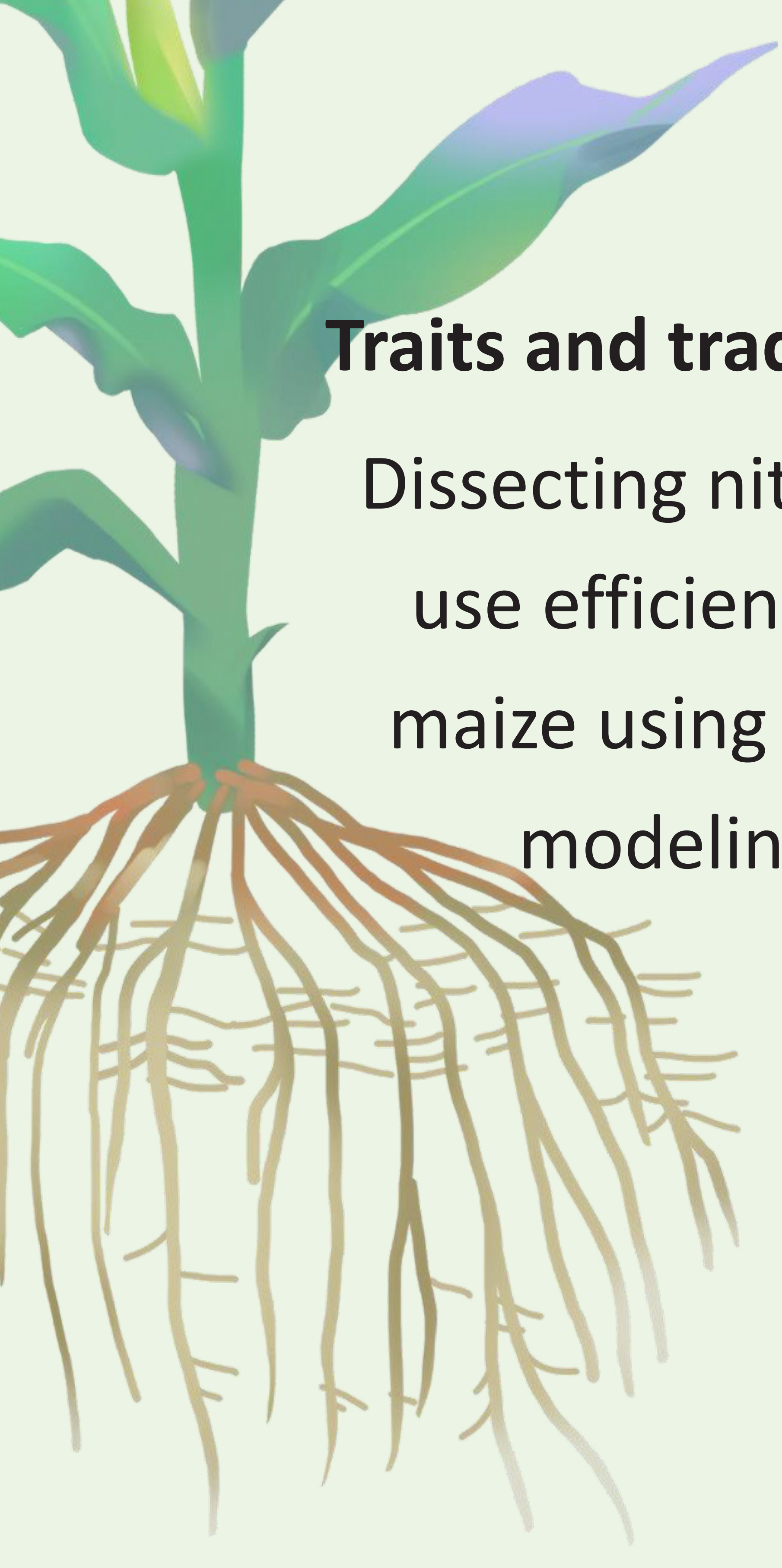

Traits and trade-offs:

Dissecting nitrogen use efficiency in maize using plant modeling

Jie Lu

Propositions

1. Plasticity cannot be ignored in future breeding programs.
   (this thesis)

2. Root system architecture is more important than shoot architecture in producing yield under resource-limited conditions.
   (this thesis)

3. Prediction should not be the main purpose of a mechanistic model.

4. Having clear stakeholders is a prerequisite for the use of scientific results.

5. Interaction with people is harder than interaction with a computer.

6. Embracing cultural diversity in a country provides social stability.

Propositions belonging to the thesis, entitled
Traits and trade-offs: dissecting nitrogen use efficiency in maize using plant modeling.

Jie Lu
Wageningen, 30 April 2024

Traits and trade-offs: dissecting nitrogen use efficiency in maize using plant modeling

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Traits and trade-offs: dissecting nitrogen use efficiency in maize using plant modeling

Jie Lu

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Abstract

Excessive nitrogen (N) can be lost to the ecosystem and result in acidification and/or eutrophication of natural ecosystems. Reducing N application in agricultural systems is necessary to mitigate environmental pollution. Breeding for crops with a high N use efficiency can contribute to maintaining or even increasing yield at lower amounts of nitrogen fertilizer applied. Nitrogen use efficiency is co-determined by N uptake and physiological use efficiency (PE, grain biomass per unit of N taken up), to which soil processes as well as plant architectural, physiological, and developmental traits contribute. This thesis aims to quantify the effects of relevant maize traits and mechanisms to nitrogen use efficiency to support maize breeding programs for sustainable maize production.

I developed and validated a functional-structural plant (FSP) model which simulates nitrogen uptake and the consequences for plant growth, as a function of a range of plant traits. I show that it is primarily the architectural and developmental traits that are relevant for N uptake and plant N use; not so much the physiological traits. Furthermore, I show that plasticity can be important not only under nitrogen-limiting conditions but also under non-limiting conditions, notably by improving nitrogen uptake. Importantly, this increase in uptake was linked to changes in the vertical distribution of roots rather than to total root system size. In addition, I show that using maize cultivars that plastically respond to plant N status by improving N uptake may mitigate any negative effects of stay-green on grain nitrogen content. Finally, I combined cluster analysis with FSP modelling to identify and quantify root system phenotypes that allow for both high yield and high N uptake. The results demonstrated trade-offs in root phenotypes for high yield and for high N uptake, between root sink strength for carbon, and root-to-leaf biomass partitioning. Using cluster analysis I identified two root phenotypes that give both high yield and high N-uptake, the combination of low root biomass and high root length density at 15 cm to 45 cm depth allowed maize to combine high yield and high N uptake.

Overall, this thesis shows combining ecological principles with current breeding criteria can add new dimensions to the process of selection for new cultivars. However, we still need to understand more about the role of trade-offs between traits to maximize plant growth while being efficient in resource use, as well as the role of phenotypic plasticity in the relationship between productivity and resource capture over a wide range of environments. Therefore, combining *in silico* and *in vivo* approaches can allow a better and more solid understanding of how individual physiological mechanisms influence both plant and crop performance.

Keywords: functional structural plant model; maize; nitrogen uptake; phenotypic plasticity; phenotyping; physiological efficiency; root system architecture.

Contents

Chapter 1

General introduction

Background

Maize is one of the major crops worldwide and serves as a crucial source for food, feed, and industrial materials. At the global level, maize grain is primarily used as feed (56% of production), non-food uses such as for biofuel (20% of production), and food (13% of production) (Erenstein et al., 2022). China is the world's second-largest producer of maize, with an annual production of 260 million tons between 2016 and 2020, according to FAOSTAT (2020). In 2018 in China, the total sown area of maize was 42.1 million hectares, accounting for 25.4% of the total crop area, and the total yield of maize was 257 million tons, accounting for 42.5% of the total yield of cereal crops (the National Bureau of Statistics of China, 2019). Even though maize can grow across all regions of China, there is a relatively concentrated narrow belt for maize production from Northeast to Southwest, called the corn belt (Li, 2009). Within the corn belt, the Huang-huai-hai region and the northeast region contribute ∼70% of the maize production in China (Li, 2009).

Maize management in China

China has a high demand for food and feed due to its population size of around 20% of the world population. Since China contains only roughly 7% of the arable land, efficient and optimized use of crop land is crucial. Application of nitrogen (N) fertilizer is an important management measure to ensure maize reaches its potential grain yield (Burney et al., 2010; van Grinsven et al., 2022). Since the 1980s, the recommendation for N application has relied on a yield-maximization strategy (Cui et al., 2008). As the world's largest consumer of N fertilizer, China has a 137% higher N input per hectare than the world average, while the average yields of the major crops are not equivalently higher (Wheat: 58% higher, rice: 53% higher, and maize: 7% higher) (Shen et al., 2023; Yu et al., 2022). This excessive use of N fertilizer causes many environmental problems. The surplus N is lost through leaching, surface run-off, ammonia volatilization or denitrification, and subsequent N_2 or N_2O volatilization to cause acidification and eutrophication of natural ecosystems and global warming (Cameron et al., 2013; Ju & Zhang, 2017). The hot spots of overuse of N fertilizer in China and major maize production regions are largely overlapping (Wang et al., 2018; Li, 2009). To eliminate the negative effects of N input on the environment, enhancing crop nitrogen uptake and optimizing the N application are essential.

Next to high N application, optimizing planting density has also been an important management strategy to improve maize yield (Sarlangue et al., 2007; Xu et al., 2017). A 8.5% to 17% yield gain is possible by optimizing planting density (Luo et al., 2023). However, too dense a canopy may lead to a reduction in grain yield and the optimal density is region-dependent (Luo et al., 2020; Xu et al., 2017). Between 1960 and 2020, planting density of maize increased in the major maize-growing region in Northern China and the Huang-huai-hai region in China (Xu et al., 2017; Luo et al., 2020, 2023).

It is possible to reduce N application to 180 kg/ha while maintaining grain yield by increasing planting density to 8.25 plant/m^2 with the use of a suitable genotype (Tian et al., 2022). However, we do not know well what combinations of plant traits are responsible for yield maintenance at reduced N application while planted at high density.

Maize breeding in China

Parallel to the increases in N application and population density, maize breeding has focused on high grain yield under those conditions. The modern maize breeding program in China was initiated in the 1950s by selecting inbred lines from local open-pollinated varieties. Then, double-cross cultivars took over in China from 1960s to 1970s. After that, single-cross maize hybrids became dominant in the 1970s in China (Li, 2009). From 1975 to 1996, China's average maize grain yield increased from 2.5 tons per hectare to 5.2 tons per hectare (Li, 2009). Around 50% improvement of grain yield from the 1970s to the 2000s is due to breeding, based on the estimation from field experiments with multiple maize cultivars released from the 1950s to the 2000s (Niu et al., 2013; Ci et al., 2011). However, with breeding for high-yielding maize over the last forty years, nitrogen use efficiency of cultivars from China decreased over time, while nitrogen use efficiency of cultivars released from the USA first decreased until 1990s but then increased slightly (Chen et al., 2021). Understanding how N use efficiency is determined by plant traits is important to assist further breeding for high-yielding cultivars that do not rely on high N input to reach high production levels.

Aims of the thesis and the larger agricultural green development program

This study is part of the Agricultural Green Development (AGD) program in China. This program aims to assess current agricultural practices in China on their sustainability and to provide insights and policy recommendations to different stakeholders at different levels. The study presented in this thesis focuses on understanding plant mechanisms and providing suggestions for future breeding targets to arrive at higher nitrogen use efficient maize cultivars allowing to reduce N application in a still intensive agricultural system.

Understanding mechanisms that influence plant traits and interactions among neighboring plants are necessary to maintain the grain yield when N application is reduced. Such understanding can further assist breeding towards a more sustainable direction. To improve this understanding, I developed an analytical tool based on current knowledge of N use in maize plants. In the following sections, I introduce the tool used in this thesis and the current understanding of relevant plant mechanisms.

Functional-structural plant modeling

In this thesis, I adopted the functional-structural plant (FSP) modeling approach to explore how plants interact with their environment including neighboring plants, and how this in turn impacts plant N uptake and grain production. This type of model typically encompasses 3D structures of plants at the organ level, with plant development governed by physiological processes and environmental factors. The explicitly described 3D structure in this type of model provides great potential to explore the effects of plant structural changes in reaction to the environment on resource capture. Unlike crop models (e.g. Chenu et al., 2017; Angulo et al., 2013), which are mainly used to predict yield for various environmental conditions or agronomic management, FSP modeling approaches are applied to explore the effects of plant traits or their combinations on resource capture under various conditions (e.g. Postma et al., 2014; Rangarajan et al., 2022; Li et al., 2021; Zhu et al., 2015). To explore the effect of novel traits on plant N use and productivity, I developed a whole-plant FSP model of maize, including root and shoot structure, carbon and nitrogen sink-source processes, and environmental factors (soil N and light) based on current knowledge of N usage within the plant. Next to simulating individual plants, this model can simulate multiple individually growing and interacting plants to represent a canopy of individual plants for a whole growing season. Due to the complexity of the interactions between plant phenotype, N uptake and use, the local environments, and interaction with other plants, identifying and quantifying the effect of related plant traits to improve understanding of plant N use is barely done.

Plant traits are affected by both environmental and genetic factors. Phenotypic plasticity causes variation in plant phenotypes from identical genotypes across different environments (Ballaré et al., 1994; Price et al., 2003; Schneider, 2022). Plants need to have plasticity to adapt to climate change (Brooker et al., 2022). However, excessive or insufficient plasticity can lead to increased heterogeneity in plant performance within the population, which may be unfavorable in agricultural systems (Chacón-Labella et al., 2019). It is still unclear to what extent plasticity can be beneficial in maize production. Furthermore, under high planting density, smaller plant sizes are found than under low planting density for identical genotypes (e.g. Shao et al., 2018). This variation in phenotype under different environments is due to resource limitations, which do not correspond to plants actively reacting to their environment. Therefore, to clarify this concept and based on the literature, in this thesis, I've further divided plasticity into two categories: active plasticity and passive plasticity (Brooker et al., 2022). Passive plasticity in this thesis refers to the plasticity due to resource limitation, while active plasticity refers to the changes in phenotype due to plants actively responding to environments (Schneider, 2022; Van Kleunen & Fischer, 2005).

The FSP modeling approach can be one of the advanced tools to understand the contribution of active plasticity to plant performance in both plant and crop stands. The effect of passive plasticity on plant performance is an emergent property of this type of model since limited resources (e.g. light) from parameter settings are assigned to each plant in the scene. Meanwhile, specific plastic responses (i.e. active plasticity) can be implemented into this type of model and when simulating multiple interacting individuals the model provides the option to upscale to explore the effect on plant performance at a crop level. However, based on our knowledge, only a few studies have explored the effect of specific plastic responses on plant performance (Bongers et al., 2018, 2019; Henke et al., 2014) while none of them considered the effect of plastic responses of whole plants at stand level.

Nitrogen use in maize plants

N use efficiency (g grain dry weight/g N applied) depends on soil attributes, plant physiological, and architectural traits including developmental root and shoot traits, as well as plasticity in those traits. N use efficiency generally contains the two major plant processes, N uptake and plant internal N use. Efficient resource capture depends on both plant root and shoot structure and their interactions with light and soil N. The physiological efficiency (PE) of internal N use is defined in the context of this thesis as the grain dry weight divided by the total nitrogen taken up (g grain dry weight/ g N in the plant). Grain N content can determine grain quality. To achieve reasonably high PE without reducing grain quality, it is crucial to consider the balance between maintaining photosynthesis and improving grain N.

The carbon (C) and N assimilation processes are interconnected and influenced by sink-source relationships. C is assimilated through photosynthesis and determines the growth of plant organs such as leaves, stems, roots and grains. During the early growth stage, the development of plant architecture lays the foundation for subsequent grain formation (Dietz et al., 2021). The vertical leaf area and N distribution determine the efficiency of light interception and use of plants in a canopy. Less leaf area tends to be located at higher leaf ranks in modern maize cultivars to enhance deeper light penetration in the canopy (Perez et al., 2019; Ma et al., 2014; Zhai et al., 2017). Deeper light penetration also optimizes canopy leaf N distribution, which enhances canopy photosynthetic capacity (Mantilla-Perez & Salas Fernandez, 2017). Optimizing canopy leaf N allocation is a key adaptive mechanism to maximize canopy photosynthesis rate and crop production under N-limited conditions during the grain filling stage in maize (Mu et al., 2016). Meanwhile, C assimilated from the shoot is also transported to the root and the C determines root growth, which directly feeds back on soil N uptake (Irving, 2015). Different maize genotypes differ in N uptake and allocation, affecting seasonal carbon assimilation through differences in light interception efficiency. There are still gaps in knowledge of which and how plant traits do interact and can contribute to affect N uptake and allocation.

The grain-filling stage is the most important stage for grain C and N accumulation (Johnson & Tanner, 1972). Grain quality in terms of protein concentration is highly associated with grain N level. In maize, N for grain filling does not only come from new N assimilation during reproductive growth (∼50%) but also incorporates N remobilization from N assimilated in vegetative organs before silking (∼50%) (Mueller & Vyn, 2016; Hirel et al., 2007; Ning et al., 2017). Leaves are the major photosynthetic organs and maintaining photosynthesis requires N. Rather than leaves, the stem is the most important temporary sink for N accumulation before grain filling and also an important source when remobilizing N during pollination, fertilization, and kernel set (Yang et al., 2017; Mueller & Vyn, 2018). Maize leaves and stems contribute approximately 45% each to the total nitrogen remobilization towards the ear, while remobilization from roots contributes only 10% (Ta & Weiland, 1992). Modern maize cultivars have a 10 to 15% greater N uptake after silking and have higher grain mass accumulation compared to older cultivars, particularly in low nitrogen conditions (Mueller & Vyn, 2016).

