light interception through a higher LAI and improved light distribution in the canopy through steeper leaf angles. In contrast, the coordination of light and nitrogen was not affected much throughout breeding. That is, there was no significant relationship between the shape of the nitrogen distribution in the canopy and the date at which a cultivar was released (Chapter 4).

6.1.4 A new empirical equation to describe the vertical leaf distribution profile of maize

The vertical distribution of leaf area is an important factor to influence light capture in canopy, which is an essential part of the development of plant and crop models (Fournier and Andrieu, 1999; Vos et al., 2010). Mathematical approaches with modelling can be very convenient and useful for estimating plant growth, in order to address questions on maize performance. The popular bell-shaped function is a robust way to describe the fully expanded leaf area of maize (Keating and Wafula, 1992; Zhen et al., 2018). However, new maize hybrids show different canopy architecture, for example, the ear leaf and adjacent leaves of modern hybrids are longer than that of older hybrids (G. Liu et al., 2021; Zhang et al., 2018). Earlier equations and their parameters, which have been used for older maize hybrids that were grown at low plant densities, will thus not accurately represent modern hybrids (Zhen et al., 2018). Therefore, the previous mathematical models need to be modified.

The modern hybrids ZD958 and XY335 were used to develop a new empirical equation that captures vertical leaf distribution (Chapter 5). The leaf dimension data (leaf length, width and area) obtained from 2015 to 2017 of all leaf ranks were used to fit the new equation and the original bell-shaped function to determine parameters using the nonlinear least squares algorithm. The independent data from 2018 of all leaf ranks were used to evaluate these two equations, resulting in improved accuracy with fewer parameters. Moreover, each parameter of the new equation can be interpreted in a biologically meaningful way. According to the characteristics of this new equation, a method of using derivation formulas to determine key leaf ranks to simplify mathematical equations was proposed in this study, that could simplify the process of data acquisition in the field.

6.2 The identified targets for improving nitrogen-use efficiency (NUE) and yield

As noted above, crop nitrogen-use efficiency (NUE) is defined as the ratio between the amount of N output in harvested products and the amount of N input applied with fertilisers and available from other sources (e.g., manure, synthetic fertilizer, symbiotic fixation and atmospheric deposition). A physiological framework for the analysis of NUE considers two components: how much N is taken up by the crop (N recovery efficiency) and how efficiently N is transformed into grain yield once it has been accumulated in the crop (N internal efficiency). NUE is a useful measure to assess the effectiveness of N fertilizers and the overall N management in agricultural systems (Noor, 2017). High NUE indicates that plants are able to optimize N uptake, minimize losses through leaching or volatilization, and efficiently allocate N to support their growth (Bonelli and Andrade, 2020; Yan et al., 2022) (though very high NUE values can be indicative of soil mining, taking more out than comes in and hence depleting soil N). By contrast, low NUE indicates that a significant amount of applied N is lost to the environment, wasting the resource, producing threats to air, water, soil and biodiversity, and generating additional greenhouse gas emissions (Zhang et al., 2015). Therefore, improving NUE is an important goal in agriculture as it can contribute to sustainable and environmentally friendly farming practices.

6.2.1 Nitrogen-use efficiency in different parts of the world

Crop NUE varies across different countries and regions worldwide (Lassaletta et al., 2014). Certain countries and regions like the USA and Europe exhibit high NUE levels (between 0.5 and 0.9 kg N kg⁻¹N, defined as ‘high’ by the EU Nitrogen Expert Panel (EUNEP, 2015)). In contrast, countries like China but also African countries tend to have low NUE (Lassaletta et al., 2014; Yan et al., 2022). Specifically, there are two primary factors that contribute to the low NUE in different regions: (1) in Africa, the low NUE is associated with both low N application rates and low yields. Often basic agronomic practices such as pest, disease and weed management or water supply are inadequate inhibiting yield responses to fertilizers (Asante et al., 2022; George, 2014). This means that NUE could be increased primarily by improving general agronomic practices; (2) In countries like China and India, the low NUE is primarily caused by excessive N fertilizer application and poor management practices. For instance, the synthetic N fertilizer application rate in China was 256 kg N ha⁻¹ in 2016 (China is currently globally the largest user of N fertilizers with 27.8% of the global N use) (Liu et al., 2020). Therefore, in these countries the key objective should be to attain

high yields while maintaining a reasonable and sustainable NUE. Research efforts that could help make this possible have been made. For instance, a 212 site-year field experiments at two planting densities of 60000 and 75000 plants/ha with the same inputs of N were conducted in China. The maize yield increased about 5.6% and the reactive N loss intensity and greenhouse gas emissions (GHG) intensity reduced by 2.2% to 10.2% across different regions of China indicating that yield increase could be achieved without extra N inputs and further negative environmental impacts if planting density was increased by 15000 plants/ha under the current condition of excessive N surplus in China (Hou et al., 2020). But given the urgency of having to reduce the input of reactive nitrogen into environment, it is imperative to develop further strategies that optimize N management, enhance crop productivity, and ensure sustainable agricultural practices. The potential ways to improve NUE were explored in Chapters 2-4 through a long-term N fertilization experiment.

6.2.2 Enhancing efficient N uptake and effective N reallocation

Enhancing NUE requires both efficient N uptake and effective N allocation and reallocation (i.e. high N harvest index, NHI) in maize (Kant et al., 2011; Ma et al., 2020). Regarding the latter, the delayed senescence of leaves under higher N availability allows for an extended period of photosynthetic activity by the canopy (Ciampitti and Vyn, 2012; Tollenaar and Lee, 2002), which contributes to a higher seasonal canopy photosynthesis and associated assimilate supply. This, in turn, leads to an increased efficiency of nitrogen utilization for biomass production. Put simply, the photosynthetic nitrogen taken up by the plants stays longer in the leaves and contributes longer to photosynthesis thus increasing assimilate production per unit nitrogen (Borrell et al., 2001; Martin et al., 2005). But there is also potentially a negative side to this stay-green (leaves staying green because senescence is delayed) phenomenon. Typically, during the reproductive stage, N compounds are broken down and released from senescing or older leaves. These released N compounds can be reallocated to the grain, where they are utilized to efficiently support grain production during the grain-filling stage and, quite importantly, contribute to the grain's protein content and associated nutritional value (Li et al., 2020; Peng et al., 2006). Therefore, in cereal crops such as maize, a trade-off exists between the “stay-green” trait and N reallocation, since when more N is reallocated to the grain, less N remains available to sustain leaf photosynthesis at a given N level. This rate of N reallocation from leaves and associated stay-greenness in turn varies genetically, i.e., there being so-called stay-green varieties, but is also affected by environmental conditions such as N availability (Abeledo et al., 2008; Kliopova et al., 2016).