Based on current knowledge, I propose to explore three categories of plant traits, including stay-green, root traits, and plasticity, as potentially relevant in influencing plant N use and N uptake through N assimilation and remobilization. In addition, I explore potential ideotypes for plant N use and N uptake since improving single traits is not always enough to reach breeding purposes. Taking multiple traits, their interactions, and environments into consideration is necessary when breeding for efficient cultivars. Ideally, breeding results in cultivars that maintain a linear productivity increase over a larger part of the yield-N input curve until achieving crop production potential (Fig. 1.1) and thereby reach the same production at lower N input. In this thesis, I explore the plant traits related to stay-green, root system functioning and plasticity that may reach same production at lower N input, using FSP modeling.

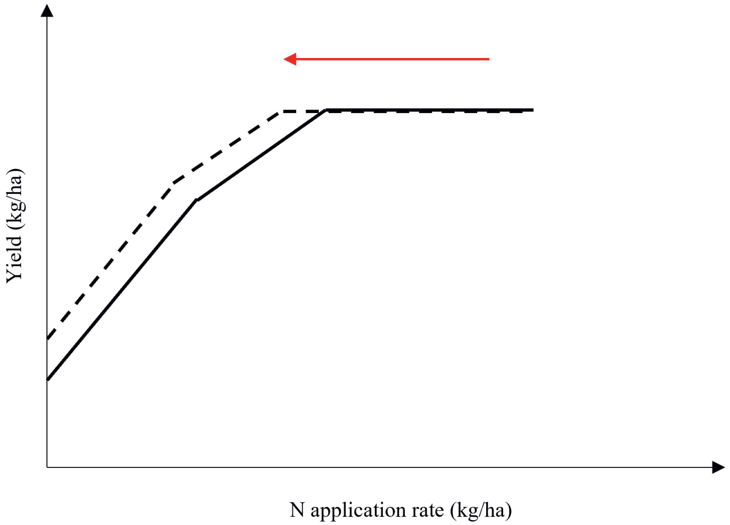

Figure 1.1: Illustration of the envisaged effect of increased nitrogen uptake efficiency on yield and the option to reduce N application. The solid line represents the yield pattern of current cultivars with N application. The dotted line represents a desirable high N uptake efficient cultivars that reaches similar yields at reduced N application, through a higher uptake of applied N. The red arrow represents the breeding effort.

Stay-green to influence plant N use

Delaying leaf senescence and thus making plants stay green for longer can increase plant yield by extending the photosynthesis period and allowing for more carbon assimilation (Masclaux-Daubresse et al., 2010; Thomas & Ougham, 2014). Multiple factors induce the initiation of leaf senescence. The stay-green genotype has a lower rate of grain development before reaching 70% of final grain weight and a later decrease in shoot N concentration than the normal genotype. This suggests that the leaf senescence may not be solely attributed to leaf aging, but may also be influenced by the rates of grain sink development or the rate of shoot N reallocation to grain (Pommel et al., 2006).

Stay-green has been treated as an important charcteristic in modern maize breeding programs from 1930 to the early 21st century (Chen et al., 2021). However, this stay-green characteristic is considered detrimental to vegetative nitrogen remobilization and grain nitrogen concentration (Kosgey et al., 2013; Mueller & Vyn, 2016; Yang et al., 2017). This is because the major photosynthetic protein in leaves, Rubisco, is a major source of nitrogen remobilization (Masclaux-Daubresse et al., 2010). Starting N remobilization early, particularly under low N conditions, is not advantageous for grain biomass accumulation while remobilizing N too late is detrimental to grain protein content. Interestingly, in modern cultivars, the decrease of grain N concentration is not always as much as expected (Mi et al., 2003). Therefore, in this thesis, we explored novel mechanisms that could compensate for the trade-off between grain yield and grain quality in stay-green cultivars.

Maize root traits to influence N use

N uptake is largely determined by traits related to root system architecture (RSA), which is defined as the spatial arrangement of root components in the soil. For maize, the RSA is directly associated with biomass accumulation and grain yield (Hammer et al., 2009). Up to 50% of the assimilates produced daily contribute to root growth (Lynch, 2007).

The maize root system comprises of two major components: the embryonic root system and the post-embryonic root system. The embryonic root system consists of a primary root and several seminal roots, while the post-embryonic root system comprises shoot-borne roots, also known as crown roots for underground nodes and brace roots for aboveground nodes. On average, there are 6 whorls of crown roots and 2 to 3 whorls of brace roots. Crown roots are mainly responsible for avoidance of lodging and water/nutrients uptake. Functions of brace roots are not fully clear but are proposed to also provide support to resist lodging and take up water and nutrients (Hochholdinger et al., 2004). Genotypes with fewer crown roots acquire N from deeper soil layers than those with more crown roots (Saengwilai et al., 2014b; Schneider et al., 2021). Manually reducing the number of the roots within a single genotype does not directly result in reducing root biomass accumulation but results in more lateral and deeper roots (Guo & York, 2019). Under low N, the maize genotypes with few but long laterals show greater rooting depth, better shoot N content, leaf photosynthesis and shoot biomass than the genotypes with many but short laterals in both empirical and modeling studies since few lateral roots can reduce the self-competition for N (Postma et al., 2014; Zhan & Lynch, 2015). In addition, due to the whole plant carbon balance, few lateral roots can result in deeper axial roots. This is especially beneficial for deeper soil N capture since N is a mobile nutrient in the soil (Postma et al., 2014).

Besides the root system architecture, root anatomic traits also play important roles in nutrient uptake. Root cortical aerenchyma (RCA) can maintain plant growth under drought and low soil fertility by reducing metabolic costs and enhancing root exploration for deeper soil layers (Lynch, 2007; Postma & Lynch, 2011). However, many such root traits are usually associated with some trade-offs. Even though few roots can promote deeper soil nutrient usage, too few crown roots can result in lodging and therefore reduce grain yield (Saengwilai et al., 2014b; Liu et al., 2012; Hostetler et al., 2022). High RCA may reduce radial transport, mycorrhizal colonization, and disease resistance (Lynch, 2007; Postma & Lynch, 2011). High-yielding genotypes are typically associated with thicker roots (Schneider et al., 2021; Yang et al., 2019), but theoretically, a thinner root with the same biomass can result in a larger root surface area that improves root nitrogen uptake (Gao et al., 2015).

Even though root traits are usually not under direct selection pressure like yield or aboveground traits, root traits are likely to be indirectly selected for. Root traits are also influenced by increasing levels irrigation or fertilization (York et al., 2015; Schmidt et al., 2016; Ren et al., 2022). The root systems of the commercial genotypes released in the 2000s were found 7 degrees more shallow, with one less nodal root per whorl, double the distance from nodal root emergence to lateral branching, and less area of individual metaxylem vessels but more metaxylem vessels compared to commercial genotypes released before 1941 (York et al., 2015). Those changes are considered as caused by the indirect selection for N stress created due to high planting density (York et al., 2015). Even with the opposite direction of changes in root angle within the Chinese maize genotype released from the 1950s to the 2000s, the evidence of indirect selection for root traits such as root angle becomes obvious (Ren et al., 2022). The root traits seem to play a crucial role in enhancing yield through indirect selection in intensive systems. Unfortunately, obtaining enough useful root data from the field remains a challenge, leading to gaps in understanding of how root traits impact plant performance. In this thesis, I make use of existing root data and design models to further understand and quantify the contribution of root traits to N uptake and plant internal N use to better develop future experiments.

Plastic responses to influence plant N use

Many root traits are highly plastic (Schneider & Lynch, 2020). Within a single genotype, a shallow root may become steeper under N deficient conditions (Trachsel et al., 2013). When treated with local high nitrogen, maize roots can actively react to the signal by increasing lateral root length in high nitrogen locations while decreasing root growth in low nitrogen locations (Yu et al., 2014a). Drought reduced lateral root branching density within one genotype but increased total root length (Zhan & Lynch, 2015). It is unclear whether root plasticity is beneficial for plant N use and uptake and how it interacts with genotypes or other factors to affect grain yield.

In this thesis, I considered two active plastic responses to plant N status. The first is related to carbon allocation between root and shoot (Brooker et al., 2022). A higher root-to-shoot ratio corresponded with better maize nitrogen use efficiency in a comparison of Chinese and US varieties (Yu et al., 2015). Nutrient deficiency generally increases the maize root-to-shoot ratio based on possible consequences of carbon allocation, while other stresses such as high plant density seem to decrease the maize root-to-shoot ratio (Mašková & Herben, 2018; Wang et al., 2019b; Fernandez & Rubio, 2015; Yu et al., 2015; Shao et al., 2019). This mechanism can be explained by the functional-equilibrium theory. This theory describes that plants allocate more carbon to the organ, which is the most relevant for capturing the most limiting resource to support its growth (Lambers, 1983; Shipley & Meziane, 2002). According to this theory, when leaf nitrogen concentration increases along with root-specific activity, the root-to-shoot ratio decreases and there is less need for additional root biomass (Scheible et al., 1997; De Groot et al., 2003; Hilbert, 1990).

The second important active plastic response considered in our study is the axial root emergence rate in response to plant N status. Fewer axial roots under low N are linked with a lower root emergence rate. Under low N, fewer axial roots have been found than in the same genotypes under high N, which can result in greater N uptake (Saengwilai et al., 2014b; Ajmera et al., 2022; Guo & York, 2019). It is still unclear how these plastic responses contribute to plant N uptake and use, and to which extent they could be considered as interesting target traits for nitrogen-use efficient maize.

Root system ideotypes for N use

A crop ideotype has been defined as a plant that can have expected performance such as yield or grain quality for specific environments. The ideal crop phenotype is usually a weak competitor to avoid redundant growth to interfere with neighbors (Donald, 1968). There were several efforts to identify crop ideotypes under certain environments or management scenarios. Meanwhile, rather than purely based on field observations, scientists started to consider modeling approaches as promising tools to further quantify the ideotype (Breitler et al., 2022; Reddy, 2021; Eker et al., 2022; Bourke et al., 2021). However, it is still challenging to identify and quantify root system ideotypes because of data availability issues, partly also because root systems are hard to observe with non-destructive methods.

The optimal maize root system phenotype for water and N acquisition in low-input systems has been proposed as “steep, cheap, and deep” (Lynch, 2013). Many traits of this root system ideotype such as deeper roots have been found experimentally beneficial for nutrient uptake (Saengwilai et al., 2014b). However, this ideotype has not taken plasticity and plant-to-plant interaction into consideration which can become crucial for medium input systems (Lynch, 2013, 2019). Based on long-term field observations, Mi et al. (2016) proposed a root system ideotype by considering nutrient uptake, lodging resistance, and heterogeneously distributed soil nutrients. In their proposed root system ideotype, shallow axial roots, long lateral roots and strong response to nutrient patches are ideal for seedling maize plants while steep, thick, few and long lateral roots on nodal roots at 1 to 4 whorls; shallow, thick, dense, more aerenchyma and long lateral roots of nodal root at 5 to 7 whorls; and strong response to nutrient patches are ideal for adult maize plants (Mi et al., 2016). In this proposed ideotype, scientists start to take plasticity in root system into consideration (Mi et al., 2016). However, due to complexity in field conditions, large variation in each of these root traits and the strong linkages among root traits, a quantitative understanding of root system ideotypes for high N uptake and high yield remain limited.

Research objectives

In this thesis, I aimed to quantify the effects of relevant maize traits and mechanisms to nitrogen use efficiency, aiming to identify target traits to support maize breeding programs for sustainable maize production. The specific research objectives are:

1. to quantify the contribution to N uptake and PE of architectural and physiological traits that vary among maize genotypes in relation to soil nitrogen conditions.

2. to quantify and explore how active plastic root system responses to plant N status contribute to N uptake, yield, and PE.

3. to explore the relevance of active plastic root system responses to plant N status in mitigating possible negative effects of an enhanced leaf life span (i.e. stay-green) on N remobilization to the grains.

4. to identify and quantify the maize root trait combinations and root system phenotypes that give high N uptake without compromising yield of maize at high density, supporting the transition to lower N input.

Outline of the thesis

In order to address the questions and aim given above this thesis consists of the following six chapters: this general introduction (Chapter 1) in which I introduce the context of the work, the main knowledge gaps and the ensuing research objective, four research chapters one for each of the above research questions (Chapters 2 to 5), and a general discussion in which I will revisit the above aim (Chapter 6).

In Chapter 2, I quantified the contribution of architectural, developmental, and physiological plant traits to maize nitrogen uptake and use, to identify potential traits breeding might target. To this end, I developed and validated a functional-structural plant model which simulates nitrogen uptake and the consequences for plant growth, as a function of a range of plant traits. I show that it is primarily the architectural and developmental traits that are relevant for N uptake and plant N use; not so much the physiological traits.

In Chapter 3, I addressed the question to which extent plant nitrogen use depends on plastic responses in biomass allocation and root formation to plant nitrogen status, and if whole-plant modeling could be instrumental in addressing this question. I show that plasticity can be important not only under nitrogen-limiting conditions but also under non-limiting conditions, notably by improving nitrogen uptake. Importantly, this increase in uptake was linked to changes in the vertical distribution of roots rather than to total root system size. This work provides quantified insight into specific beneficial plastic responses and how these plastic responses influence nitrogen usage. The information and methods in this study indicate directions to breeding for plasticity aimed at more resource-use-efficient cultivars.

In Chapter 4, I applied the functional-structural maize model to explore to which extent such plastic responses interact with stay-green characteristics in affecting grain N. I found that the absence of leaf senescence contributed to grain yield under high N, while under low N, the combination of plasticity and stay-green improved grain yield. Plastic responses reduced the negative effect of stay-green on grain N concentration by improving N uptake, especially under low N. Using maize cultivars that plastically respond to plant N status by improving N uptake may therefore mitigate any negative effects of stay-green on grain quality.

In Chapter 5, I combined cluster analysis and functional-structural maize modeling to identify and quantify root and root system phenotypes that combine both high yield and high N uptake, aiming to ultimately propose ideotypes that are N-use efficient under high planting density allowing for a reduction in N input without a yield penalty. The results demonstrated trade-offs in root phenotypes when selected based solely on high yield or high N uptake. Then, I identified two root phenotypes that combined both high yield and high N-uptake by altering large root sink strength or high root-to-leaf ratio response. I found that genotypes with higher grain yield and N uptake have lower total root biomass but higher root length density between 15 cm to 45 cm soil depth when compared to those with lower grain yield and N uptake. I illustrate that cluster analysis of FSP modeling results can be used to identify improved root system phenotypes under various environments which can be useful information when defining targets for breeding.

In Chapter 6, I discussed how the modeling studies of this thesis contribute to promote the understanding in breeding for high nitrogen use efficient maize cultivars, including the specific role of FSP modeling in dealing with trade-offs and plasticity. Furthermore, I provided potential applications and possible challenges of the FSP model developed in this study in two aspects. Firstly, the FSP model has potential for combination with genomic studies to assist breeding for high nitrogen use efficient maize. Secondly, the FSP model can be used to identify novel mechanisms both for maize grown in monoculture and in mixtures that could improve nitrogen use efficiency in a system.

Chapter 2

Identifying and quantifying the contribution of maize plant traits to nitrogen uptake and use through plant modeling

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Abstract

Breeding for high nitrogen use efficient crops can contribute to maintaining or even increasing yield with less nitrogen. Nitrogen use is co-determined by N uptake and physiological use efficiency (PE, biomass per unit of N taken up), to which soil processes as well as plant architectural, physiological and developmental traits contribute. The relative contribution of these crop traits to N use is not well known but relevant to identify breeding targets in important crop species like maize. To quantify the contribution of component plant traits to maize N uptake and use, we used a functional-structural plant model. We evaluated the effect of varying both shoot and root traits on crop N uptake across a range of nitrogen levels. Root architectural traits were found to play a more important role in root N uptake than physiological traits. Phyllochron determined the structure of the shoot through changes in source: sink ratio over time which, in interaction with light and temperature, resulted in a significant effect on PE and N uptake. Photosynthesis traits were more relevant to biomass accumulation rather than yield, especially under high nitrogen conditions. The traits identified in this study are potential targets in maize breeding for improved crop N uptake and use.

Introduction

In the past five decades, nitrogen fertilizer application has increased globally along with an increased requirement for food production (Glass, 2003; De Vries et al., 2013). However, more than 60 % of the nitrogen applied is lost through leaching, surface run-off, denitrification and volatilization (Raun & Johnson, 1999; Wang & Li, 2019). For maize, like for many other crops, the maximum production per unit of N fertilizer applied is considered to be genotype-specific (Tsai et al., 1992). Therefore, breeding for high overall crop nitrogen use efficiency at reduced nitrogen inputs is one of the approaches to allow farmers to reduce nitrogen fertilizer input while maintaining high production levels.

Overall crop nitrogen use efficiency is a complex trait and the combined result of several underlying processes during crop growth. Nitrogen use efficiency can be decomposed into two main components: nitrogen uptake efficiency and a measure for utilization efficiency of nitrogen called physiological efficiency (PE) (Van Keulen, 1982). Nitrogen uptake efficiency is expressed as kg N taken up per kg N in the soil; PE is the efficiency with which the N taken up is converted into grain biomass, in kg grain per kg N taken up. In maize, major genetic variation in nitrogen use efficiency under non-limiting nitrogen conditions relates to differences in nitrogen uptake efficiency. Under limiting nitrogen, the genetic variation in nitrogen use efficiency is mainly determined by differences in PE (Gallais & Coque, 2005; Xu et al., 2012a; Li et al., 2015). However, it is still unclear which plants traits are underlying the observed difference in PE and N uptake efficiency.

A range of root system architectural, anatomical and physiological traits are associated with nitrogen uptake (Mi et al., 2010, 2016). Fewer crown roots increased 95% of rooting depth located from 26.7cm to 34.4cm in a field experiment in South Africa. This increase of rooting depth contributed to more nitrogen uptake from deeper soil layers and was found especially beneficial under low nitrogen conditions (Saengwilai et al., 2014b). A lower number of crown roots has been associated with a larger nodal root diameter. Fewer but thicker roots were found to result in better growth in a low N environment (Schneider et al., 2021). In addition, increasing the amount of root cortical aerenchyma (RCA), which is related to root tissue density (Postma & Lynch, 2011; Saengwilai et al., 2014a) can increase the rooting depth by reducing metabolic costs of roots through reducing respiration. Besides architectural and anatomical traits, physiological traits such as those related to both high and low affinity transporters of nitrate play a role in nitrogen uptake (Lazof et al., 1992; Parker & Newstead, 2014; York et al., 2016). In experiments, those architectural and physiological traits usually interact, depending on the genotype that is used, and display plastic responses to environmental factors. Therefore, it is difficult to quantify experimentally the effects of individual traits on efficient N use through their effect on N uptake efficiency.

Traits related to shoot architecture, leaf photosynthetic traits and nitrogen reallocation further contribute to nitrogen use efficiency by affecting PE. Traits such as leaf angle distribution along the stem and leaf number determine light distribution within the canopy and as a consequence light interception, leaf area, plant height and yield accumulation (Huang et al., 2017). Photosynthetic traits such as the maximum photosynthesis rate of leaves also differ among maize cultivars (Chen et al., 2013). Maximum photosynthesis rate can be linked with leaf nitrogen content since leaf nitrogen is the major component to construct photosynthetic enzymes such as rubisco (Vos et al., 2005; Mu et al., 2016). When grains start growing, there is competition for nitrogen between grains to form the protein for the next generation and leaves to continue photosynthesis. The ability to maintain a high leaf photosynthesis rate by keeping rubisco levels high is called “stay green”. “Stay green” cultivars tend to have a higher yield combined with a reduced nitrogen remobilization efficiency and a reduced protein concentration in the seeds (Masclaux-Daubresse et al., 2010; Mi et al., 2003). These traits determine PE, and experimentally it is hard to disentangle their effects on both components of PE and maize crop performance.

Crop growth models can help to reveal to which extent individual plant traits contribute to nitrogen use efficiency (Semenov et al., 2007; Asseng et al., 2001). However, in most traditional crop models many plant traits and processes relevant to PE are not explicitly included. Here a modeling approach is adopted, called functional-structural plant (FSP) modeling, that does allow to include such traits and related processes. FSP models simulate stand performance based on individual plants, their growth, physiological functioning and above and below ground 3D architecture when growing in a crop stand and competing with each other for resources (Vos et al., 2010; De Vries et al., 2021). The objective of this study was to quantify the contribution to N uptake and PE of architectural and physiological traits that vary among maize genotypes and in relation to soil nitrogen conditions, using the FSP modeling approach.

Methods

We expanded an existing whole plant FSP model (De Vries et al., 2021; Evers & Bastiaans, 2016) by including plant and soil processes related to nitrogen uptake and utilization. We then used experimental data from six maize cultivars released in different years between the 1970s and the 2000s grown in a field in 2011 to parameterize the model for maize and the experimental data from these six maize cultivars grown in the field in 2010 to evaluate the model. After that, we performed a series of simulation analyses to quantify the contribution of a range of plant traits to N uptake and PE, especially the traits with great potential for further study.

Model description

Our 3D maize FSP model represents development and growth of both root and shoot, as a function of competition for light and soil nitrogen within and between plants (Fig. 2.1; Fig. A.1). Since our goal was to analyze plant behavior, rather than to predict crop performance for specific weather and/or soil conditions like in crop models, in our model the representation of daily light and temperature conditions and the dynamics of soil nitrogen is simpler than in such typical crop models (details below). The model was developed in the GroIMP platform (Hemmerling et al., 2008). To represent maize root systems, seminal roots are initiated as a function of thermal time and the diameters of different root orders are treated as fixed values based on Pagès et al. (2014). Second order (lateral roots on axial roots) and third order (axial roots) root segments are explicitly simulated in 3D while the higher order roots (i.e., lateral roots on lateral roots) are only numerically simulated as carbon sinks but is not considered to enhance N uptake (De Vries et al., 2021). Thus, the model simulates mechanistically plant shoot and root development and growth as driven by respectively temperature, light and soil N and plant carbon and nitrogen sink-source relationships in 3D.

Light response curves were adopted here to simulate photosynthesis at the leaf level as a function of light absorbed by the leaf. After assimilation organic carbon is allocated to each organ to simulate organ growth based on individual organ sink strength which is defined as the sigmoid function of organ potential dry mass and growth duration (Yin et al., 2003). Therefore, unlike in crop models, the leaf expansion rates in our model are co-determined by leaf sink strength, available carbon and thermal time, in a same manner stem, root and grain growth are modeled. Grain growth starts after the initiation of the final leaf. Root carbon sink strength is considered a fraction of leaf sink strength (De Vries et al., 2021). Non-structural root carbon is considered to be negligible (1% of root dry weight) in our model (Wu et al., 2019). Nitrogen, taken up from the soil and reallocated from senescing leaves, is an important component for photosynthetic enzymes. Therefore photosynthetic nitrogen is a crucial determinant of leaf photosynthetic capacity and linked to the light response curve in our model. The N remaining after the photosynthetic nitrogen and the amount of nitrogen required as structural N for organ growth is allocated is stored in a N pool ($Nsource$, g/plant). This nitrogen stored in the N pool is available in the next time step for allocation to leaves, stems and grains.

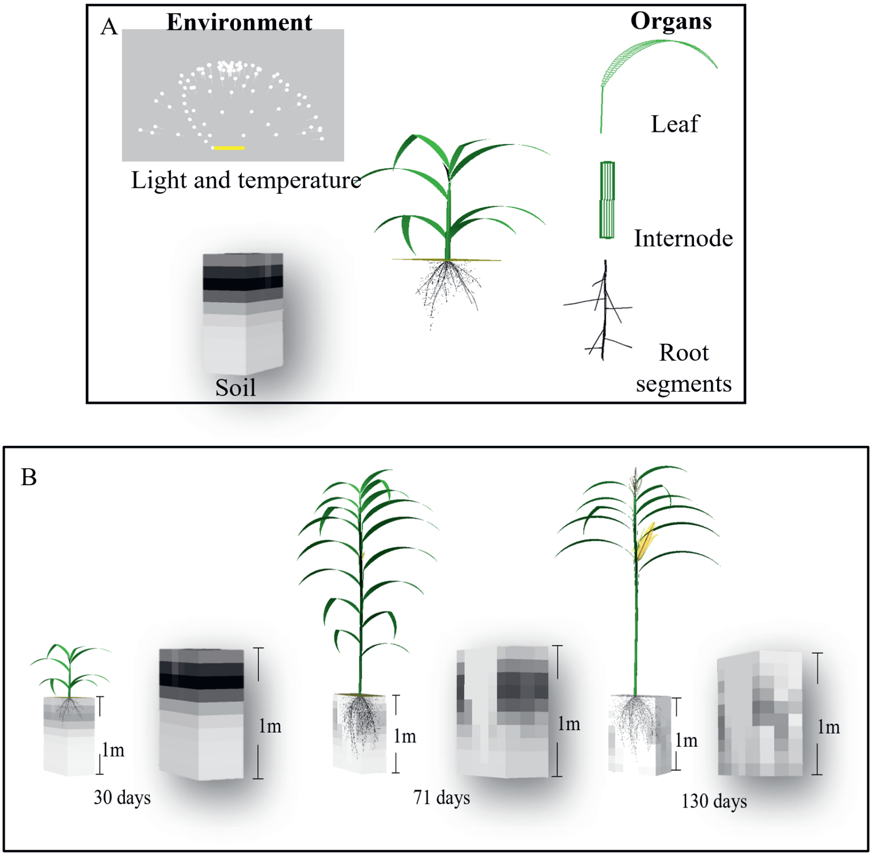

Figure 2.1: Overview of the maize FSP model including the environmental components (soil cells, temperature and light) and organ types (individual leaf, internode and root segments) of the plant model (A) and model output in terms of plant growth and remaining N in the soil profile at three moments during the maize plant development (B). In the soil cells, darker soil cell colors represent cells with higher soil N concentration. Days are days after emergence.

Light and temperature in model

Actual daily light and temperature data were obtained from the Jilin weather station. Since the daily data with erratic temperature and radiation values could easily obscure the subtle effects we aimed to quantify (Li et al., 2021), we used as input the annual course in average daily temperature (T_{avg}, eq. 2.1) fitted to the measured daily average temperatures at the experimental site in 2010.

$$T_{avg} = a + b \times \sin \frac{2\pi(D_y - c)}{365} \tag{2.1}$$

Where a (°C) is the yearly average temperature, b (°C) is the temperature amplitude and c is the day of year with the daily average temperature.

In a similar way the annual course in incoming daily radiation was used as a function of the latitude and day of the year and transmissivity of the atmosphere (Spitters, 1986; Evers et al., 2010), fitted to the daily incoming radiation data from 2010.

To simulate plant growth under field conditions, direct incoming radiation was simulated by using an array of 24 directional light sources based on latitude and day of the year while diffuse light was simulated by using an array of 72 directional light sources positioned in a hemisphere (Evers et al., 2010; Buck-Sorlin et al., 2011). Light capture was simulated by the reverse Monte-Carlo ray tracing algorithm in GroIMP (Hemmerling et al., 2008).

Thermal time and plant development

The phenological development of maize plants from sowing to maturity was expressed as a function of thermal time (tt) (°Cd). Thermal time (eq. 2.2) in the model was calculated from daily average temperatures (T_{avg}, °C) and a maize base temperature (T_b, °C). T_b was set to 10 °Cd for these maize cultivars (Chen et al., 2013). No optimum temperatures for development where considered as in the experiments used to calibrate and check the model daily maximum temperatures rarely were above reported optimum temperatures for development of maize (30.8 °Cd) (Sánchez et al., 2014).

$$tt = (T_{avg} - T_b) \times days \tag{2.2}$$

Soil N

The soil was composed of cubic soil cells of which the size ($Cellsize$, m) was 0.1x0.1x0.1 m. Simulated soil depth was set to 1 m to account for N percolating beyond the rooting zone as observed rooting of the simulated cultivars was maximally 0.6 m. No further root biomass accumulation was observed after silking in the field experiment (Shao et al., 2018). Therefore, root elongation was assumed to stop when all leaves were fully expanded. In contrast to many maize cultivars, root segments are rarely found beyond 0.6 m depth for the maize cultivars and growing conditions for which we conducted the analyses (Ning et al., 2014). As all data used for verification was under no-water-stress conditions and for simplicity, we did not explicitly consider the effects of precipitation or soil temperature.

For individual soil cells at each simulation step (t, day), soil nitrogen ($SoilN(t)$, μmol/m^3) was a function of the amount of N applied at the start of the simulation ($Napp$, μmol/m^3), the additional N application ($Napp2$, μmol/m^3) at certain date ($DNapp2$), the initial soil N ($Ninit$, μmol/m^3), mineralization (Nm, μmol/m^3) and leaching (Nl, μmol/m^{-3}) and uptake by the roots present in the cell (Nu,μmol/m^3). The $SoilN(t)$ is described in eq. 2.3 (Fig. A. 2).

$$SoilN(t)_{i,j,k} = Nm(t)_{i,j,k} - Nl(t)_{i,j,k} - Nu(t)_{i,j,k} + \begin{cases} Ninit_{i,j,k} + Napp_{i,j,k} & (t = 0) \\ SoilN(t-1)_{i,j,k} + Napp2_{i,j,k} & (t = \text{DNapp2}) \\ SoilN(t-1)_{i,j,k} & (t \neq 0 \ \ \& \ \ t \neq \text{DNapp2}) \end{cases} \tag{2.3}$$

Where i, j, and k are the Cartesian coordinates of a soil cell, of which k indicates the vertical coordinate, and $Cvol$ (m^3) is the cell volume. The initial and applied N were assumed to be distributed homogeneously in the top 0.3 m soil. The initial amount of N in the soil cells deeper than 0.3 m were set to 0.

The net N change at any point in time in a soil cell at depth k through leaching ($Nl(t)_{i,j,k}$) was always the sum of loss to the cell at depth $k + Cellsize$ directly below, and gain from leaching from the soil cell at depth $k - Cellsize$ directly above. To reduce the computing time, no lateral N movement between soil cells was considered. Leaching finally accumulated in the bottom soil layer from which no leaching loss was calculated (eq 2.4, 2.5).

$$Nl(t)_{i,j,k} = \frac{1}{2}\alpha \times SoilN(t)_{i,j,k} \tag{2.4}$$

$$SoilN(t+1)_{i,j,k+Cellsize} = SoilN(t)_{i,j,k+Cellsize} + Nl(t)_{i,j,k} \tag{2.5}$$

where α is soil permeability to water and hence to nitrogen (Addiscott & Whitmore, 1991).

To simulate N made available by mineralization in the top 0.3 m soil (Stanford & Smith, 1972), organic nitrogen (Norganic, μmol/m^3) was assumed to gradually mineralize and provide extra N that can be taken up by the root (eq. 2.6, 2.7, 2.8).

$$Nm(t)_{i,j,k} = \beta \times Norganic(t)_{i,j,k}, k \leq 0.3m \tag{2.6}$$

$$SoilN(t+1)_{i,j,k} = SoilN(t)_{i,j,k} + Nm(t)_{i,j,k} \tag{2.7}$$

$$Norganic(t+1)_{i,j,k} = Norganic(t)_{i,j,k} - Nm(t)_{i,j,k} \tag{2.8}$$

where β is the relative mineralization rate of available soil organic nitrogen.

N uptake

N uptake by the root system was calculated as the cumulative uptake across all soil cells (eq. 2.13). For each soil cell separately N uptake was calculated for the root segments in that soil cell. For per root segment uptake, Michaelis-Menten kinetics were used to simulate N uptake through the high affinity transport system ($HATS$, μmol/(m$^2 \cdot$ day), Eq. 2.9) and a linear relation with soil N to simulate N uptake through the low affinity transport system ($LATS$, μmol/(g $\cdot$ day), eq. 2.10) (Siddiqi et al., 1990). eq.2.12 represents nitrogen uptake (μmol/day) for each root segment per simulation through the two N transport systems. E_N (dimensionless, eq. 2.11) represents a negative feedback of plant nitrogen concentration on root nitrogen uptake by the combined transport systems. Based on previous studies, this negative feedback was added to N uptake in the model (Forde & Clarkson, 1999; Bertheloot et al., 2011; Barillot et al., 2016).

$$HATS(t) = \frac{I_{max} \times (SoilN(t) - Nmin)}{Km + (SoilN(t) - Nmin)} \tag{2.9}$$

$$LATS(t) = K_2 \times (SoilN(t) - Nmin) \tag{2.10}$$

$$E_N = e^{-P \times Nc_{plant}} \tag{2.11}$$

$$Nuptake(t, n, x) = E_N \times (HATS(t) \times A(n, x) + LATS(t) \times rootDW(n, x)) \tag{2.12}$$

$$Nu(t) = \sum_n \sum_x Nuptake(t, n, x) \tag{2.13}$$

Where x is the root segment rank within a root and n is the root primordium number. $Nmin$ (μmol/L) is the minimum N concentration for nitrogen uptake. I_{max} (μmol/(m$^2 \cdot$ day)) is the maximum influx rate of N. Km (μmol/m^3) is the soil nitrogen concentration at half I_{max}. Both parameter values were derived from York et al. (2016). K_2 (μmol/(g $\cdot$ day)) is the constant rate of $LATS$ activity, which was derived from Pace & McClure (1986). A (m^2) is the surface area of an individual root segment and $rootDW$ (g) is the dry weight of the individual root segment. P (dimensionless) is the coefficient for the negative feedback of plant N concentration on root nitrogen uptake.

Nc_{plant} (g N/g DW) is the whole plant nitrogen concentration.