The reallocation of N from vegetative tissues assumes significant importance for grain growth, especially under abiotic stress conditions such as drought or nitrogen deficiency, which can trigger leaf senescence (Liu et al., 2022; Molla et al., 2019). Not just the magnitude but also the vertical pattern of N reallocation across leaves within the canopy (i.e., the extent to which N is reallocated from upper or lower leaves) is influenced by the N availability, as I showed in Chapter 2. Notably, leaves positioned higher up in the canopy become increasingly important sources of N reallocation when N availability from the soil is limited. As the availability of N from the soil decreases, the proportion of N uptake during the reproductive stage that contributes to grain N accumulation diminishes. Consequently, plants progressively rely on N reallocation from upper leaves, even if it entails a relatively larger opportunity cost in terms of photosynthesis (Chapter 2). However, further research is needed to determine how the balance between dry matter yield and grain protein content are mediated by the N reallocation and its effects on canopy photosynthesis, potentially through plant simulation modelling, since the physiological processes underlying yield quality are not so well understood and quantified.

6.2.3 Improving canopy photosynthesis

Canopy photosynthesis is influenced by the interplay of light capture and the efficiency with which captured light is used for carbon assimilation (Evans, 1993). In maize production, optimizing canopy structure has practical implications for increasing canopy light interception and improving productivity. Application of N fertilizers is one of the most powerful measures to regulate canopy structure by influencing leaf expansion, leaf lifespan and leaf orientation (Luo et al., 2020). For instance, N-deficient plants tend to have smaller and narrower leaves, stunted growth, and shorter canopies compared to N-sufficient plants (Hammer et al., 2009; Ouyang et al., 2021). These factors strongly influence the plant's capacity to intercept light, the uniformity of light distribution within the canopy, and the efficiency of light-induced photosynthesis (Evans and Clarke, 2019). In addition, when N is limited, plants may adaptively adjust their canopy architecture by modifying leaf angles, or reallocating resources (Ma et al., 2014). These responses in turn affect canopy photosynthesis. Erect architectures (i.e., smaller leaf angles and upright leaves), for example, increase the light transmittance to lower leaves, resulting in more light interception in the lower canopy, reducing the degree of shading of lower leaves. This increased light penetration may also delay leaf senescence, which is partly driven by shading (Boonman et al., 2006). Furthermore, canopy architecture varies among maize hybrids. For instance, modern maize varieties tend to be taller than older varieties but have a lower ear position, along with a more upright leaf orientation (G. Liu et al., 2021).

At the canopy level, the canopy N profile, the light profile and their relationship play a crucial role in determining canopy productivity (Anten et al., 1995; Hirose and Werger, 1987). As noted earlier in this chapter, due to the strong relationship between light saturated photosynthesis and leaf N content, the gradient of leaf N content in the canopy contributes to the NUE at the whole-plant level (Chen et al., 2016). Vertical light and nitrogen distributions in a canopy can be described by an exponential function of the downward cumulative leaf area index with an extinction coefficient for light (K_L) and for photosynthetically active leaf nitrogen (K_N) (Anten et al., 1995; Hirose, 2005). As noted, theoretical studies have suggested that canopy photosynthesis would be maximized if a vertical nitrogen distribution follows the light gradient within the canopy, i.e. $K_N: K_L=1$ (Field, 1983; Hirose and Werger, 1987). However, the ratio between K_N and K_L was found to be around 0.34 in rice canopies, and 0.37 in wheat canopies (Gu et al., 2017; Hikosaka et al., 2016). Interestingly, a positive relationship between K_N/K_L and RUE was found across different rice varieties, indicating that the coordination of light and nitrogen distribution is conducive to the improvement of RUE and yield (Gu et al., 2017; Ouyang et al., 2021). Studies that compared K_N and K_L values across species found a positive relationship; species with high K_L values tended to have higher K_N values and this correlation held both across crops and wild herbaceous plants (Anten 2016; Hikosaka et al. 2016). This suggests there might be selective advantage to coordinating the nitrogen and light distributions. However, few studies have reported the K_N and K_L ratios in maize canopies. The vertical distribution patterns of light and nitrogen of six cultivars released between 1950 and 2004 were quantified and analysed in Chapter 4. In contrast to results for other herbaceous species, the nitrogen distribution within maize canopies could not be described with a negative exponential function (i.e., one that is similar to the light distribution in the canopy (Monsi and Saeki, 2005), because the upper-middle leaves rather than the top-most leaves had the highest SLN. Moreover, the coordination between light and nitrogen in maize canopy was not associated with maize breeding history from the 1950 to 2004 in Chinese cultivars. This lack of an association raises an intriguing question: does the coordination of light and nitrogen distribution in the canopy represent an unutilized breeding target to further enhancing yield and NUE in maize or is it less important than what theoretical studies assume? This question could be addressed through a combination of a combination of crop (systems biology) modelling and further experiments like the one conducted in Chapter 4.

6.3 Implications and future perspective

6.3.1 The genotype \times environment \times management (G \times E \times M) interactions

One of the complexities that need to be considered in developing agronomic strategies and/or breeding targets comes from what has been denoted as genotype \times environment \times management (G \times E \times M) interactions. Crop management practices such as fertilizer application (M) affect crop performance through modifications of the environment (M \times E) (Chapter 2). The implications of these changes in environment (E) in turn depend on plant traits (E \times G) which changes as a consequence of artificial selection and breeding (Chapter 4). Plants respond to these environmental changes through plasticity. The phenotypic plasticity is the capacity of a plant to produce a diversity of phenotypes to adjust their phenotype under different environmental conditions (Chapter 3). Thus, to develop strategies to increase maize yields in China, it is important to quantify the key factors contributing to the genotype \times environment \times management (G \times E \times M) interactions.

To understand the relationship between maize canopy architecture and function, and the genotype \times environment \times management interactions, it is necessary to integrate the collected field data and crop modelling (Fournier et al., 2005; Yin et al., 2022). However, the theoretical studies on optimization of canopy nitrogen use have tended to be static (e.g. they treat N distribution or LAI as a static phenomenon while in reality these arise from a dynamic process of leaf production, senescence and loss), and they do not always consider explicit architecture (Hikosaka, 2005). Using plant simulation modelling such as functional-structural plant (FSP) modelling the growth and development of the three-dimensional (3D) plant structure based on physiological mechanisms can be simulated, integrating knowledge on plant functioning and morphology as modulated by the environment (De Vries et al., 2021; Vos et al., 2010). This makes FSP models ideal tools to address questions on the mechanisms behind the distributions of leaf properties and light capture in maize plants. In this thesis, the observed data from the experiments could be used to calibrate the parameters and evaluate such FSP models (Chapter 5). The plant architecture data (leaf area, leaf angle, internode length, internode width) and leaf N contents were collected on individual leaf position. The simulated data combined with measured plant architecture data (Chapter 5) could be used to quantify the interaction between plant architecture and light capture in maize canopies under different N availabilities across leaf, plant and canopy scales. We expect that our results will be important for evaluating the performance of maize plant plasticity under different environment conditions, but

also can guide breeders how to choose suitable cultivars by analysing the difference of $G \times E \times M$ between old and new cultivars.

6.3.2 Coordination of grain quantity and quality

While much of the crop physiological research has focussed on yield quantity (usually the amount harvested biomass), yield quality might be at least as important. For food, yield quality largely entails the content of nutritional elements, such as proteins, vitamins or minerals per unit harvested product, though other e.g. aesthetic aspects such as size, colour and shape may also matter. As global living standards are on average increasing, emphasis on having enough nutritious food rather than simply having enough food, becomes increasingly important, emphasizing the need for crop physiology to pay more attention to quality. For instance, China has achieved an impressive status as the world's second-largest producer and consumer of maize in recent times (FAO, 2020). However, improvement of maize quality aspects has not received the widespread attention it deserves. Therefore, exploring the factors that influence corn quality and comprehending the physiological mechanisms that affect its yield are of utmost importance. This research topic holds great significance in establishing a robust theoretical foundation for achieving high-yield and high-quality specialized maize production.

The assimilates and proteins associated with photosynthesis are accumulated and stored in the endosperm and scutellum of the embryo through reallocation and grain filling during the growth and development stages, playing a pivotal role in the formation of maize yield and quality (Jobling, 2004; Sabelli and Larkins, 2009). The formation of maize yield and quality is influenced by various factors, including genotype, environment, and production management (Duvick, 2005; Ray et al., 2019). For instance, the grain protein content decreased while starch content increased when maize crops were grown at higher planting density, due to the assimilation ability of carbon and nitrogen affected by intra-plant competition (Maddonni and Otegui, 2006). Moreover, enhancing N nutrition during the grain-filling stage has a significant impact on promoting the synthesis and accumulation of proteins and amylose (Kaplan et al., 2019; Nasielski et al., 2019), thereby influencing the process of grain yield and quality formation.

During the past several decades, maize grain yield has increased dramatically, while grain nitrogen concentration has declined in modern hybrids (Chen et al., 2014; Scott et al., 2006). The decline in grain N could at least partly be attributed to the leaves tending to stay-green at maturity in high-yielding hybrids (Mueller et al., 2019). This stay-green characteristic relies on increased post-silking N absorption from the soil and

decreased N reallocation from vegetative organs (Borrell et al., 2001). As discussed earlier in this chapter and in Chapter 2, there is a trade-off between leaf N reallocation and leaf photosynthesis rate during post-silking stages. Maintaining N in the canopy through delayed senescence ensures higher canopy photosynthesis, but at the same time entails that less N is reallocated to grain. Previous studies demonstrated a strong negative association between N reallocation and post-silking N uptake (Z. Liu et al., 2021; Wei et al., 2019). However, the plant traits related to the coordination between grain yield and grain nitrogen concentration, and between post-silking N uptake and N remobilization are largely overlooked. It is supposed that higher N reallocation together with higher N and DM accumulation are the target traits to improve the modern stay-green cultivars to increase grain N without penalty in grain yield (Chen et al., 2015; Ray et al., 2020).

6.4 Concluding remarks

In this thesis I explored how maize plants respond to N availability. The aim was to obtain crop physiological understanding of the morphological and physiological traits that are responsible for differences in adaptive abilities to N availability and to determine how these traits may have changed through the course of breeding. Ultimately, I hope that the knowledge thus obtain can contribute to increasing yields and especially nitrogen-use efficiency (NUE) in maize. The following conclusions can be drawn:

- (1) Nitrogen availability determines the vertical patterns of accumulation, partitioning, and reallocation of dry matter and nitrogen in maize.
- (2) Maize plants tend to cope with low N by maintaining leaf area, and they achieve this by increasing specific leaf area (SLA) throughout the plant but only during the vegetative growth phase.
- (3) The higher yields that are obtained with modern cultivars do not appear to rely on a coordinated light and N distribution within the canopy.

Moreover, the findings of this thesis may provide insight for both farmers and maize breeders to enhance NUE and yields. For farmers, the appropriate N fertilizer (i.e. N1 treatment in this thesis, 150kg N/ha) can maintain high-yield and decreasing the environmental impact. Breeders intentionally selected for erect architecture so that grain yield per unit land area could increase along with increased planting density tolerance. However, the maize breeding goals should not only include high yields, but also high grain quality (grain N concentration) that meet production demands.