A certain amount of nitrogen is fixed in the root to form root structure to support growth of the root system. This amount of nitrogen is assumed not-available for the shoot. In the model, it is a fixed fraction ($fNroot$, g N/g DW) of the root dry weight of the plant accumulated during each simulation step. The rest of the nitrogen is available to the shoot (eq. 2.15). When during a time step the amount of newly acquired and stored N cannot fulfill the N demand for growth of the root, all N is fixed in the root and none of the N taken up in a time step becomes available to the shoot. This leads to a reduction in the shoot N concentration. In case N was available as stored N from the previous time step this is partly allocated to the root and partly to the shoot based on their relative sink strength for N.

$$\Delta RootN(t) = fNroot \times \Delta rootDW(t) \tag{2.14}$$

$$Nu_{shoot} = MAX(Nu - \Delta RootN, 0) \tag{2.15}$$

Grain nitrogen

Once the plant enters the reproductive stage, part of the available nitrogen is allocated with priority to the grains (Masclaux-Daubresse et al., 2008, 2010), using a linear relationship between leaf N and grain N concentration in maize (Fernandez et al., 2021). Under low nitrogen, the grain nitrogen influx rate was less than the rate under sufficient nitrogen (Ning et al., 2017). Therefore, to account for balancing the plant N flux between photosynthetically active leaf tissue and grain protein accumulation, the fraction of nitrogen ($fNgrain$, g N/g DW) allocated to the grains was considered to be a function of leaf nitrogen concentration from the previous time step ($Nleaf(t-1)$, g N/g DW).

$$fNgrain(t) = a_g + b_g \times Nleaf(t-1) \tag{2.16}$$

The total N in the grains at any point in time ($GrainN(t)$) is the accumulation of

$$\Delta GrainN(t) = fNgrain(t) \times \Delta GrainDW(t) \tag{2.17}$$

a_g is the intercept of the relation between $fNgrain$ and $Nleaf$, representing at which leaf nitrogen concentration no nitrogen is allocated to the grains. b_g is the slope of this relation.

Photosynthetic nitrogen

During the vegetative stage, the nitrogen available to the shoot functions as photosynthetic nitrogen and is distributed over the leaves and internodes ($StemN_{photo}$, g) according to their nitrogen demand. The organ (i.e. leaf and internode) demand for photosynthetic nitrogen ($DNarea_{photo}$, g/m^2) is the smallest of either 1) the nitrogen demand ($DNareal_{photo}$, g/m^2) as a function of the light gradient in the canopy and the target nitrogen concentration ($LN0$, g/m^2) of fully lit leaves (eq. 2.18) (Hikosaka et al., 2016) or 2) the nitrogen demand ($DNareaa_{photo}$, g/m^2) as a function of the individual organ's age ($AgeD$, day) (eq. 2.19).

$$DNareal_{photo}(i,t) = LN0 \times Fabs^{kNkL} \tag{2.18}$$

$$DNareaa_{photo}(i,t) = LN0 - \gamma \times AgeD \tag{2.19}$$

$$DNarea_{photo}(i,t) = MIN(DNareal_{photo}(i,t), DNareaa_{photo}(i,t)) \tag{2.20}$$

Where i represents an individual photosynthetic organ (i.e. individual leaf or internode). $Fabs$ is the fraction of incoming light absorbed by that organ. $kNkL$ is the ratio between nitrogen and light extinction coefficients, and set equal to 0.368 (Hikosaka et al., 2016). γ is the coefficient of demand of photosynthetic nitrogen controlled by age and set equal to 0.02 (Chen & Mi, 2018).

Similar to the carbon sink and source relationship in the model, we integrated a nitrogen sink and source relationship for photosynthetic nitrogen. If available organ photosynthetic nitrogen ($avaN_{photo}$, g) was more than organ demand (DLN, g), the amount of nitrogen allocated to each photosynthetic organ was equal to its demand for nitrogen. The surplus nitrogen was then assumed to be stored in the stem as a part of a $Nsource$. If the total amount available was less than the total demand, the amount available was allocated over all organs, based on the organ nitrogen demand times the relative nitrogen sink strength of that organ (eq. 2.21).

$$avaN_{photo}(i,t) = \begin{cases} DLN(i,t) & \sum avaN_{photo}(i,t) \geq \sum DLN(i,t) \\ \frac{DLN(i,t)}{\sum_i DLN(i,t)} \times avaN_{photo}(t) & \sum avaN_{photo}(i,t) < \sum DLN(i,t) \end{cases} \tag{2.21}$$

As above, i represents an individual photosynthetic organ (leaf and stem) and t is the unit time (*day*).

An asymptotic exponential relationship was used to estimate photosynthesis rate (eq. 2.22). This relationship is widely used in different crop models, such as e.g. SUCROS (Archontoulis & Miguez, 2015):

$$An = Amax \times (1 - e^{\frac{-\alpha \times PAR}{Amax}}) - Rd \tag{2.22}$$

where $Amax$ ($\mu mol\ CO_2/(m^2 \cdot s)$) represents the maximum photosynthetic rate, α represents the initial slope of the curve at low irradiance levels, Rd ($\mu mol\ CO_2/(m^2 \cdot s)$) is the dark respiration rate and PAR ($\mu mol/(m^2 \cdot s)$) represents photosynthetically active radiation.

The $Amax$ of photosynthetic active organs was related to specific leaf (or stem) nitrogen (SLN, g/m^2) through a modified logistic model (Sinclair, 1989; Vos et al., 2005).

$$Amax = \lambda \times (\frac{2}{1 + e^{-2.7 \times (SLN(i,t) - 0.25)}} - 1) \tag{2.23}$$

Where λ ($\mu mol/(m^2 \cdot s)$) is the maximum value of $Amax$ under non-limiting SLN. $SLN(i,t)$ is calculated by $avaN_{photo}(i,t)$ and the surface area of the ith leaf or internode.

Stem nitrogen

During the stem extension period, a proportion of the available nitrogen is allocated to the internodes to maintain a fixed internode N mass fraction ($fNstem$, g N/g DW). This nitrogen is considered to be incorporated in the stem structure and therefore cannot be reallocated. Additionally, stems contain nitrogen for photosynthesis ($StemN_{photo}$, see section Photosynthetic nitrogen) which is assumed to be available for reallocation when the generative phase has started (Ning et al., 2017). Since the stem is also assumed to act as a buffer organ, N stored in the pool for later use ($Nsource$, g/plant) is also assumed to be contained in the stem until required elsewhere:

$$\Delta StemN(t) = fNstem \times \Delta stemDW(t) + \sum_i StemN_{photo}(i,t) + Nsource(t) \tag{2.24}$$

Leaf nitrogen

All nitrogen in leaves is considered to contribute to photosynthesis, including leaf structural nitrogen. When leaf nitrogen drops below a critical leaf nitrogen ($LeafN_{critical}$, g/m^2), the leaf sheds regardless of whether it reached its life span. Once a leaf is shed, all nitrogen except for leaf structural nitrogen ($LeafN_{struct}$, g/m^2) is available for use elsewhere and photosynthesis of that leaf stops. Both values of leaf structural nitrogen and leaf critical nitrogen were derived from Chen et al. (2016b).

Carbon allocation between root and shoot

Based on the functional equilibrium theory (Lambers, 1983; Shipley & Meziane, 2002), plants systematically invest extra carbon when exposed to resource limitation, to support the growth of organs relevant to acquire the more limiting resource more effectively to optimize whole-plant growth. This concept is adopted here by implementing a relationship between root-to-leaf biomass partitioning ratio ($rlratio$) and $Nleaf$, which was assumed constant across plant developmental stages. This relationship was implemented in the model to control carbon allocation between shoot and roots for each time step (See Model description section) in response to leaf N levels.

$$rlratio(t) = a_r + b_r \times e^{-c_r \times Nleaf(t-1)} \tag{2.25}$$

where a_r represents the minimum value of $rlratio$, and b_r and c_r are the coefficients for the negative feedback between leaf N concentration and $rlratio$ (Fig. A.3, Fig. A.4). The coefficients were derived for the two cultivars (Zhengdan958 and Xianyu335) on which we had most information and are for this study considered cultivar independent as no clear difference were found between these two cultivars.

Field experiment

A field experiment was conducted in Siping (43°17'N, 124°26'E), Jilin province, China, in 2010 and 2011, reported in Chen et al. (2013, 2014a). A split plot design was used with 4 replications in 2010 and 5 replications in 2011. Nitrogen treatments were the main plot and genotypes were the subplots. The selection of maize cultivars used to parameterize and evaluate the model were Zhengdan958 (ZD958), Xianyu335 (XY335), Zhongdan2 (ZD2), Danyu13 (DY13), Yedan13 (YD13) and Nongda108 (ND108) which were all released between the 1970s and the 2000s in China. Three nitrogen levels were applied: 240kg/ha (HN), 120kg/ha (MN) and 0kg/ha (LN). For HN and MN, half the nitrogen was applied before sowing and the other half was applied at the V8 stage (the eighth leaf fully expanded). Maize seeds were hand-sown at a density of 6 plants/m^2 and a row and plant distance of 0.6 and 0.28 m, respectively. The crops were rain-fed. Precipitation was 580 and 401 mm in 2010 and 2011, respectively (Chen et al., 2015a).

Biomass and nitrogen concentration for each organ (leaf, stem and grain) were collected separately for three plants of each cultivar within each nitrogen treatment and replicate, at both silking and maturity stages in both years. Biomass was oven dried at 70 °C until dry weight was constant. N concentration of separate organs (i.e. leaf, stem and grain) was then measured by the semi-micro Kjeldahl method (Nelson & Sommers, 1973).

Photosynthesis light response curves were derived for ear leaves of the six maize cultivars per plot at grain filling stage under HN using a portable photosynthesis system (Li6400; LI-COR, Lincoln, NE, USA). The light intensity was set to 0, 20, 50, 100, 200, 500, 1000, 1300, 1600 or 2000 $\mu mol/(m^2 \cdot s)$. After measuring photosynthesis rates, the ear leaf was removed, dried and measured for biomass, leaf area and nitrogen mass concentration.

At grain filling stage, roots of the six cultivars grown at HN and LN in 2011 and at HN in 2010 were excavated by sampling a 60 cm wide by 28 cm long by 60 cm deep soil block that was divided into three 20cm layers. Per layer, roots were washed with a 0.4mm sieve and axial roots and lateral roots were separately scanned (Epson1600, India). WinRhizo (version Pro 5.0, Canada) was used to analyze root traits (such as root diameters, root length). After scanning, root samples were dried and weighed.

Model parameterization

The values of the model parameters were derived from the 2011 experimental data. The cultivar specific parameters a_g and b_g in eq. 2.16 to define grain nitrogen concentration in response to leaf N concentration were derived from relations fitted by linear regression using the“lm” function in R. Maize grain N concentration is quite stable during grain development (Ning et al., 2017; Fernandez et al., 2021). The data of grain N concentration and leaf N concentration at physiological maturity from the 2011 field experiment were used here to represent the N balance between grain and leaf during grain development.

Nested models (mle2) in the R package “bbmle” were used to derive photosynthesis related parameters. Multiple models were fitted to obtain the best parameter set. The models either assumed that all three parameter values for photosynthesis (eq. 2.22) differed between cultivars, only two of them differed and one was shared, only one of them differed and two were shared, or all three were shared among cultivars. The Akaike information criterion (AIC) was used to select the best model. By definition, smaller AIC values represent better overall fits with adjustment on parameter number. If the difference in AICs between two models is less than 2, the two models can be considered equivalent and therefore, in this case, the simpler model was chosen (Bolker, 2008). Values for other parameters were determined from the experimental data for each cultivar and listed in Table A.2.

Model evaluation

Temperature and incoming radiation (Table A.1) were derived from local weather data during the 2010 growing season. The "nls" function in R version 4.0.2 (Milliken, 1990; R Core Team, 2021) was used here to fit the temperature and incoming daily global radiation patterns for 2010 (eq. 2.1) to test modelled N uptake, yield and physiological efficiency (PE) versus observed data. PE in this study is defined as:

$$PE = \frac{Yield(g/plant)}{TotalNuptake(g/plant)} \tag{2.26}$$

Soil nitrogen leaching and mineralization have been taken into account in this model which means that at each time step the amount of nitrogen in a soil cell is one of the components to determine available soil nitrogen in that cell for a next time step. Therefore, nitrogen fertilizer application as done in the 2010 experiment was adopted in the model (half of the N applied before sowing and half at V8 stage). In addition, based on the recorded dates from 2011, plants were set to emerge 17 days after sowing, for model evaluation purposes. All the environmental and management parameter values are listed in Table A.1. Simulations were run under the three different nitrogen treatments (HN, MN and LN) by converting nitrogen applications from the field experiment to model input settings (Table A.3). The parameter values of $Ninit$ and Nm were assumed based on final plant N uptake of unfertilized plants (treatment LN).

Simulation runs were replicated five times to take into consideration the variation in output caused by the light model; an inherently stochastic computation. Each run consisted of a single plant. To reduce border effects, above ground canopy light conditions were created by cloning the plant 10 times in both x and y directions using the replicator functionality of GroIMP. Belowground, periodic boundaries were used so root segments that laterally extended beyond the plot boundary entered the cell on the opposite side (De Vries et al., 2021).

The relative root mean square error (rRMSE) was used to estimate differences between observed and simulated values for N uptake, yield and PE:

$$rRMSE = \sqrt{\frac{1}{n}\frac{\sum_{i=1}^{n}(Sim_i - Obs_i)^2}{\hat{Obs}^2}} \tag{2.27}$$

Where Obs_i and Sim_i are the observed and simulated values and n is the number of observed and simulated values.

Simulation experiment

A simulation experiment was done to quantify the relevance of genotype-specific plant architectural, developmental and physiological traits for N uptake, yield and PE (physiological efficiency). To run the simulation experiment, sowing date was set to Julian day 129 (May 9th) based on the 2010 field experiment and total growth duration was set at 154 days at which grain biomass was stable. The parameter values of temperature and light derived from the 2010 weather data were used as input. Plant density and nitrogen fertilizer application were taken from the 2010 experiment as well. In this simulation experiment, the earlier established parameter values from the cultivar ZD958 were used as default scenario since the parental line of ZD958, Zheng58, is widely used in current Chinese maize breeding program and ZD958 has been well studied in China.

We chose a range of plant traits potentially having a major effect on PE, to test by first individually changing values of root-trait related parameters $initD$, average ratio of the diameter of the daughter root to that of the mother root (RDM), $RootNum$, root tissue density (RTD), I_{max} and K_2 which were all genotype-specific (Table 2.1) and photosynthesis and leaf growth related parameters: λ, b_g, final leaf number ($LeafNum$; both cases with and without an effect on the time of silking were included), the time between leaf appearance (*phyllochron*) which affects time of silking without affecting leaf number; and finally grain growth related parameters: potential grain biomass ($wmax$, g), grain growth duration (te, °Cd), grain growth rate and grain nitrogen influx rate (Table 2.1). To account for the fact that two plant traits of interest (axial root diameter and time to silking) in the model are the result of component parameters, two combinations of model parameters were used (Table 2.1). The combination of $initD$ and RDM represented axial root diameter. The combination of phyllochron and total leaf number represented the final leaf number on the plant with a given time to silking. Not all traits tested appear in the equations presented above, such as LeafNum and Phyllochron. Such parameters directly drive plant development without the need for an equation; this is fundamental to FSP modeling. The complete model code is available online (https://git.wur.nl/lu068/cn-maize).

Table 2.1: List of plant traits tested in the parameter sensitivity test, their default values and numbers of related equations.

Description	Related model parameter	Eq.	Unit
Maximum influx rate of soil nitrogen for HATS activity	Imax	2.9	μmol/(m^2 $\cdot$ day)
Constant rate of LATS activity	K_2	2.10	μmol/(g $\cdot$ day)
Root diameter for whole root system	initD	-	m
Axial root diameter	initD and RDM	-	m
Lateral root diameter	RDM	-	m
Maximum root number	RootNum	-	-
Root tissue density	RTD	-	g/dm^3
Final leaf number	LeafNum	-	-
Time lag between two successive leaf appearance	Phyllochron	-	°Cd
Silking time	LeafNum and Phyllochron	-	-
Maximum photosynthesis under non-limiting leaf nitrogen	λ	2.23	-
Potential grain dry weight	wmax	-	g
Grain growth duration	te	-	°Cd
Nitrogen influx rate in response to leaf nitrogen concentration	b_g	2.16	g N/g DW

Parameter sensitivity

In order to identify and quantify the effects of changes in any plant trait alone or in combination, we changed the value of the related parameters by +10% and -10% and recorded the change in output parameter values (N uptake, yield, PE). To achieve a +10% change in axial root diameter, we increased the *initD* by +10% and then decreased *RDM* by 10% to keep lateral root diameter the same. To achieve a +10% change in the final leaf number without affecting time to silking, we increased the final leaf number by 10% and then decreased phyllochron by 10% to maintain silking time. The opposite was done to achieve a -10% change in both plant traits.

Sensitivity was calculated as:

$$Sensitivity = \frac{Y_i - Y_0}{Y_0} \times \frac{X_0}{X_i - X_0} \tag{2.28}$$

where Y_0 is the output value with default input parameters and Y_i is the output value with a change in input parameters. X_0 represents the default input parameter and X_i represents the input parameter with a change. If the value of sensitivity was larger than 1 or smaller than -1, the output was considered to be sensitive to changes in the input parameter. When values remained between -1 and 1, the output was considered to be insensitive. A positive value meant that when an input parameter increased or decreased by 10%, the change in output values was in the same direction. A negative value meant a change in output in the opposite direction.

Sensitivity was checked for three key output variables: 1) seasonal N uptake (g N/plant), 2) yield (g grain/plant), and 3) PE or physiological efficiency (Grain (g)/N (g)), which is the ratio of yield and N uptake.

The sensitivity tests were run under high (Napp = 5.714 μmol/m^3) and low (Napp = 0 μmol/m^3) N input conditions to see the potential ability of the tested plant trait values to contribute to N uptake, yield and PE. The plant N concentrations at maturity for both N levels are shown in Fig. A.5. The 'ggplot2' package (Wickham, 2009) was used to produce figures.

Results

Model parameterization

Based on observed daily average temperature and daily global radiation of the growing season in 2010, curves were established as model input (Fig. A.6; Table A.1). The initial slope of the light response curve at low irradiance levels, α (0.0506 $\pm$ 0.0023) and dark respiration Rd (2.43 $\pm$ 0.245 μmol/(m$^2 \cdot$ s)) in the photosynthesis model (eq. 2.22) were the same for all cultivars (Fig. A.7; Table A.4). In contrast, the λ in the relation of $Amax$ to specific leaf nitrogen (eq. 2.23) was found cultivar specific. In the model, λ was set from 21.2 for ND108 to 31.8 for XY335 based on field measurement of ear leaf photosynthesis under HN in 2011 (Table A.2).

The parameters a_g and b_g, (eq. 2.16) defining the relation between grain and leaf nitrogen concentration were also found cultivar specific parameters (Fig. A.8). On the basis of the 2011 field data (Table A. 2), a_g ranged from -0.0128 (XY335) to 0.00621 (ND108) and b_g from 0.433 (ND108) to 1.97 (XY335).

Model evaluation

We found an overall satisfying correspondence between modelled and observed N uptake with rRMSE equal to 0.233 (Fig. 2.2A). Interestingly, the model slightly underestimated the N uptake for certain cultivars under low N, while overestimated N uptake for other cultivars under high N. This could indicate the current model is lacking potentially relevant processes such as plastic responses to N level. The overall correspondence of simulated and observed yield values was acceptable with rRMSE equal to 0.174 (Fig. 2.2B). Simulated and observed physiological efficiency corresponded well with an overall rRMSE of 0.135 (Fig. 2.2C).

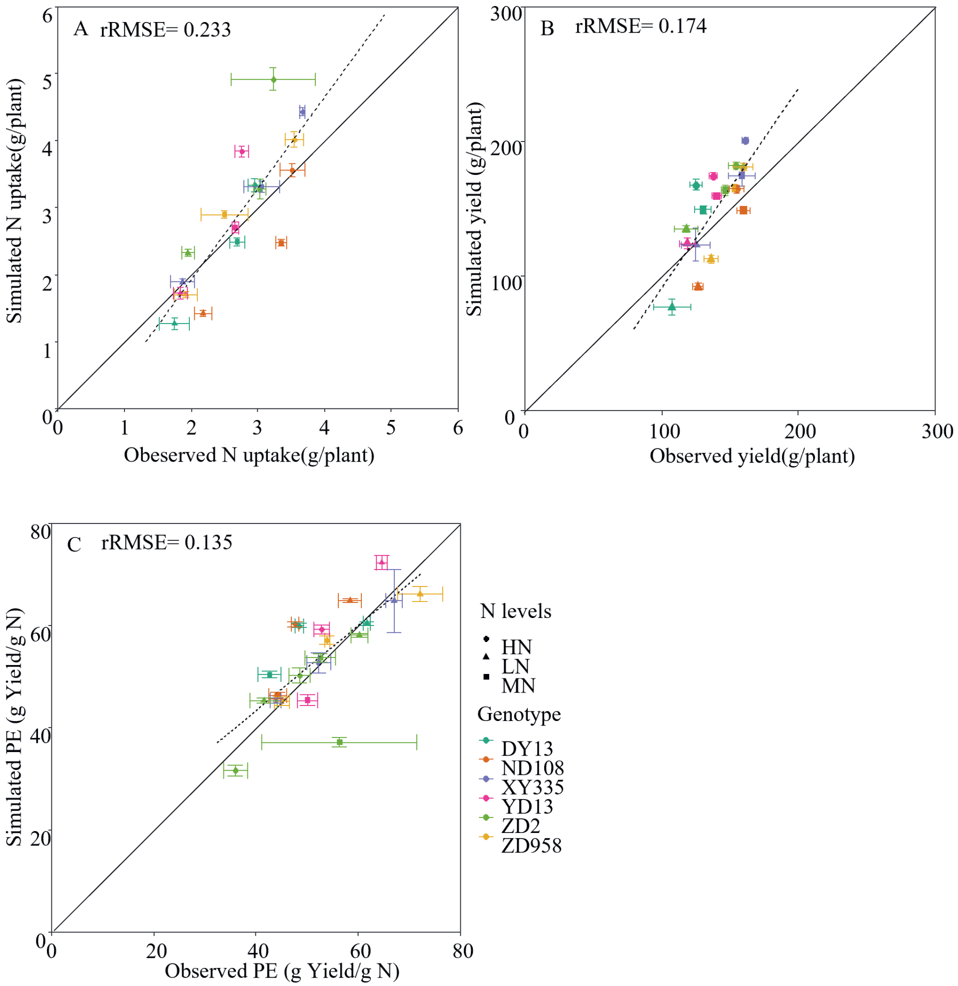

Figure 2.2: Scatter graphs of simulated versus observed N uptake (A), yield (B) and PE (C) for six maize cultivars grown at high (240kg N/ha, HN), medium (120kg N/ha, MN) and low (0kg N/ha, LN) N fertilizer application rates. Observed data are from experiments in 2010 reported in Chen et al. (2014a), where points and error bars represent means ± SE (n=4).

Parameter sensitivity

N uptake mechanism

Nitrogen uptake, PE and yield were not sensitive (i.e. sensitivity values were between 1 and -1) to changes in parameter values related to high or low affinity N transport (Fig. 2.3). Changing the high-affinity related parameter I_{max} by 10% resulted in sensitivity values ranging from -0.86 to 0.59 for N uptake, yield and PE under both high and low

N conditions (Fig. 2.3A, B, C). Changing the low-affinity related parameter K_2 by 10% (eq. 2.10) gave sensitivity values from -0.51 to 0.33 for N uptake, yield and PE under both high and low N conditions (Fig. 2.3D, E, F).

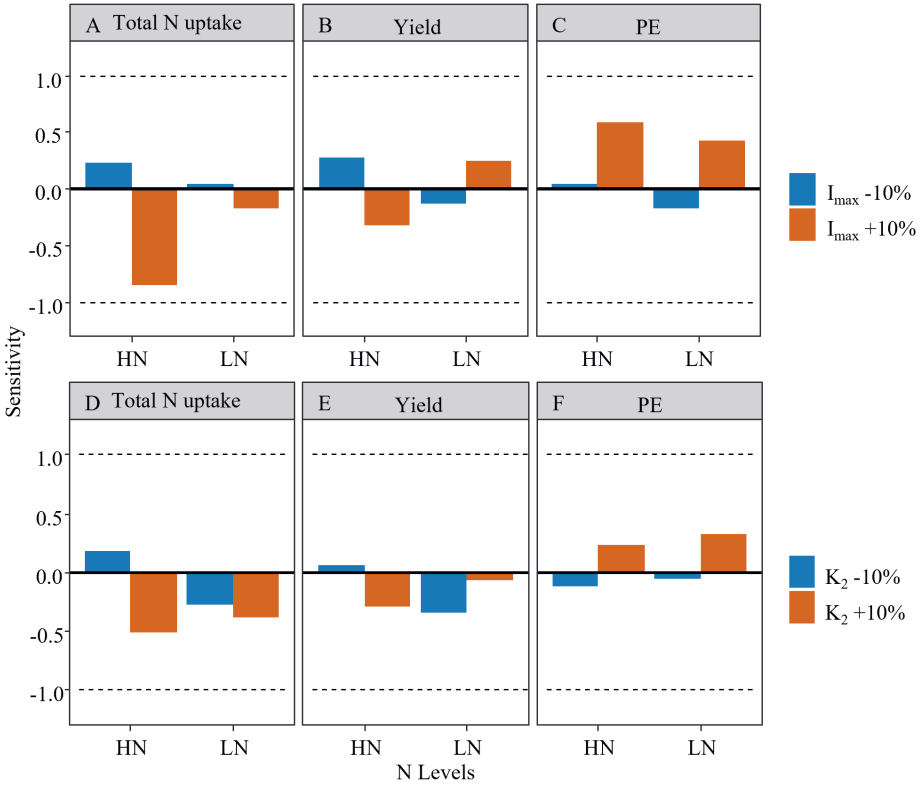

Figure 2.3: Sensitivity values of total N uptake (g/plant, A, D), yield (g/plant, B, E) and PE (g yield/g N, C, F) under high nitrogen condition (240kg/ha, high N) and low nitrogen condition (0kg/ha, low N) to changes in N uptake capacity related traits: I_{max} (A, B, C) and K_2 (D, E, F). All analyses were using parameter values of cultivars ZD958 as default. The dashed lines represent model sensitivities of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of an output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

Root morphology

Nitrogen uptake was sensitive to changes in diameters of all root classes (values between -1.06 and -1.9, Fig. 2.4A). The negative sensitivity values meant that the response in N uptake was in the opposite direction of the change in parameter value. In other words, reducing root diameter enhanced N uptake more than proportionally, and, increasing root diameter reduced N uptake. When decreasing diameters of all root classes under high N, the change in N uptake was less than proportional (-0.814). For yield a similar major negative sensitivity was observed when changing diameters of all root classes under low N (-1.58 and -1.56, Fig. 2.4B) meaning that increasing root diameter reduced yield and decreasing root diameter enhanced yield. PE did not show sensitive to changes in diameters of all root classes by 10% under both N levels (from 0.29 to 0.96, Fig. 2.4C). Nitrogen uptake and yield were more sensitive to changes in diameters of all root classes under low N than under high N (Fig. 2.4A, B). Only under low N, nitrogen uptake (-1.36) and yield (-1.62) were enhanced by a decrease in axial root diameter alone (Fig. 2.4D, E). N uptake (-1.02) and yield (-1.50) were sensitive to decreasing only the diameter of secondary and tertiary roots under low N while increasing the diameter of these roots had no major effect on either N uptake or yield (Fig. 2.4G, H, I).

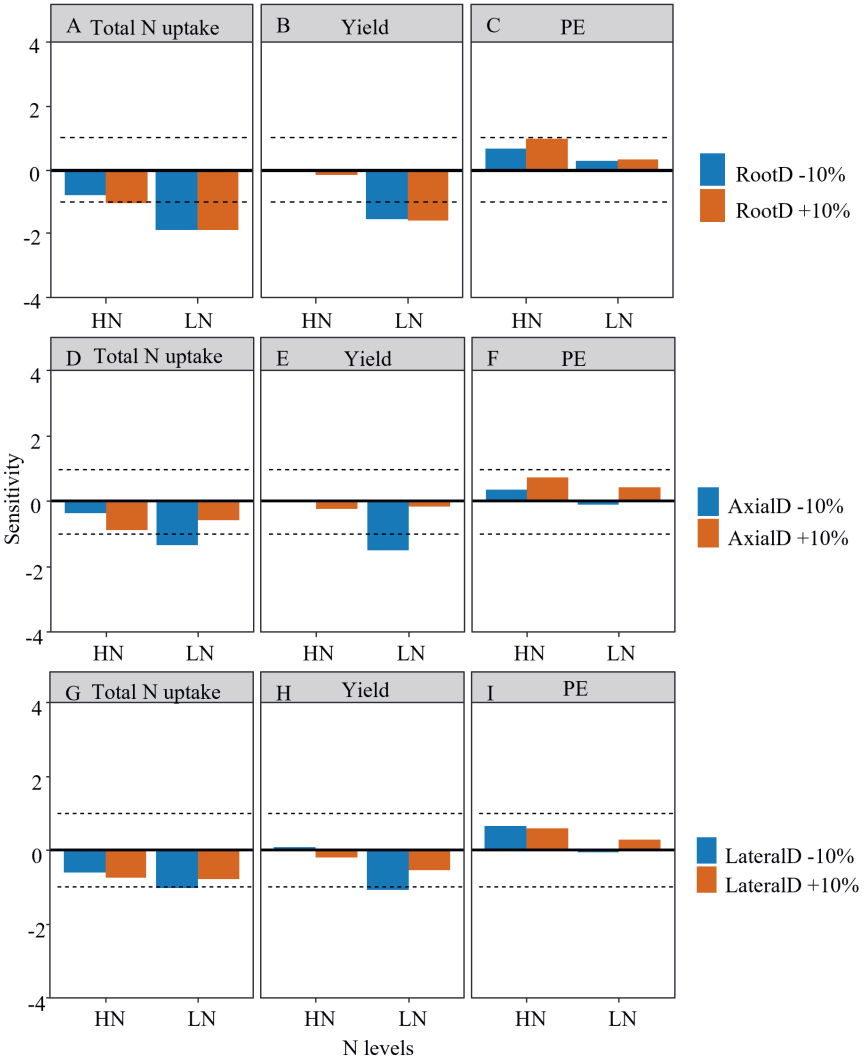

Figure 2.4: Sensitivity values of total N uptake (g/plant, A, D, G), yield (g/plant, B, E, H) and PE (g yield/g N, C, F, I) under high nitrogen condition (240kg/ha, high N) and low nitrogen condition (0kg/ha, low N) to changes in root diameter related traits: all root diameter (RootD; A, B, C), only axial root diameter (AxialD; D, E, F), or only lateral root diameter (LateralD; G, H, I). The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of an output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

The sensitivity of yield to the root diameter parameter values under low N was likely due to the increase in carbon costs per m root length when diameters of all root classes were increased. As these costs increased, less root length was produced leading to reduced nitrogen uptake during vegetative growth. This caused slower leaf area increase leading in turn to a lower leaf area per plant towards grain filling, which led to less carbon assimilation and allocation to the grains (Fig. 2.5B; A.9B, D).

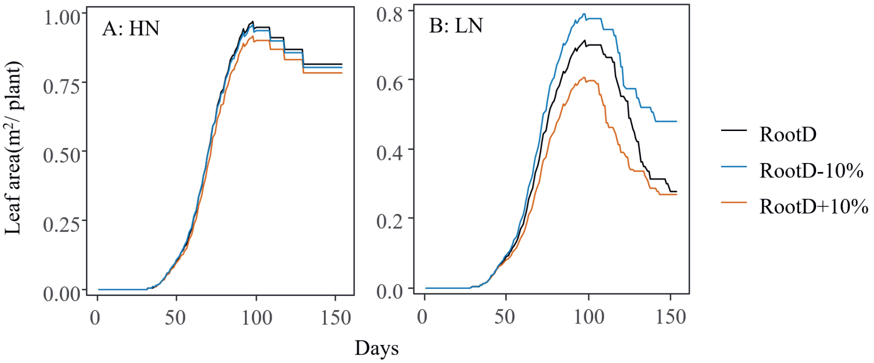

Figure 2.5: Dynamics in green leaf area per plant over the growing season when changing root diameter (RootD) under high nitrogen condition (High N, A) and low nitrogen condition (low N, B). Value ranges of y axis for high N condition is from 0 to 1 m^2 and for low N condition is from 0 to 0.7 m^2. Blue and orange lines represent green leaf are per plant for trait values that are respectively 10% higher of lower than their default in black lines.

Changing the maximum axial root number compared to the background genotype from 60 down to 28 had no effect on nitrogen uptake, yield and PE. However, when reducing maximum axial root number below 28, nitrogen uptake was increased especially under low N (Fig. 2.6A). Under low N, also yield increased with decreasing maximal axial root number, especially at numbers below 27 (Fig. 2.6B). Nitrogen uptake was slightly sensitive (-1.02) to the change root tissue density when increasing the value under low N (Fig. A. 10A). Yield and PE were not sensitive to changes in root tissue density (Fig. A. 10B, C).

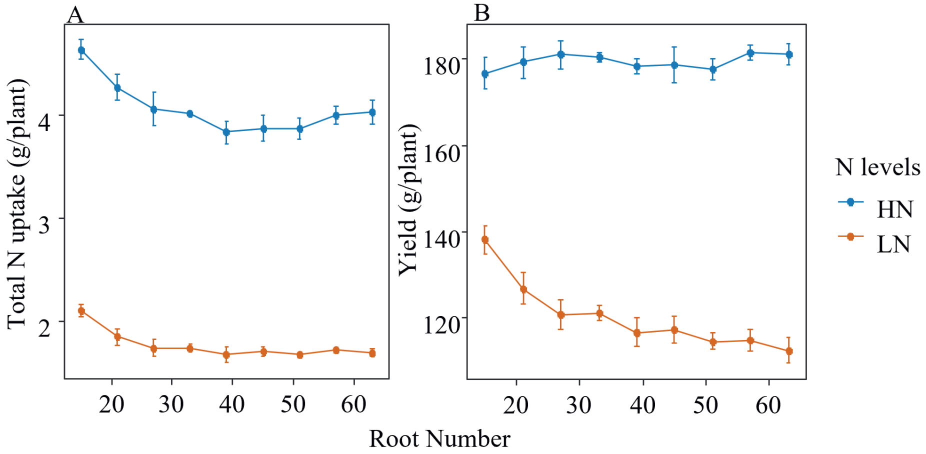

Figure 2.6: Simulated total N uptake (g/plant, A) and yield (g/plant, B) per plant across a range of maximum root numbers ($RootNum$) under both high N (blue) and low N (orange). Values represent means ± SE (n=5).