References

- Abeledo, L. G., Calderini, D. F., & Slafer, G. A. (2008). Nitrogen economy in old and modern malting barleys. *Field Crops Research*, 106(2), 171-178.
- Anten, N. P. R., Schieving, F., & Werger, M. J. A. (1995). Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C 3 and C 4 mono-and dicotyledonous species. *Oecologia*, 101, 504-513.
- Archontoulis, S. V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N. G., & Struik, P. C. (2011). Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. *Field Crops Research*, 122(3), 186-198.
- Asante, P. A., Rahn, E., Zuidema, P. A., Rozendaal, D. M., van der Baan, M. E., Läderach, P., ... & Anten, N. P. (2022). The cocoa yield gap in Ghana: A quantification and an analysis of factors that could narrow the gap. *Agricultural Systems*, 201, 103473.
- Bonelli, L. E., & Andrade, F. H. (2020). Maize radiation use-efficiency response to optimally distributed foliar-nitrogen-content depends on canopy leaf-area index. *Field Crops Research*, 247, 107557.
- Boonman, A., Anten, N. P., Dueck, T. A., Jordi, W. J., van der Werf, A., Voesenek, L. A., & Pons, T. L. (2006). Functional significance of shade-induced leaf senescence in dense canopies: an experimental test using transgenic tobacco. *The American Naturalist*, 168(5), 597-607.
- Borrell, A., Hammer, G., & Van Oosterom, E. (2001). Stay-green: A consequence of the balance between supply and demand for nitrogen during grain filling?. *Annals of applied biology*, 138(1), 91-95.
- Chen, Y., Xiao, C., Chen, X., Li, Q., Zhang, J., Chen, F., ... & Mi, G. (2014). Characterization of the plant traits contributed to high grain yield and high grain nitrogen concentration in maize. *Field Crops Research*, 159, 1-9.
- Chen, Y., Xiao, C., Wu, D., Xia, T., Chen, Q., Chen, F., ... & Mi, G. (2015). Effects of nitrogen application rate on grain yield and grain nitrogen concentration in two maize hybrids with contrasting nitrogen remobilization efficiency. *European Journal of Agronomy*, 62, 79-89.
- Chen, Y., Wu, D., Mu, X., Xiao, C., Chen, F., Yuan, L., & Mi, G. (2016). Vertical Distribution of Photosynthetic Nitrogen Use Efficiency and Its Response to Nitrogen in Field-Grown Maize. *Crop Science*, 56(1), 397-407.
- Ciampitti, I. A., & Vyn, T. J. (2012). Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crops Research*, 133, 48-67.
- de Vries, J., Evers, J. B., Kuyper, T. W., van Ruijven, J., & Mommer, L. (2021). Mycorrhizal associations change root functionality: a 3D modelling study on competitive interactions between plants for light and nutrients. *New Phytologist*, 231(3), 1171-1182.
- Dimkpa, C. O., Fugice, J., Singh, U., & Lewis, T. D. (2020). Development of fertilizers for enhanced nitrogen use efficiency—Trends and perspectives. *Science of the Total Environment*, 731, 139113.
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in agronomy*, 86, 83-145.
- EUNEP, (2015). Nitrogen Use Efficiency (NUE) an indicator for the utilization of nitrogen in food systems. Wageningen University, Alterra, Wageningen, Netherlands.
- Evans, J. R. (1993). Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. *Functional Plant Biology*, 20(1), 55-67.

- Evans, J. R. (1989). Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia*, 78(1), 9-19.
- Evans, J. R., & Clarke, V. C. (2019). The nitrogen cost of photosynthesis. *Journal of Experimental Botany*, 70(1), 7-15.
- FAO, 2020. <https://www.fao.org/faostat/en/#compare>. [WWW Document].
- Field, C. (1983). Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia*, 56, 341-347.
- Fournier, C., & Andrieu, B. (1999). ADEL-maize: an L-system based model for the integration of growth processes from the organ to the canopy. Application to regulation of morphogenesis by light availability. *Agronomie*, 19(3-4), 313-327.
- Fournier, C., Durand, J. L., Ljutovac, S., Schaufele, R., Gastal, F., & Andrieu, B. (2005). A functional-structural model of elongation of the grass leaf and its relationships with the phyllochron. *New Phytologist*, 166(3), 881-894.
- Gallais, A., Coque, M., Le Gouis, J., Prioul, J. L., Hirel, B., & Quillere, I. (2007). Estimating the Proportion of Nitrogen Remobilization and of Postsilking Nitrogen Uptake Allocated to Maize Kernels by Nitrogen-15 Labeling. *Crop science*, 47(2), 685-691.
- Gaudin, A. C., McClymont, S. A., & Raizada, M. N. (2011). The nitrogen adaptation strategy of the wild teosinte ancestor of modern maize, *Zea mays* subsp. *parviglumis*. *Crop science*, 51(6), 2780-2795.
- George, T. (2014). Why crop yields in developing countries have not kept pace with advances in agronomy. *Global Food Security*, 3(1), 49-58.
- Gu, J., Chen, Y., Zhang, H., Li, Z., Zhou, Q., Yu, C., ... & Yang, J. (2017). Canopy light and nitrogen distributions are related to grain yield and nitrogen use efficiency in rice. *Field Crops Research*, 206, 74-85.
- Hammer, G. L., Dong, Z., McLean, G., Doherty, A., Messina, C., Schussler, J., ... & Cooper, M. (2009). Can changes in canopy and/or root system architecture explain historical maize yield trends in the US corn belt?. *Crop Science*, 49(1), 299-312.
- Hikosaka, K. (2005). Leaf canopy as a dynamic system: ecophysiology and optimality in leaf turnover. *Annals of botany*, 95(3), 521-533.
- Hikosaka, K., Anten, N. P.R., Borjigidai, A., Kamiyama, C., Sakai, H., Hasegawa, T., ... & Ito, A. (2016). A meta-analysis of leaf nitrogen distribution within plant canopies. *Annals of Botany*, 118(2), 239-247.
- Hirose, T. (2005). Development of the Monsi-Saeki theory on canopy structure and function. *Annals of botany*, 95(3), 483-494.
- Hirose, T., & Werger, M. J. A. (1987). Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia*, 72, 520-526.
- Hou, P., Gao, Q., Xie, R., Li, S., Meng, Q., Kirkby, E. A., ... & Chen, X. (2012). Grain yields in relation to N requirement: Optimizing nitrogen management for spring maize grown in China. *Field Crops Research*, 129, 1-6.
- Hou, P., Liu, Y., Liu, W., Liu, G., Xie, R., Wang, K., ... & Li, S. (2020). How to increase maize production without extra nitrogen input. *Resources, Conservation and Recycling*, 160, 104913.
- Huang, J., Pray, C., & Rozelle, S. (2002). Enhancing the crops to feed the poor. *Nature*, 418(6898), 678-684.
- Jobling, S. (2004). Improving starch for food and industrial applications. *Current opinion in plant biology*, 7(2), 210-218.