Shoot traits

Nitrogen uptake, yield and PE were not sensitive to changes in leaf number with or without including an effect on the time of silking (Fig. 2.7A-F). Changing phyllochron changes the moment of silking without affecting leaf number; and nitrogen uptake was sensitive to changes in phyllochron under low N. This indicated that increasing phyllochron enhanced N uptake under low N. Similarly, decreasing phyllochron reduced N uptake (Fig. 2.7G). N uptake was sensitive to decreased phyllochron but only under high N indicating decreasing phyllochron reduced N uptake under high N (Fig. 2.7G). A 10% change in phyllochron, irrespective of the direction of that change, resulted in an effect on PE in the opposite direction, meaning increasing phyllochron reduced PE and decreasing phyllochron increased PE (sensitivity values ranged from -1.53 to -0.83; Fig. 2.7I).). Yield was not sensitive to changes in phyllochron.

N uptake under high N was sensitive to decreasing the maximum photosynthetic capacity at non-limiting specific leaf nitrogen (λ), meaning that decreasing λ lowered N uptake under high N. However, under low N, nitrogen uptake, yield and PE were not sensitive (Fig. 2.8). Even though yield was not sensitive to λ, total biomass was, especially to a decrease in λ, and especially during early development at both N rates (Fig. 2.9). These positive sensitivity values indicate that increasing λ enhanced biomass accumulation and decreasing λ reduced biomass accumulation during early growth, by increasing photosynthetic capacity ($Amax$) (eq. 2.19). Since the effect disappeared at later growth stages, no sensitivity in yield to λ was observed.

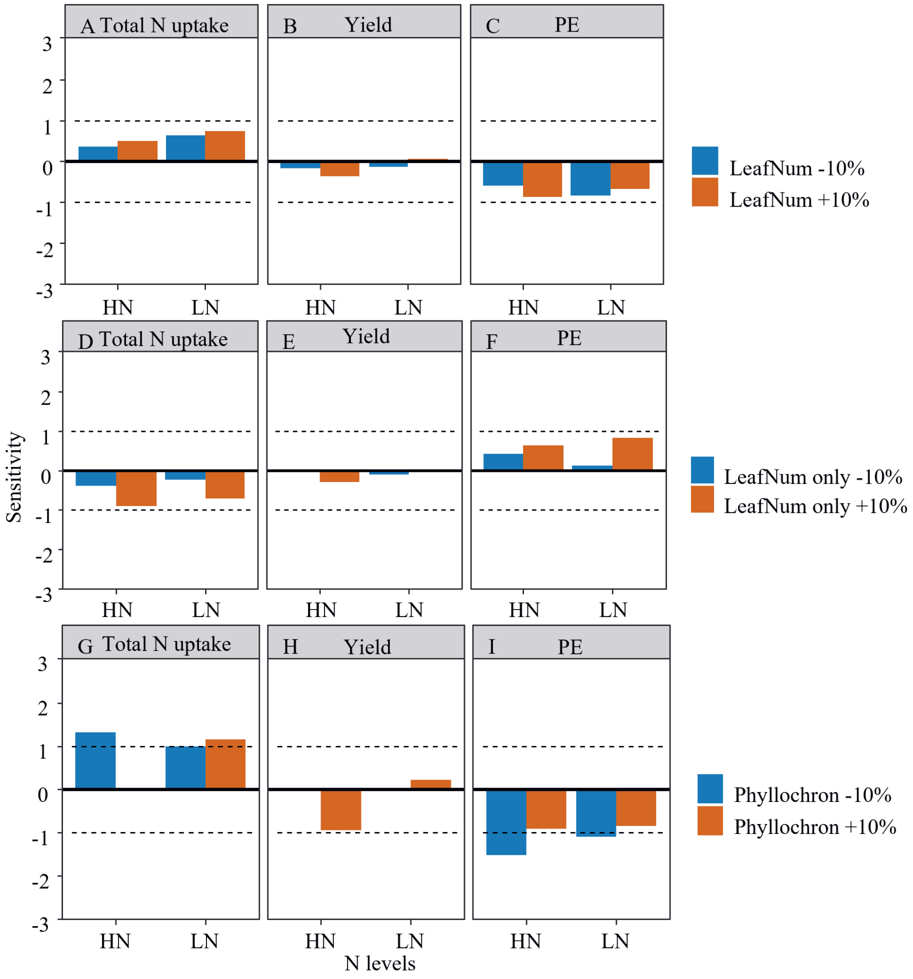

Figure 2.7: Sensitivity values of total N uptake (g/plant, A, D, G), yield (g/plant, B, E, H) and PE (g yield/g N, C, F, I) under high nitrogen application (240kg/ha, high N) and low nitrogen application (0kg/ha, low N) to changes in leaf number and time to silking related traits: leaf number (and as a consequence time of silking) (A, B, C), leaf number without affecting silking time (D, E, F), and phyllochron (and as a consequence time of silking) (G, H, I) under both high N condition and low N condition. The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of the output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

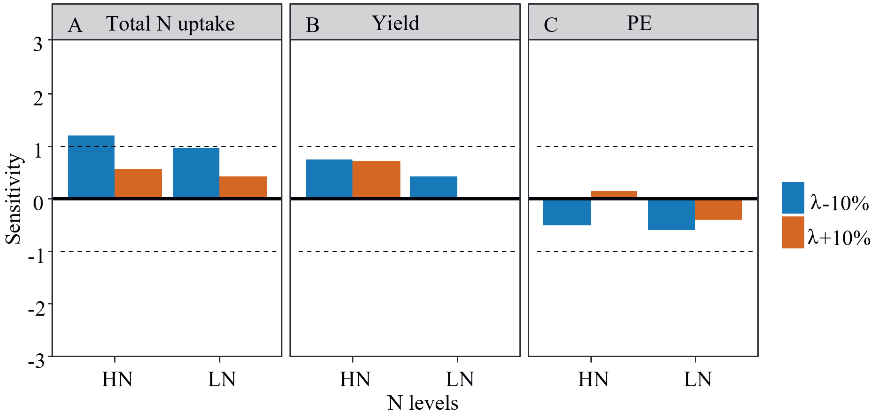

Figure 2.8: Sensitivity values of total N uptake (g/plant, A), yield (g/plant, B) and PE (g yield/g N, C) under both high nitrogen application (240kg/ha, high N) and low nitrogen application (0kg/ha, low N) to changes in the maximum photosynthesis under non-limiting leaf nitrogen λ. The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of the output parameter to respectively a 10% decrease or increase in a traits value compared to its default .

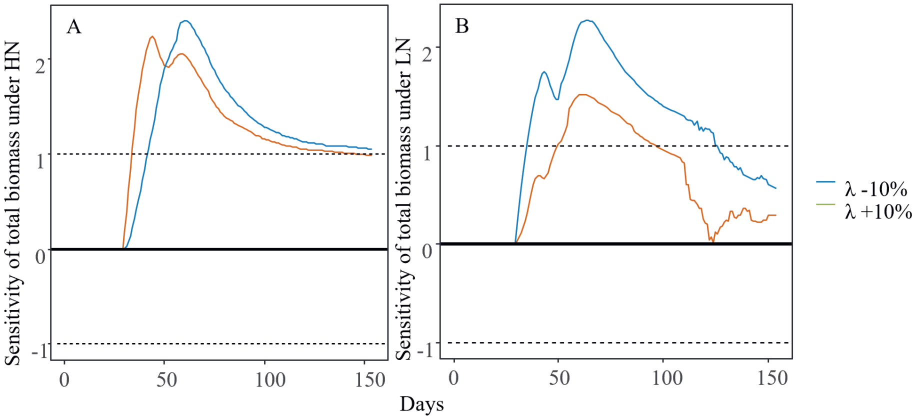

Figure 2.9: Sensitivity values of total biomass over time under both high nitrogen application (A) and low nitrogen application (B) to changes in maximum photosynthesis under non-limiting leaf nitrogen (λ). The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange lines represent sensitivity of the output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

Nitrogen reallocation

Nitrogen uptake, PE and yield were not sensitive to parameters related to grain growth rate and grain nitrogen influx rate (Fig. A.11). Under both N conditions, changing potential individual grain biomass ($wmax$) by 10% resulted in sensitivities ranging from -0.346 to 0.556 for N uptake, yield and PE, while changing grain growth duration (te) by 10% gave sensitivities between -0.378 to 0.209 for N uptake, yield and PE. Finally, sensitivity to changes in the N influx rate of grains (b_g) ranged from -0.885 to 0.607.

Discussion

Our model approach can capture observed differences in nitrogen uptake, yield and PE at the same magnitude for all N conditions and maize cultivars tested well enough for the purpose of testing trait effects (Fig. 2.2). In addition, our model reasonably well estimated the differences among cultivars even though only nine (out of more than 60) plant parameters were used to differentiate between the cultivars and this was especially true when nitrogen was not limiting. There was a significant overestimation of N uptake for cultivar ZD2 which was the oldest cultivar in the test panel, released in the 1970s. Under non-limiting nitrogen conditions, this cultivar combines a smaller root diameter with a higher specific root length compared to more recently developed cultivars such as ZD958 (Chen et al., 2014a; Ning et al., 2015). The overestimation of N uptake for ZD2 indicates that additional processes may have to be included in the current model to improve the representation of old cultivars. A possible difference between old and more recent cultivars is in their root anatomical characteristics. These may interact with soil chemical and physical characteristics through, for instance, penetration strength of roots, which in turn impacts N uptake. The differences between observed and simulated N uptake may thus related to the trade-off between penetration strength and soil N forage of thin roots. Soil texture and root penetration strength were not part of this study. To capture plant architectural, developmental and physiological genotype-specific traits related to PE, our model is a suitable tool.

Maize traits related to N uptake and PE

The role of root architectural and physiological traits in N uptake and PE

Our simulations suggested that root traits such as root diameter are more relevant to N uptake and PE than physiological traits related to root N uptake mechanisms, especially under low N conditions. Differences in I_{max} have been reported between different regions along roots from highest at root tips to lowest at the root base, as well as between axial or lateral roots (Lazof et al., 1992; Sorgona et al., 2011). However, such observations were typically made in experiments at the scale of individual root segments, and usually over a short time span. Differences in I_{max} were also identified among maize genotypes at the root system scale, but these were found only weakly correlated to nitrogen uptake rate at plant level (Pace & McClure, 1986). This is also the conclusion from the current modeling exercise.

In an earlier modeling study (Dunbabin et al., 2004) plasticity in ion-uptake mechanisms was more relevant for N uptake in herringbone type root systems typical for dicots, than in dichotomous type root systems such as in maize. Similarly, within the range of measured I_{max} for different maize root classes, shoot biomass was not sensitive to I_{max} over soil nitrate ranging from 20kg/ha to 200kg/ha (York et al., 2016). In general, the capacity for nitrogen uptake plays a more important role when competition or compensation for N between roots of a same plant is low, due to low root density. In addition, a lower root length density in the top soil layer (0-20 cm) was found with higher root system efficiency of N for modern maize cultivars (released after 1990s) compared with old maize cultivars (Chen et al., 2014a). Combining with our simulation results (Fig. 2.3), all together indicated the competition for soil N among root segments with a plant plays a more important role than capacity of N uptake for individual root segment. Since maize plants usually have dense root systems, it would be less important to consider capacity for nitrogen uptake such as I_{max} as a primary target trait in a maize breeding program aimed at high nitrogen use maize genotypes. In contrast, breeding for traits allowing better soil exploration will be very relevant.

Root diameter inheritance in our model is based on the principles of the ArchiSimple model (Pagès et al., 2014). Therefore, decreasing root diameter caused carbon investment to go into additional root length, extending the root system into previously unexplored soil allowing additional N uptake. This reduced competition among root segments for limited soil nitrogen within each soil cell, through a reduction of root surface area per cell. In *in vivo* studies, longer root hairs can increase nitrogen uptake under low N which also means roots with small diameter can increase their N depletion zone (Saengwilai et al., 2021). In line with our findings, a smaller axial root diameter was found to provide an adaptation to low N conditions (Gao et al., 2015). In addition, quantitative trait loci for smaller root diameter were associated with higher N uptake and finally higher yield (Coque et al., 2008). However, since primary root diameter can be correlated with anatomic traits which affect penetration strength, N use efficient genotypes generally have larger root diameters than N use inefficient genotypes under low nitrogen in field conditions (Yang et al., 2019) which is contradicting our results. In addition, the diameter of basal roots were positively correlated with root length under field conditions indicating the importance of penetration strength (Wu et al., 2016). Both of the examples hint at a trade-off between root expansion and root penetration strength not included in our current model. Further work on the role of root diameter on N use should therefore consider competition for resources between plants in relation to soil compactness and related requirements for penetration strength of roots. Alternatively breeding towards plants with a lower primary root number (Fig. 2.6) and lower root tissue density (RTD) could lead to reduced carbon costs at equal penetration strength allowing for root system expansion.

Trade-off between biomass accumulation and yield

Phyllochron determines the rate of creation of new sinks for carbon and nitrogen. Together with final leaf number, phyllochron determines the duration of vegetative growth, which controls the carbon sink:source ratio during plant growth. Increasing phyllochron led to individual leaf area of lower leaves becoming larger, since fewer sinks competed for resources in the early growth stage. In line with our findings, a positive correlation was found between phyllochron and leaf size in rice (Rebolledo et al., 2012; Miyoshi et al., 2004). Based on our model assumption that root sink strength is a fraction of leaf sink strength, increasing both traits improved root growth. However, both phyllochron and final leaf number hardly affected simulated yield. Increasing phyllochron can increase leaf area during vegetative growth, which meant a larger assimilate requirement to develop vegetative structures. Also, the final grain growth is controlled by both grain sink strength and available assimilates. Therefore, an increase in phyllochron ended up with no significant increase in yield. Similarly, increasing leaf number also hardly affected yield since yield formation did not only depend on source assimilation, but also on grain sink strength.

Yield and PE were not sensitive to leaf photosynthetic capacity related to leaf N while total biomass was sensitive only at early growth stages (Fig. 2.8B, C; Fig. 2.9). The combination of a reduction in leaf N sink strength with developmental stage with changes of biomass accumulation at early growth stages, may have led to limited changes in photosynthetic capacity. Sink-related traits have been reported to play an important role in limiting both plant growth and grain development in annual crops (Borrás et al., 2004; Serrago et al., 2013; Long et al., 2006). However, we observed that simply increasing grain sink strength, by increasing potential grain growth rate ($wmax$ and te), also hardly affected final yield. Thus, sink and source rather co-determine final yield, and only accounting for the function of just the sink for or the source of grain carbon hardly improves yield.

Breeding for an increase in PE

PE (kg grain/ kg N in the plant) is a complex trait that is affected by the nutrients present in the plant, it distribution among organs, photosynthetic traits and sink potentials. Component traits like photosynthesis related traits can have higher heritability than compound traits such as yield, can have stronger genetic correlation and are easier to measure across a wide range of genotypes during early plant growth (Gallais & Coque, 2005). Therefore, a FSP model like ours is a valuable tool to dissect PE into plant architectural and functional traits like photosynthetic capacity, and to identify which of these could be future targets in breeding programs.

In order to breed for a more nutrient use efficient maize, root morphological traits such as root diameter are potentially interesting breeding targets. The root system architecture is directly associated with biomass accumulation and grain yield for maize (Hammer et al., 2009). Genetic variation in root diameter has been reported (Yang et al., 2019; Coque et al., 2008) giving scope for breeding. However, there are still gaps in our understanding of trade-offs as thinner roots have the potential to explore a larger soil volume by increasing root length while it may equally reduce its penetration strength into more compacted soil. Phyllochron and the maximum photosynthesis under non-limiting leaf nitrogen (λ, eq. 2.23) also appeared to be interesting plant traits. Even if phyllochron seems to be of minor importance for final yield, this trait determines vegetative biomass accumulation during early growth and thus N uptake of the plant. Therefore, breeding for higher values of this traits can potentially improve nitrogen uptake of plants since larger leaf area positively correlates with root biomass especially under N-limiting condition. Genetic variation exists for the phyllochron, and this is not always associated with final leaf number (Lacube et al., 2020; Padilla & Otegui, 2005). Therefore, it is possible to breed for this traits independent from leaf number. Similar to phyllochron, λ can be an interesting trait to breed for to increase N uptake of maize, through enhancing biomass accumulation. Genes identified for photosynthetic capacity in maize directly resulted in changes in biomass accumulation and N uptake (Li et al., 2020b; Wang & Li, 2019). Our model indicates plant traits that seem important based on our understanding of whole plant growth and development, may help to target breeding for nitrogen use efficiency. However, combining results from our model and previous studies, we would argue that simply improving a limited number of plant traits cannot result in a significant increase in grain yield. For such an increase likely several plant traits will simultaneously have to be changed. For instance, yield was not found sensitive to either grain sink strength or λ.