- Kant, S., Bi, Y. M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of experimental Botany*, 62(4), 1499-1509.
- Kaplan, M., Karaman, K., Kardes, Y. M., & Kale, H. (2019). Phytic acid content and starch properties of maize (*Zea mays* L.): Effects of irrigation process and nitrogen fertilizer. *Food chemistry*, 283, 375-380.
- Keating, B. A., & Wafula, B. M. (1992). Modelling the fully expanded area of maize leaves. *Field Crops Research*, 29(2), 163-176.
- Kliopova, I., Baranauskaitė-Fedorova, I., Malinauskienė, M., & Staniškis, J. K. (2016). Possibilities of increasing resource efficiency in nitrogen fertilizer production. *Clean technologies and environmental policy*, 18, 901-914.
- Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., & Garnier, J. (2014). 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters*, 9(10), 105011.
- Lemaire, G., van Oosterom, E., Jeuffroy, M. H., Gastal, F., & Massignam, A. (2008). Crop species present different qualitative types of response to N deficiency during their vegetative growth. *Field Crops Research*, 105(3), 253-265.
- Li, G., Wang, L., Li, L., Lu, D., & Lu, W. (2020). Effects of fertilizer management strategies on maize yield and nitrogen use efficiencies under different densities. *Agronomy Journal*, 112(1), 368-381.
- Liu, G., Yang, H., Xie, R., Yang, Y., Liu, W., Guo, X., ... & Li, S. (2021). Genetic gains in maize yield and related traits for high-yielding cultivars released during 1980s to 2010s in China. *Field Crops Research*, 270, 108223.
- Liu, Y., Heuvelink, G. B., Bai, Z., He, P., Xu, X., Ma, J., & Masiliūnas, D. (2020). Space-time statistical analysis and modelling of nitrogen use efficiency indicators at provincial scale in China. *European Journal of Agronomy*, 115, 126032.
- Liu, Z., Hu, C., Wang, Y., Sha, Y., Hao, Z., Chen, F., ... & Mi, G. (2021). Nitrogen allocation and remobilization contributing to low-nitrogen tolerance in stay-green maize. *Field Crops Research*, 263, 108078.
- Liu, Z., Sha, Y., Huang, Y., Hao, Z., Guo, W., Ke, L., ... & Mi, G. (2022). Efficient nitrogen allocation and reallocation into the ear in relation to the superior vascular system in low-nitrogen tolerant maize hybrid. *Field Crops Research*, 284, 108580.
- Luo, L., Zhang, Y., & Xu, G. (2020). How does nitrogen shape plant architecture?. *Journal of experimental botany*, 71(15), 4415-4427.
- Ma, D., Yu, X., Ming, B., Li, S., Xie, R., & Gao, J. (2020). Maize nitrogen use efficiency improved due to density tolerance increase since the 1950s. *Agronomy Journal*, 112(3), 1537-1548.
- Ma, D. L., Xie, R. Z., Niu, X. K., Li, S. K., Long, H. L., & Liu, Y. E. (2014). Changes in the morphological traits of maize genotypes in China between the 1950s and 2000s. *European Journal of Agronomy*, 58, 1-10.
- Maddoni, G. A., & Otegui, M. E. (2006). Intra-specific competition in maize: Contribution of extreme plant hierarchies to grain yield, grain yield components and kernel composition. *Field Crops Research*, 97(2-3), 155-166.
- Martin, A., Belastegui-Macadam, X., Quilleré, I., Floriot, M., Valadier, M. H., Pommel, B., ... & Hirel, B. (2005). Nitrogen management and senescence in two maize hybrids differing in the persistence of leaf greenness: agronomic, physiological and molecular aspects. *New phytologist*, 167(2), 483-492.

- Massignam, A. M., Chapman, S. C., Hammer, G. L., & Fukai, S. (2012). Effects of nitrogen supply on canopy development of maize and sunflower. *Crop and Pasture Science*, 62(12), 1045-1055.
- Molla, M. S. H., Nakasathien, S., Ali, M. A., Khan, A. S. M. M. R., Alam, M. R., Hossain, A., ... & El Sabagh, A. (2019). Influence of nitrogen application on dry biomass allocation and translocation in two maize varieties under short pre-anthesis and prolonged bracketing flowering periods of drought. *Archives of Agronomy and Soil Science*, 65(7), 928-944.
- Monsi, M., & Saeki, T. (2005). On the factor light in plant communities and its importance for matter production. *Annals of botany*, 95(3), 549-567.
- Mueller, S. M., Messina, C. D., & Vyn, T. J. (2019). Simultaneous gains in grain yield and nitrogen efficiency over 70 years of maize genetic improvement. *Scientific Reports*, 9(1), 9095.
- Nasielski, J., Earl, H., & Deen, B. (2019). Luxury vegetative nitrogen uptake in maize buffers grain yield under post-silking water and nitrogen stress: A mechanistic understanding. *Frontiers in Plant Science*, 10, 318.
- Noor, M. A. (2017). Nitrogen management and regulation for optimum NUE in maize—A mini review. *Cogent Food & Agriculture*, 3(1), 1348214.
- Ouyang, W., Yin, X., Yang, J., & Struik, P. C. (2021). Roles of canopy architecture and nitrogen distribution in the better performance of an aerobic than a lowland rice cultivar under water deficit. *Field Crops Research*, 271, 108257.
- Peng, S., Buresh, R. J., Huang, J., Yang, J., Zou, Y., Zhong, X., ... & Zhang, F. (2006). Strategies for overcoming low agronomic nitrogen use efficiency in irrigated rice systems in China. *Field Crops Research*, 96(1), 37-47.
- Ray, K., Banerjee, H., Dutta, S., Hazra, A. K., & Majumdar, K. (2019). Macronutrients influence yield and oil quality of hybrid maize (*Zea mays* L.). *PloS one*, 14(5), e0216939.
- Ray, K., Banerjee, H., Dutta, S., Sarkar, S., Murrell, T. S., Singh, V. K., & Majumdar, K. (2020). Macronutrient management effects on nutrient accumulation, partitioning, remobilization, and yield of hybrid maize cultivars. *Frontiers in plant science*, 11, 1307.
- Rizzo, G., Monzon, J. P., Tenorio, F. A., Howard, R., Cassman, K. G., & Grassini, P. (2022). Climate and agronomy, not genetics, underpin recent maize yield gains in favorable environments. *Proceedings of the National Academy of Sciences*, 119(4), e2113629119.
- Sabelli, P. A., & Larkins, B. A. (2009). The development of endosperm in grasses. *Plant physiology*, 149(1), 14-26.
- Scott, M. P., Edwards, J. W., Bell, C. P., Schussler, J. R., & Smith, J. S. (2006). Grain composition and amino acid content in maize cultivars representing 80 years of commercial maize varieties [*Zea mays* L. Iowa; North Carolina]. *Maydica* (Italy), 51, 417-423.
- Tian, X., Engel, B. A., Qian, H., Hua, E., Sun, S., & Wang, Y. (2021). Will reaching the maximum achievable yield potential meet future global food demand?. *Journal of Cleaner Production*, 294, 126285.
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field crops research*, 75(2-3), 161-169.
- Vos, J., Evers, J. B., Buck-Sorlin, G. H., Andrieu, B., Chelle, M., & De Visser, P. H. (2010). Functional–structural plant modelling: a new versatile tool in crop science. *Journal of experimental Botany*, 61(8), 2101-2115.
- Wei, S., Wang, X., Li, G., Jiang, D., & Dong, S. (2019). Maize canopy apparent photosynthesis and ¹³C-photosynthate reallocation in response to different density and N rate combinations. *Frontiers in Plant Science*, 10, 1113.

- Welcker, C., Spencer, N. A., Turc, O., Granato, I., Chapuis, R., Madur, D., ... & Tardieu, F. (2022). Physiological adaptive traits are a potential allele reservoir for maize genetic progress under challenging conditions. *Nature Communications*, 13(1), 3225.
- Yan, X., Xia, L., & Ti, C. (2022). Temporal and spatial variations in nitrogen use efficiency of crop production in China. *Environmental Pollution*, 293, 118496.
- Yin, X., Gu, J., Dingkuhn, M., & Struik, P. C. (2022). A model-guided holistic review of exploiting natural variation of photosynthesis traits in crop improvement. *Journal of Experimental Botany*, 73(10), 3173-3188.
- Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, 528(7580), 51-59.
- Zhang, Z., Zhang, X., Lin, Z., Wang, J., Xu, M., Lai, J., ... & Lin, Z. (2018). The genetic architecture of nodal root number in maize. *The Plant Journal*, 93(6), 1032-1044.
- Zhen, X., Shao, H., Zhang, W., Huo, W., Batchelor, W. D., Hou, P., ... & Zhang, F. (2018). Testing a bell-shaped function for estimation of fully expanded leaf area in modern maize under potential production conditions. *The Crop Journal*, 6(5), 527-537.

SUMMARY

Summary

Population growth, shortage of water and land resources, and impacts of climate change all make the situation of world food security more and more acute. In order to meet the growing demand for food, agronomic management practices and breeding are the two main important avenues to increase production per unit area of maize, a prime food crop species. Nitrogen (N) application is one of the most effective means to increase yields in maize, since N plays a key role in the physiological processes that determine crop growth and seed production. However, the application of N fertilizer has several negative effects on the environment, agriculture, and human health as well, and thus high nitrogen-use efficiency (NUE, high yields per unit of N applied) becomes important. To enhance NUE and yields while limiting N fertilization, the response of maize morphological and physiological traits needs to be explored. On the basis of background and research questions presented in **Chapter 1**, in this thesis, we focused on quantifying plant architecture, nitrogen and biomass distribution across leaf positions, stems, and other organs in maize. The main objective was to understand the spatial and vertical distribution of light and nitrogen in relation to N availability in terms of the morphological and physiological traits within the canopy, and how these traits may have changed by maize breeding.

In **Chapter 2**, the accumulation, partitioning, and reallocation of dry matter (DM) and nitrogen (N) under different N availabilities at both the single leaf and plant scales were quantified. The findings revealed that grain N relies on reallocation more than grain DM. Regardless of N availability, a portion of grain N has been obtained through reallocation from vegetative organs. Moreover, this dependency of grain N on N reallocation from vegetative parts (i.e., the fraction of grain N coming from reallocation) was higher at lower soil N availability. But the absolute amount of N reallocated to grains was highest at intermediate N availability. The leaf ranks from which most DM and N were reallocated, gradually increased from the bottom to upper ranks as N availability decreased. This could reflect a balance between N availability for reallocation, being higher in upper than in lower canopy leaves and opportunity costs in terms of losses in photosynthesis of reallocating N also being highest in upper canopy leaves. Together, our results on the dynamics of N and DM uptake and reallocation during the reproductive phase are important for plant and crop models that require these processes for accurate predictions of maize performance. The observations in this study provide opportunities to improve N management in precision farming and provide data support for crop models to simulate N treatment effect assessments.