Potential next steps in modeling

Plastic responses of plant traits to environmental conditions play an important role in root growth and development and thus root system architecture (Schneider, 2022). Our current approach only included a limited number of generic plastic responses to nitrogen like making the allocation of produced assimilates to roots or shoot dependent on leaf N concentration and making the N uptake rate (both the low and high affinity transport) dependent on plant N concentration. However, a number of potentially also relevant plastic reactions in response to plant and soil nitrogen queues have not been included in our approach, due to a lack of suitable empirical data to account for genotype-specific difference in plasticity. To prioritize plasticity by its importance on plant performance for further study, results of sensitivity analysis can be used. For instance, based on results of the sensitivity analysis (Fig. 2.4; Fig. 2.6), changes in root diameter and root number were significant in plant N uptake and yield, especially when N application was reduced. Differences in these traits between various N conditions within a same genotype have also been observed through experiments (Saengwilai et al., 2014a; Schneider et al., 2021). Both *in silico* and *in vivo* results together indicates the importance of plasticity in these two traits on plant N use and is worth further study. Therefore, for future studies with FSP models, plastic responses such as in root diameter can be considered for integration to identify and quantify effects of variation available among genotypes. After implementing the relevant plant mechanisms, conditions like daily temperature, radiation and soil N dynamics can then be considered as input to further predict plant performance more precisely. If simulations would show potential for improved nitrogen uptake or internal use efficiency these genotype-specific parameters could then be considered relevant target traits for breeding towards more efficient N usage maize across environments.

Chapter 3

Root plasticity improves nitrogen use when nitrogen is limiting

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Abstract

Plant phenotypic plasticity plays an important role in plant resource acquisition under limited nitrogen (N) conditions and may have a role under non-limited N conditions. However, the contribution of plastic responses to N use at higher N application levels has not been quantified, leaving it unclear whether plastic responses should be considered potential targets for phenotypic selection. Here, a combined modeling and experimentation approach is taken to assess the potential role of plasticity in maize for N uptake and for the conversion efficiency of N taken up into yield (called physiological efficiency, PE). Based on a small experiment, we considered two traits: root-to-leaf biomass allocation ratio and axial root emergence rate. In a virtual experiment we individually enabled or disabled both plastic responses for maize stands grown across six N levels to quantify the effect of plasticity on N use. Both plastic responses contributed to maintaining N uptake and plant productivity at N limited conditions compared to stands with plastic responses disabled. We conclude that plastic responses quantified in this study may be a potential target trait in breeding programs for greater N uptake across N levels while it may only be important for PE under N-limited conditions in maize. Given the complexity of breeding for plastic responses *a priori* model analysis seems a useful tool to highlight which plastic traits to target for enhanced plant performance in specific environments.

Introduction

Nitrogen use in plants

Nitrogen (N) is one of major elements driving plant development and growth. Due to the often excessive N fertilizer application rates, nitrogen use efficiency (NUE) of maize is low and in for instance China has substantially reduced over the past decades (Chen et al., 2021). The associated increase in N leaching is causing environmental pollution. While reduced inputs is the only viable solution to alleviate excessive fertilisation, breeding to improve plant N uptake ability, improve internal use efficiency or both could allow further N fertiliser input reduction. Breeding for phenotypic plasticity may be promising especially when N input is reduced below optimal input levels since the availability of soil resources is dynamic in space and time, and plastic responses to N availability may promote resource capture at the lowest carbon cost (Schneider & Lynch, 2020). However, not all phenotypic plasticity is beneficial in all environments and the current understanding of the effects of phenotypic plasticity on different aspects of NUE is very limited.

NUE is determined by two major underlying processes: the uptake and the internal use of nitrogen. The latter is called physiological efficiency (PE), and is defined as the efficiency with which the N once taken up is converted into grain biomass, in kg grain per kg N taken up (Van Keulen, 1982). NUE and its components strongly depend on the root system phenotype (Schneider & Lynch, 2020; Mi et al., 2010).The plant level usage of N and the carbon and nitrogen balance contribute to grain biomass formation and grain N allocation. The current understanding of how phenotypic plasticity affects N uptake, PE and ultimately grain biomass, is limited but relevant to breeding for sustainable farming.

Plastic responses in maize

Phenotypic plasticity is the ability of a single genotype to respond to growing conditions by adjusting its phenotype, which includes morphological, developmental and physiological traits (Sultan, 2000; DeWitt & Scheiner, 2004; Schneider, 2022).We distinguish passive and active phenotypic plasticity. Passive plasticity manifests as plant trait values that directly result from resource limitations, such as smaller leaves and other organs, due to limited light or nitrogen availability. On the other hand, active plasticity is the physiological tuning of plant traits in response to competition, via environmental signals such as the light spectrum as modified by neighbouring leaves, and root exudates in the soil given off by neighbouring roots. Through active plasticity, plants tailor their phenotype to make optimal use of the resources available by avoiding competition with other plants

(Van Kleunen & Fischer, 2005; Schneider, 2022). Passive and active plastic responses usually occur both, which increases the complexity of studying plastic responses in plants and their contribution to plant performance. For important crop species like maize, plastic responses to N have been reported for various shoot and root traits (Galizia et al., 2020; Yang et al., 2022; Yu et al., 2014b).

Variation exists in the ratio between root and leaf dry weight in response to resource limitation, such as N deficiency (Fernandez & Rubio, 2015; Mašková & Herben, 2018; Wang et al., 2019b). Leaf N concentration is an important element determining photosynthetic capacity of the leaf. When leaf N is limiting photosynthesis, plants increase carbon allocation to the roots to improve the capacity to take up N. Therefore, carbon allocation to the roots is strongly negatively correlated with leaf N concentration (Scheible et al., 1997; De Groot et al., 2003). The ratio between carbon allocation to the roots and to the leaves is actively regulated, and therefore a prime example of active plasticity. This plastic response to N can be described by the functional-equilibrium theory (Poorter et al., 2012), also called optimal partitioning theory. This theory postulates whole-plant control of carbon allocation to organs, ensuring resource investment to the tissues responsible for the uptake of the most limiting resource (Brouwer, 1962; Lambers, 1983; Shipley & Meziane, 2002). Active plastic responses such as regulation of carbon allocation, and passive plasticity jointly shape a phenotype and thus cannot easily be distinguished in greenhouse or field experiments. How and to which extent N uptake or PE in maize depends on plasticity under different growing conditions, is not fully understood.

In addition to the response of carbon allocation between the shoot and roots, the production of fewer crown roots results in deeper rooting, leading to greater N uptake (mg/plant) under low N conditions(Saengwilai et al., 2014b; Schneider et al., 2021; Ajmera et al., 2022; Rangarajan et al., 2022). Besides taking up nutrients and water, few axial roots means a greater risk for plant lodging, especially when shoot biomass is large (Liu et al., 2012; Hostetler et al., 2022). A low number of axial roots are produced under low N indicating a reduced root emergence rate, without a clear relation to root elongation rates (Maizlish et al., 1980; Gaudin et al., 2011; Khan et al., 2019). This shows N plays a more important role in controlling the rate of root emergence rather than the rate of root elongation. Since the rate of root emergence can actively respond to N availability, the rate of axial root emergence is another active plastic response. Plasticity in root traits may enable a single maize genotype to respond adaptively to a wide range of N availabilities. The consequence of these plastic responses for N uptake or PE in maize has never been quantified.

Plant modeling

Greenhouse and field experiments, as well as meta-analyses, have been instrumental to study and create an understanding of plasticity effects on plant performance (Geiler-Samerotte et al., 2013; Gratani, 2014; Matesanz et al., 2021). Those methods are however constrained to existing genotypes and their suite of traits and plastic responses. In addition, plastic effects are usually analysed at certain moments in development while plant plasticity is dynamic in response to changes in the environment over the growth season. Simulation modeling that can explicitly capture plant traits, plastic responses, and the consequences for plant functioning, is a useful analytic tool for this purpose. Therefore, in this study, we adopted a functional structural plant (FSP) model, which can simulate individual plants, their growth, physiological functioning and both above-ground and below-ground 3D architectures in a crop setting (Vos et al., 2010; De Vries et al., 2021). This type of model can simulate individual organ growth for individual plants when growing in a canopy and interacting with other plants in that canopy. Therefore, it allows to study the effects of single organ-level plastic responses to plant nitrogen status on N uptake or PE, by changing the responses individually or in any combination; something not possible with traditional approaches. FSP modeling has been widely used to explore the effects of novel traits on resource capture under various conditions (e.g. Postma et al., 2014; Rangarajan et al., 2022; Li et al., 2021; Zhu et al., 2015). Also, FSP modeling can be used as tool to distinguish passive and active plasticity. Passive plasticity is an emergent property in the FSP model, caused by competition for resources (light and soil N), as resource acquisition differs between simulated plants. Active plasticity, in contrast, can be implemented in an FSP model by implementing plant traits not as fixed parameters but as a function of an internal resource status or an external signal (Bongers et al., 2018). In this study, we applied the FSP modeling approach to explore how changes in the magnitude of active plasticity affect plant and crop performance. Our objective was to assess the role of plasticity in maize for N uptake and physiological efficiency using FSP modeling combined with experimental data on plastic responses to plant N status.

Materials and Methods

To explore the effects of the active plastic responses on N uptake and yield, first a hydroponic experiment on maize was conducted to quantify the response of both root-to-leaf biomass partitioning and root emergence to plant N status. Then, the relationships describing the observed plastic responses were implemented into an existing maize FSP model, which we further parameterized and evaluated using two independent field experiments with multiple nitrogen levels conducted in several years. To quantify the contribution of plasticity to maize N uptake, plant productivity and PE, two virtual experiments were run: one for maize stands with and without the plastic responses enabled at a range of soil nitrogen availabilities comparable to the field experiments; a second with multiple responses strengths in response to high, medium and low soil N.

Setup of greenhouse experiment

To quantify the plastic responses to plant N, maize plants (*Zea mays L.* cv. LG30223) were grown hydroponically in a greenhouse at Wageningen University and Research, Wageningen, The Netherland. As plant morphological traits are largely conserved across species (Fort et al., 2013; Wang et al., 2017), we assumed plastic responses within the species are similar. Therefore , the responses derived from the cultivar used here, can represent the responses of the cultivars used in the field experiments. The greenhouse was set to a 12-hour daylength, with 25 °C / 20 °C day / night temperature and 40 % relative humidity. Approximately 200 seeds were placed at 1 cm depth in moist, coarse quartz sand. Seven days after sowing (7 DAS), 114 individuals of a similar size were selected and placed for 5 days with 3 plants per 7.8 L aerated hydroponic containers with ample nutrition (Table B.1) with set to 5.5. Then, 75 uniformly looking plants were selected and put individually on a 7.8 L aerated hydroponic container at pH 5.5. The experiment followed a split-plot design with three replicates as blocks, five N treatments as main plots randomised within the blocks, and five harvest times as sub plots randomised within the main plots. The three replicates were placed perpendicular to the light gradient present in the greenhouse compartment. The single plants on containers essentially grew as stand-alone plants, so not in a canopy setting.

Treatments

The treatments were targeted to create plants differing in shoot N concentrations between 3.5% and 1.6%. To arrive at these plant N levels, biomass accumulation was assumed to follow an exponential growth relation in the 5 weeks the plants were grown, where maize dry weight (DW, g) accumulation was described by (eq. 3.1) (Yin et al., 2003).

$$DW(t) = DW(0) \times e^{a \times t} \tag{3.1}$$

and where DW(0) is the initial DW, a is a fitted parameter that differs among nitrogen treatments and t represents time in days after sowing (DAS).

Nitrogen treatments were then made to attain constant plant nitrogen concentrations ranging between 3.5% of the plant dry weight at the highest nitrogen level expected to produce 25 g of DW and 1.6% of the plant dry weight at the lowest nitrogen level expected to produce 5 g DW both after 4 weeks. The other nitrogen treatments were then distributed in between. The NH_4NO_3 (mol) required for each nitrogen treatment and added to the medium twice a week was then calculated as eq. 3.2.

$$NH_4NO_3 = \frac{b \times (DW(t+x) - DW(t))}{2u} \tag{3.2}$$

where b (g N/ g DW) represents the target plant nitrogen concentrations, x (days) represents the time between N applications and u represents the molar mass of nitrogen (g N/mol). The calculated application rates were maintained for five weeks. After each addition of nitrogen, the pH was adjusted to 5.5.

Measurements

To obtain the growth data at organ level, plants from all three replicates in five nitrogen levels were harvested every seven days for five successive weeks starting at 12 DAS (Harvest 0). At each harvest, above ground biomass was divided into leaf blades and stems including leaf sheaths. The number of axial roots was counted and the root biomass was collected separately from the shoot. All samples were dried for 48 hours at 70 ^oC and then weighed.

The leaf, stem and root samples were analysed for tissue N concentration. To that aim, the samples were ground in a bill mill and 200-300 mg ground plant material was digested with a mixture of H_2SO_4Se and salicylic acid (Novozamsky et al., 1983). The actual digestion was started by H_2O_2 and in this step, most of the organic matter was oxidized. After decomposition of the excess H_2O_2 and evaporation of water, the digestion was completed by concentrated H_2SO_4 at elevated temperature (330^oC) under the influence of Se as a catalyst. After these digestion steps total N in the sample was measured spectrophotometrically with a segmented-flow system (San++ System, Skalar, Netherlands).

Derivation of plastic responses

In this study, root to leaf biomass allocation and axial root emergence rate to N status were considered as the two target active plastic responses. We based our implementation of plasticity on root to leaf biomass allocation on the functional equilibrium theory. Leaf N concentration ($LeafNc$, g N/g DW) was taken as the determinant of the root-to-leaf allocation ratio. We decided to use root-to-leaf allocation ratio rather than root-to-shoot, since shoot biomass contains an increasing fraction of stem biomass over time, which does not contribute to photosynthesis. A negative exponential curve was derived from the data that described the relationship between a root-to-leaf partitioning coefficient nRL and $LeafNc$ (eq. 3.3). In the model, nRL is used as a coefficient that modulates the root sink strength in the FSP model. This results in a larger fraction of assimilates being allocated to the roots from the plant assimilate pool when nRL is high. nRL varied between a maximum at the lowest observed leaf N concentration and a minimum at very high leaf N concentrations (Fig. B.1). Since there was no data on nRL for very low leaf nitrogen concentrations, we defined a minimum leaf nitrogen concentration ($minLeafNc$, g N/g DW) below which we assumed no further increase of nRL.

$$nRL = \begin{cases} a_l \times e^{-b_l minLeafNc} + 1 & (leafNc \leq minLeafNc) \\ a_l \times e^{-b_l LeafNc} + 1 & (leafNc > minLeafNc) \end{cases} \tag{3.3}$$

Parameters a_l (dimensionless) and b_l ($g\ DW/g\ N$) determine level and the curvature of the response curve and are referred to as the coefficients for plasticity (Table. 3.1). This response in biomass allocation to N limitation has been widely reported (Shipley & Meziane, 2002; De Groot et al., 2003).

Table 3.1: Parameter values of identified plastic responses

Plastic responses	Parameter	Mean values	Standard errors
RL related	a_l	2.75	0.611
	b_l	-100	14.2
	$minLeafNc$	0.0112	
ER related	a_r	0.094	0.0137
	b_r	-0.000551	0.000339
	$maxShootNc$	0.033	0.00188
	$minShootNc$	0.014	0.00191

The second plastic response we incorporated was the axial root emergence rate (ER, roots *per degree day*). This rate affects carbon allocation within the root system, i.e. allocation to individual roots of the carbon that has been allocated to the roots, as determined by the first plastic response. Plant N status is an important driver of axial root formation (Gaudin et al., 2011; Saengwilai et al., 2014b). In this study, a function (eq. 3.4) of shoot nitrogen concentration ($ShootNc$, g N/g DW) was applied to describe the plastic response in ER. Since we had information on a range of shoot N concentrations from our experiment, we defined a maximum shoot N concentration ($maxShootNc$, g N/g DW) and a minimum shoot N concentration ($minShootNc$, g N/g DW), beyond which we assumed no further increase or decrease in ER.

$$ER = \begin{cases} a_r \times minShootNc + b_r & (ShootNc \leq minShootNc) \\ a_r \times ShootNc + b_r & (minShootNc < ShootNc < maxShootNc) \\ a_r \times maxShootNc + b_r & (ShootNc \geq maxShootNc) \end{cases} \quad (3.4)$$

Where parameter a_r defines the increase is root emergence rate per unit of shoot N and b_r is the intercept of the response which represents root emergence rate per degree day in the theoretical situation of no shoot N. Both parameters are referred to as coefficients for plasticity (Table. 3.1).

Field experiment for model parameterization

A field experiment with 5 replications was conducted in Siping (43°17'N, 124°26'E), Jilin province, China, in 2011, as fully described in Chen et al. (2013, 2014a). Two of the nitrogen conditions, high N (240 kg/ha) and low N (0 kg/ha), were used for model parameterization. Maize plants, cultivar Zhengdan958 (ZD958), were hand-planted at a density of 6.0 plants/m^2, obtained by a row distance of 60 cm and a plant distance within the row of 28 cm. The experiment was rainfed and the precipitation during the growth season was 401 mm (Chen et al., 2015b).

Biomass and N concentration were collected separately for each shoot organ (leaf, stem and grain) of three plants within each replication and for each N treatment at both silking and physiological maturity stages. Biomass (leaf, stem and grain) was separately oven-dried at 70 °C until dry weight was constant. N concentration was then measured by the semi-micro Kjeldahl method (Nelson & Sommers, 1973).