In **Chapter 3**, a trade-off strategy in terms of radiation capture and radiation-use efficiency (RUE) under nitrogen- deficient conditions was explored in maize plants. Specifically, how maize deals with this trade-off, through responses in specific leaf nitrogen (SLN), leaf area (LA), and their underlying traits during vegetative and reproductive growth. Maize plants tended to maintain LA and decrease SLN during vegetative stages (i.e., favouring maintaining light capture over maintaining RUE), but both LA and SLN decreased comparably during reproductive stages. Moreover, individual leaf size, lifespan and specific leaf area (SLA) within maize canopy contribute to shaping canopy SLA. Canopy-SLA decreased sharply during vegetative stages and slightly during reproductive stages, mainly because senesced leaves in the lower canopy had a higher SLA than upper leaves that remain. Maize plants tended to increase SLA to adapt to N-deficient conditions, and this could partly offset the negative effects of N deficiency on LA. However, the faster leaf senescence in N-deficient plants negatively affected average canopy SLA as it were the lower canopy leaves had high SLA values. These two contrasting effects - producing higher SLA leaves but also dropping high SLA leaves faster - may explain why canopy SLA in the reproductive phase no longer differed among N treatments.

In **Chapter 4**, the canopy architecture, vertical light and nitrogen distribution patterns, and grain yields of maize cultivars released between 1950 and 2004 were analysed. The genetic gain in grain yield (i.e., the slope of the regression between yield and year of release) was about $109 \text{ kg ha}^{-1} \text{ year}^{-1}$ across these cultivars. The yield improvement from older to newer cultivars was strongly associated with changes in canopy architecture, resulting in increased light interception through higher leaf area index (LAI) and improved distribution of light in the canopy due to steeper leaf angles. The vertical light distribution throughout the canopy was relatively more uniform in modern cultivars, as indicated by a decreased extinction coefficient (K_d) from 0.61 in 1950 to 0.39 in 2004 at a rate of $0.67\% \text{ year}^{-1}$. However, the coordination of light and nitrogen in the canopy did not differ among old and new cultivars. The shape of nitrogen distribution in the canopy showed no relationship with the release date of the cultivars. These results showed that breeding has increased yields during the past decades of breeding history, and the genetic yield improvement has been associated with changes in morphological and physiological traits. Understanding these relationships can lead to more targeted breeding strategies to optimize canopy architecture and enhance RUE and overall plant productivity in maize.

In **Chapter 5**, to improve plant modelling tools that would allow us to analyse maize growth and production in relation to distributions of light, nitrogen and leaf angle, we

developed a new empirical equation to quantitatively describe the vertical distribution of leaf dimensions within the maize canopy. All parameters in the new empirical equation can be interpreted in a biological meaningful way. Compared with previous bell-shaped functions, the new equation reduces the number of parameters required to characterize the leaf morphology, which simplifies numerical calculations. The equation was fitted with leaf morphological data and evaluated against independent data, showing higher prediction accuracy than earlier models. Furthermore, by analysing the characteristics of the new equation with the growth and development of maize plants, we found that, instead of using all leaf positions, only four key leaf positions need be used to build the equation, greatly simplifying data acquisition in the field. The results should help develop maize plant models more efficiently to improve our understanding of leaf-growth dynamics and the consequences for maize productivity.

Chapter 6 addresses the research objectives of this thesis (Chapter 1) and reflects on the new insights presented in this thesis. This chapter summarized and discussed the main findings obtained in Chapter 2-5 and the current scientific challenges and future perspectives. In conclusion, the vertical distribution of leaf area, canopy architecture, light and nitrogen distribution under N availability lead to the identification of several traits that can be exploited for enhancing maize production and nitrogen-use efficiency.

APPENDIX

Acknowledgements

About the Author

List of Publications

PE&RC Training and Education Statement

Acknowledgements

I would like to express my sincere gratitude to all the individuals who have supported and guided me throughout the completion of this thesis. Their contributions have been invaluable, and I am deeply thankful for their assistance and encouragement all the time. My sincerest gratitude goes to my supervision team: Prof. Niels. P. R. Anten, Prof. Jochem. B. Evers, from Center for Crop System Analysis, Wageningen University & Research (WUR), and Prof. Shaokun Li, Prof. Ruizhi Xie from Chinese Academy of Agricultural Sciences (CAAS). You are all great scientists with your own expertise, from which I have learned and benefited a lot.

Dear **Niels**, I am grateful for your trust from the very beginning of my PhD even though we had not met before. You are a very responsible supervisor and always there when I need help. I appreciated your careful guidance and constructive advice on my research work. Your critical comments and suggestions on my manuscript not only greatly improved the quality of my research work but also my scientific writing. I also thankful for the opportunity you provide for me to attend international conference, which help me grow as an independent researcher.

Dear **Jochem**, your patience and encouragement help me build my confidence. I remember we had a meeting to discuss references, I had no clue and was at a loss. You told me frankly that you don't know the answer either. Nobody knows everything, you let me focus on the research questions and then using modelling or experiment approaches to solve them. I can honestly say I don't know, and it's not something to be ashamed of. Thanks for understanding my anxiety, Jochem, you gave me the courage to be honest with myself.

My gratitude also goes to my Chinese supervisors, Prof. Dr **Shaokun Li**, you have not only been my supervisor for both my master's and PhD studies, but you have also played an important role in shaping my growth and development as a researcher. We started my master's project in 2014 at Crop Science Institute of CAAS, and now the PhD project ends in 2023, we have known each other for 9 years. You always be my side when I need your help, like my parents. Thank you for supporting me to join the PhD program between WUR and CAAS, as a sandwich PhD. In the past, I really couldn't believe that I could have the opportunity to study abroad. I have been to many countries and experienced different cultures. Thank you for giving me such an opportunity and making my life colourful.

Dear **Ruizhi Xie**, thanks for your academic help and financial support. It is your encouragement and support that prompted me to start my PhD. You are very confident about my research topics and dataset, and always encourage me to explore the underlying mechanisms by analysing the detailed data. Without your guidance and encouragement during the past few years, it would be impossible for me to achieve this. I still vividly recall your words: “Hardships are only temporary; once they’re in the past, the future shines brightly.” These words have become a guiding light, reminding me to persevere through challenges, knowing that brighter days lie ahead. Your belief in my capabilities has instilled in me a newfound courage. I am braver than before because I know you have my back.

Dear **Bo Ming**, thanks for your suggestions and comments on my PhD thesis. When you read literature that related to my research topic, you always share with me. Your assistance has not only aided in expanding my knowledge but has also served as a wellspring of inspiration for shaping the storyline of my paper. You like my brother, caring about my research project and giving me some life advice. I can express my real thoughts in front of you because I know you are a reliable friend. Thanks to you, I have never felt disconnected from CAAS, despite having been at WUR for the past three years.

When things come to an end, I always think of the beginning. Dear **Yaoyao Li**, I still remember the time we spend at the Gongzhuling Experimental Station in Jilin Province. We collected data together in the field almost every day during maize growth stages. The weather in July and August is hottest, and this period is also the busiest stage of our work. You are a reliable friend and colleague. We sharing happiness and sorrows at that time. Thanks for your hard work and responsibility, lighten my burden. I will always cherish the friendship that we have.

Many thanks to my dear CSA colleagues, both current and previous, it is nice to work with you. I like to say special thank you to Nicole Wolffensperger and Petra Rozema for helping me arrange the documents for applying visa and extension. I extend my sincere appreciation to my PhD fellows and friends. Thank you very much for the moments we shared together. The parties and canteen talks make my PhD journey filled with laughter and relaxation. Jin Wang and Jiayi Nie, thank you for being my paranymphs.

As a sandwich PhD student, I also appreciate all support I received from Claudius van de Vijver and Mingjun Zhang, I appreciated everything you do to support and improve the WUR-CAAS program. I would like to thank my friends in WUR-CAAS program. The journey would be less enjoyable without your companionship.

Finally, I want to express my deepest gratitude to my family. Thanks for your unconditional love, trust, and support all the time. I love you all.

About the Author

Panpan Fan was born on March 17th, 1990 in Shandong province, located in Northern China. In 2010, she started her bachelor in seed science and engineering at Tianjin agricultural university. After completing her four-year undergraduate program, she decided to further her study and enrolled in the MSc program in crop science institute, Chinese Academy of Agricultural Sciences (CAAS). In 2017, she joined “Wageningen University & Research (WUR)-CAAS joint PhD program” and start her PhD journey, under the supervision of Prof. Niels P. R. Anten, Prof. Shaokun Li, and Prof. Jochem B. Evers. She stayed in the Netherlands for 6 months in 2019, and had a pleasant experience with nice and kind colleagues at Centre of Crop System Analysis group of WUR. It provided great encouragement and motivation to finish her thesis. After one year data collection in China, she got a 2-year scholarship from the Chinese Scholarship Council and went to the Netherland for a second time. During her PhD, she worked on the spatial and temporal distribution of nitrogen within the maize canopy.

Her contact email address

Work email: fan.panpan@wur.nl

Private email: fanpanpan1211@163.com

List of Publications

Fan, P., Anten, N. P. R., Evers, J. B., Li, Y., Li, S., Ming, B., & Xie, R., (2023). Changes in the vertical distribution of light and nitrogen within the maize canopy during decades of breeding. (*Submitted*)

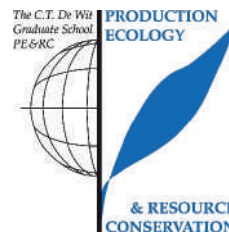
Fan, P., Ming, B., Evers, J. B., Li, Y., Li, S., Xie, R., & Anten, N. P. R. (2023). Nitrogen availability determines the vertical patterns of accumulation, partitioning, and reallocation of dry matter and nitrogen in maize. *Field Crops Research*, 297, 108927.

Fan, P., Ming, B., Anten, N. P. R., Evers, J. B., Li, Y., Li, S., & Xie, R. (2022). Plastic response of leaf traits to N deficiency in field-grown maize. *AoB Plants*, 14(6), plac053.

Fan, P. P., Li, Y. Y., Evers, J. B., Ming, B., Wang, C. X., Li, S. K., & Xie, R. Z. (2020). A new empirical equation to describe the vertical leaf distribution profile of maize. *The Journal of Agricultural Science*, 158(8-9), 676-686.

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review/project proposal (6 ECTS)

- Effects of crop management on maize canopy photosynthesis in old and modern genotypes

Post-graduate courses (8 ECTS)

- Progress in agricultural science and technology; CAAS (2017)
- Multivariate analysis; PE&RC and WIMEK (2020)
- Basic statistics; PE&RC and WIMEK (2020)
- Crop physiology and climate change; PE&RC and University of Florida (2022)
- Environmental signalling in plants; Summer School Utrecht (2022)

Competence strengthening/skills courses (7.55 ECTS)

- Effective academic development; WUR and CAAS (2017)
- Ethics in plant and environmental sciences; WGS (2020)
- Research data management; WUR Library (2020)
- Reviewing a scientific manuscript; WGS (2021)
- Searching and organising literature for PhD; WUR Library (2021)
- Scientific writing; Wageningen in' to Language (2021)
- Efficient writing strategies; Wageningen in' to Language (2021)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.8 ECTS)

- PE&RC First year retreat (2019)
- PE&RC Day (2019)
- PE&RC Last year retreat; (2022)

Discussion groups/local seminars or scientific meetings (4.5 ECTS)

- Frontier literature in plant physiology (2019)
- SKiPR-sharing knowledge in photosynthesis research (2021-2023)
- Photosynthesis discussion group (2021-2023)

International symposia, workshops and conferences (3.8 ECTS)

- 2nd Global conference on agriculture; oral presentation; Berlin, Germany (2022)
- 5th European maize meeting; poster presentation; Bologna, Italy (2023)

BSc/MSc thesis supervision (3 ECTS)

- Effects of nitrogen application rate on the spatial-temporal variation of leaf SPAD reading in the maize canopy

The research described in this thesis was financially supported by the cooperative PhD program between Chinese Academy of Agricultural Sciences and Wageningen University & Research, the National Key Research and Development Program of China, and the China Scholarship Council (CSC202003250113). Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design by Shen Li and Panpan Fan
Layout by Renate Siebes | Proefschrift.nu
Printed by ProefschriftMaken.nl