To parameterize leaf photosynthetic capacity in the model, photosynthesis-light response curves of the ear leaves were obtained at grain filling stage only for plants grown under high N using a portable photosynthesis system (Li6400; LI-COR, Lincoln, NE, USA). The light intensities were set to 0, 20, 50, 100, 200, 500, 1000, 1300, 1600 and 2000 μmol/(m$^2 \cdot$ s). After measuring photosynthesis rate and leaf area, the ear leaf was removed, dried and measured for biomass and N mass concentration.

To parameterize root architecture in the model, at grain filling stage, roots were excavated from plants grown at high N and low N by sampling a 60 cm deep soil block representing the area of one plant (60 x 28 cm) with the plant in the middle. The block was divided into three 20 cm layers. Roots were washed with 0.4 mm sieve and axial roots and lateral roots were separately scanned (Epson1600, India). WinRhizo (version Pro 5.0, Canada) was used to analyse root traits including diameters of axial and lateral roots and total root length. After scanning, root samples were oven dried and weighed as the other biomass samples.

Field experiment for model evaluation

Field experiments with four replicates were conducted over four consecutive years (2010 to 2013) at Fu-jia-jie, located near Siping (43°17'N, 124°26'E), Jilin Province, China, as fully described in Chen et al. (2015b). Maize cultivar ZD958 was used in all experiments. Five levels of nitrogen were applied: 240 kg/ha (N240), 180 kg/ha (N180), 120 kg/ha (N120), 60 kg/ha (N60) and 0 kg/ha (N0). For N60, all nitrogen fertilizer was applied before sowing while for the other N treatments, half of the N fertilizer was applied before sowing and the other half at the V8 stage (i.e. when the eighth leaf was fully expanded). Maize was hand planted at 6.0 plants/m^2 with 60 cm row distance and 28 cm plant distance within the rows. Also these experiments were rainfed and the precipitation during the growth seasons for the four consecutive years were 580, 401, 407 and 502 mm (Chen et al., 2015b). Total shoot N concentration was measured by a modified Kjeldahl digestion method at physiological maturity (Nelson & Sommers, 1973). At physiological maturity, two 4 m long rows were harvested to measure yield.

Model description

An existing maize FSP model driven by light and nitrogen was used based on concepts presented earlier (https://git.wur.nl/lu068/cn_maize_plasticity; De Vries et al., 2021; Evers & Bastiaans, 2016). This 3D maize model can mechanistically simulate shoot and root growth and development based on organ-level carbon and nitrogen sink-source relationships, light interception and nitrogen uptake. Besides the sink-source dynamics that make existing organs grow in size, plant development (the creation of new organs over time) and organ geometry (shape and orientation of leaves and roots) co-determine the 3D structure of the plant. To study the impact of plastic responses to plant N on N uptake and PE, the two plastic responses, root-to-leaf biomass partitioning coefficient and ER, quantified in the greenhouse experiment (see Section Measurement) were implemented into the maize FSP model.

Since the goal of our plant model is to analyze plant growth and development in multi plant stands as a function of N availability rather than to predict yield like crop models do, we used average rather than actual daily data to represent temperature and incoming radiation during the growing season. To calculate daily average temperature, an equation that describes temperature as function of day of year was used. In a similar way, a description of incoming daily radiation was represented by a function of radiation with the latitude, day of the year and transmissivity of the atmosphere (Spitters, 1986; Evers et al., 2010). The “nls” function in R (Bates & Watts, 1988) was used to fit data for temperature and incoming radiation for the four experimental years on these two equations (Fig. B.3, Fig. B.4). The direct incoming radiation was simulated by using an array of 24 directional light sources and the diffuse light by using an array of 72 directional light sources that were positioned into a hemisphere and calculated at a daily basis (Evers et al., 2010). Light capture by leaves was simulated by the reverse Monte-Carlo ray tracing algorithm in GroIMP (Hemmerling et al., 2008). Leaf photosynthesis was simulated by a photosynthesis-light response curve described using an asymptotic exponential relationship (Archontoulis & Miguez, 2015). Assimilated carbon was allocated to each organ to simulate organ growth based on individual organ sink strength which was defined as the sigmoid function of organ potential dry mass and growth duration (Yin et al., 2003).

Maximum photosynthesis rate highly depends on specific leaf N (Sinclair, 1989; Vos et al., 2005). Therefore, a modified logistic function was used to link carbon assimilation and photosynthetic N. N distribution among photosynthetic organs was the result of N sink strength and N available within the plant. N sink strength was determined by both the light interception gradient in the canopy and organ age. Structural N in stem, root and grain was expressed as fractions of organ biomass that cannot be redistributed. N taken up by the roots and not required for root growth (root structural N) was all allocated to the shoot. For a leaf, when N content (g/m^2) was below a critical value, the leaf died regardless of whether it reached its life span and part of its N was relocated to the plant N pool. However, N leaf content (g/m^2) embedded in leaf structures could not be reused upon leaf death (Chen et al., 2016b). The shoot N remaining after assigning the photosynthetic N and the amount of N to support organ growth was stored in an N pool. N stored in the pool, photosynthetic N and N newly acquired were used in the next time step for allocation to leaves, stems and grains.

The soil was composed of cubic soil cells of 15x15x15 cm. Within a single soil cell, the three sources of soil N were 1) N leached in from the cell above (mimicking the movement of N with water, without explicitly simulating water itself), 2) N mineralization (top two cells only) and 3) N application (top two cells only). Two source of N loss were 1) leaching of N to the cell below, and 2) N uptake by the roots. Lateral flows of N in the soil were not accounted for. Michaelis-Menten kinetics were used to simulate N uptake through the high-affinity transport system and a linear relation with soil N to simulate N uptake through the low-affinity transport system (Siddiqi et al., 1990). Simulated soil depth was set to 0.9 m to account for N percolating to a depth beyond the rooting zone. Root segments have been rarely found beyond 60 cm depth for the maize cultivars and growing conditions for which we conducted the analyses (Ning et al., 2014).

Model parameterization

For the plastic response of root-to-leaf ratio to leaf N concentration (eq. 3.3), only the second, third and fourth harvests of the greenhouse experiment were used to fit models since the seed nitrogen and initial 5 days growth on ample N nutrition still strongly influenced the N concentration at first harvest while carbon reallocation to grains became significant at the final (fifth) harvest.

Nested models (mle2) in the R package "bbmle" were used to derive plasticity coefficients a_l and b_l (eq. 3.3) of the root-to-leaf allocation ratio as a function of leaf N concentration and a_r and b_r (eq. 3.4) of axial root emergence rate as a function of thermal time(Bolker & Bolker, 2017). Model selection was done to obtain the best parameter set, aiming to find out whether the plastic responses depended on the growth stage of the plants or the N treatments. We tested models for eq. 3.3 with all plasticity coefficients different among harvests, or with only one of them different and others shared, or with two of the coefficients different and another one shared, or with all plasticity coefficients shared among growth stages (Table B.2). For the axial root emergence response (eq. 3.4), the "lm" function in R was applied to parameterize the coefficients of plasticity for axial root emergence rate in response to shoot nitrogen concentration. The plant parameters in the model were based on data collected in the field in 2011 only, since for this year we had the most data (Table 3.2).

Table 3.2: List of ZD958 parameter values derived from experimental data obtained during 2011 growth season (Chen et al., 2013)

Parameters	Description	ZD958
final Phytomer	Leaf number	22
seedMass	Endosperm mass in the seed	295
a_g	Intercept of grain N concentration to leaf N concentration (N g/DW g)	0.00121
b_g	Slope of grain N concentration to leaf N concentration (N g/DW g)	0.778
fNstem	Stem structural N concentration (N g/DW g)	0.0039
λ	Asymptote of maximum photosynthesis rate ($\mu mol/(m^2 \cdot s)$)	24.7
initD	Initial root Diameter (m)	0.00154
RTD	Root tissue density (g/cm^3)	90
RDM	Ratio in diameter of axial and lateral root	0.305

Model evaluation

To test the suitability of the model for the objectives of this study, we compared model output (N uptake and plant productivity) to observed values from a data set encompassing four years and five nitrogen treatments, taken from the field experiment (Section "Field experiment for model evaluation"). To represent the different years in the model, light, temperature and sowing date parameters were used for each year (Table 3.3). To represent the experimental N fertilizer application levels of 0, 60, 120, 180 and 240 kg/ha, we used N input levels of 0, 1429, 2857, 4286 and 5714 μmol/L soil, in addition to 1650 μmol/L of initial soil nitrogen content in the top 30 cm. The root mean square error (RMSE) was used to quantify differences between observed and simulated values for N uptake and plant productivity:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Obs_i - Sim_i)^2} \tag{3.5}$$

Table 3.3: List of weather and management parameter values derived from experimental data obtained during growth season from 2010 to 2013.

Paramters	Description	2010	2011	2012	2013
startingDayOfYear	Sowing date	127	128	124	121
a_T	Year average temperature (^oC)	9.121	7.616	9.255	9.805
b_T	Seasonal variation in daily average temperature (^oC)	15.72	17.05	14.83	14.88
c_T	Day of the year when temperature is the yearly average temperature (^oC)	104	106	106	104
Transmissivity	Percentage of incoming radiation that is transmitted through the atmosphere	0.3716	0.3566	0.3749	0.3686
InitialN	Initial soil nitrogen before N fertilizer application ($\mu mol/L$)	1650	1650	1650	1650

Simulation experiments

The light and temperature values from 2010 were used in further simulation experiments since this year represented the most suitable conditions for maize growth among the four experimental years (Chen et al., 2015b). The simulation plot was set to 3 rows of 3 plants each, with 0.6 m between rows and 0.3 m between plants. To minimize border effects in the simulations the plot was copied 10 times in both x and y directions using the replicator functionality of GroIMP. By using the 3 by 3 plant simulation plot individual plants never had their identical digital copy next to them in any direction (Fig. B.5). We repeated the runs three times to assess the variation caused by stochastic elements in the model, notably in the ray tracing and in the orientation of plants. We tested two scenarios. In scenario one we quantified the contribution of plasticity to N uptake, plant productivity and PE at a range of six N levels. In scenario two, we varied the strength of the plastic responses, rather than merely turning them on or off, to quantify the strength needed to get effects on N uptake, plant productivity and PE, at a selection of three N levels.

Scenario 1: Simulations were set up in which N uptake and plant productivity were assessed at varying soil nitrogen levels with each of the plastic responses either enabled or disabled. Six N application levels were used (0, 1429, 2857, 4286, 5714 and 7144 μmol/L) to represent six field N fertilizer applications (0, 60, 120, 180, 240 and 300 kg/ha) with initial soil nitrogen set as 1650 μmol/L (70 kg/ha). These N applications are within the realistic ranges for field conditions in China and also led to realistic levels for plant N status (Fig. B.6). N uptake (g/plant), and plant productivity (g/plant) were the model outputs used to quantify the contribution of plasticity across N treatments. From these two output variables, physiological efficiency (PE), was calculated. In addition, since the plastic responses affected root-to-leaf weight ratio and root number, both outputs were assessed for high N (5714 μmol/L), moderate N (2857 μmol/L) and low N (0 μmol/L). Also the mean of total per plant N uptake over the whole growing season and the root length density (RLD) for each 15 cm soil layer were saved as the model output for these N levels. In simulations with plastic responses disabled, fixed values for the nRL and ER were used. These were derived from the highest nitrogen treatment in the greenhouse experiment, as these two plastic responses were assumed to play no role at high N.

To further understand plastic effects on N uptake under high N, differences in relative RLD (rRLD) per 15 cm soil layer (eq. 3.6) and N uptake per soil layer between ER response enabled and both responses disabled were calculated under typical high N (5714 μmol/L or 240 kg/ha) and low N (0 μmol/L) conditions.

$$rRLD(i) = \frac{RLD(i)}{\sum_i RLD(i)} \tag{3.6}$$

where i is an index representing the different soil layers (i =1 to 6).

Scenario 2: To analyse the strengths of the plastic responses, simulations were performed with 1650 µmol/L of initial soil N, to which three levels of N were added equivalent to 0, 120 and 240 kg/ha. The plasticity strength values we tested, were chosen such as to create differences in response at low N conditions. This was done since plasticity relevant to enhancing N acquisition plays a larger role at low rather than high plant N. At high N, plasticity to avoid shading is likely more important in canopies (Sugiura & Tateno, 2011; Poorter et al., 2012) but shoot plasticity is not in the scope of the current study. The relative strength of the response curve for nRL was set to five levels (plasticity disabled, -50%, default, +100% and +300%). To calculate these new strengths, the experimentally derived value of parameter al was multiplied by 0, 0.5, 1, 2 or 4 respectively (eq. 3.3; Fig. B.7A). Similar to nRL, the relative strength of the ER response was set to five levels (disabled, -75%, -40%, -25%, default and +40%) by multiplying the experimentally derived value of parameter a_r by respectively 0, 0.25, 0.6, 0.75, 1 and 1.4 respectively. The strength of ER was set as a constant when plant N was higher than 0.033 g N/g DW (eq. 3.4; Fig. B.7B; Table 3.1). Simulations were run for each response strength under the three N levels, with only one of the plastic responses enabled. To better compare strength effects under different nitrogen levels, relative N uptake and plant productivity values ($relativeY_i$, dimensionless,) were calculated as follows:

$$relativeY_i = \frac{Y_i}{Y_{default}} \tag{3.7}$$

Where Y_i represents the absolute simulated mean of N uptake or plant productivity based on the various response strengths under different N levels. $Y_{default}$ represents the absolute simulated mean value of either N uptake or plant productivity based on the experimentally derived response strength.

Statistical analysis

All statistical analyses were done using R version 4.0.2(R Core Team, 2021). To explore the effect of ER response on N uptake and RLD per soil layer, the unpaired mean and standard error of difference of RLD and N uptake per soil layer between ER response enabled and both responses disabled were calculated under high N and low N. The ‘ggplot2’ package (Wickham, 2009) was used to produce figures.

Results

Plastic responses

The data from the greenhouse experiment showed that with decreasing leaf nitrogen concentration, a larger fraction of carbon was allocated to the roots (Fig. 3.1), while the relation between the root allocation fraction (nRL) and leaf nitrogen was independent of growth stage (Table B.2). The parameters al, bl and minleafNc (eq. 3.3) were 2.75, -100 and 0.0112 g N/g DW, respectively for the studied cultivar (Fig. 3.1A; Table 3.1).

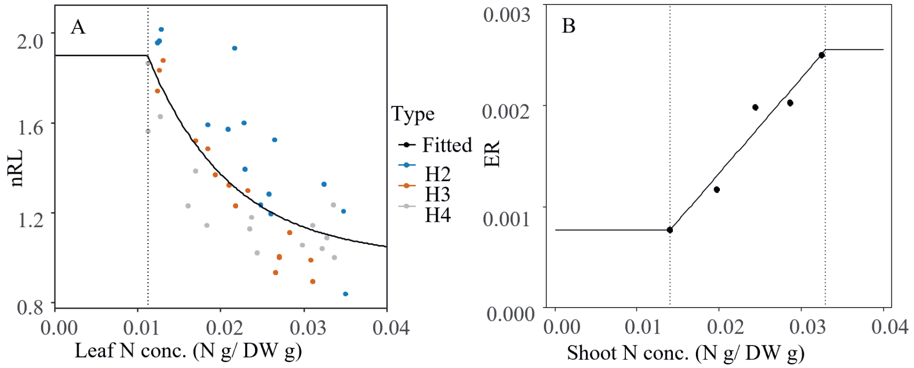

Figure 3.1: The plastic responses as implemented in the FSP model. Panel A represents the plastic response in root-to-leaf ratio in carbon allocation (nRL) to leaf N concentration. The dashed vertical line represents the minimum leaf nitrogen concentration observed in the experiment, below which level we assumed a constant value below this point. The solid line represents the fitted model described in eq. 3.3. H2, H3 and H4 respectively represent the second harvest, third harvest and fourth harvest. Panel B represents the plastic response in root emergence rate (ER) to shoot N concentration. The vertical lines here represent minimum and maximum values of aboveground N concentration observed from the greenhouse experiment. In the model (eq. 3.4), we assumed when plant N concentration above or below the observed range, there is no further increase or decrease.

With increasing shoot nitrogen concentration, the emergence rate for nodal roots (ER, eq. 3.4) increased (Fig. B.2). From the greenhouse experiment, a positive linear relationship was quantified between the number of roots that emerged per degree day and shoot nitrogen concentration. Per degree day (a_r, eq. 3.4) 0.000859 and 0.00255 roots emerged for the observed minimum and maximum shoot nitrogen concentrations of 0.0140 g N/g DW and 0.0330 g N/g DW, respectively (Fig. 3.1B; Table 3.1).

Model evaluation

After implementing the plastic responses to leaf and shoot N concentration, we found an overall satisfying correspondence between modelled and observed N uptake across four years under five N levels with a RMSE value equal to 0.541 g/plant (Fig. 3.2A). The correspondence between simulated and observed plant productivity across four years under five N levels was less strong with a RMSE values of 41.4 g/plant. For the higher N levels, the model did not capture the increase of plant productivity with an increase of N application well. For lower N levels correspondence was stronger (Fig. 3.2B).

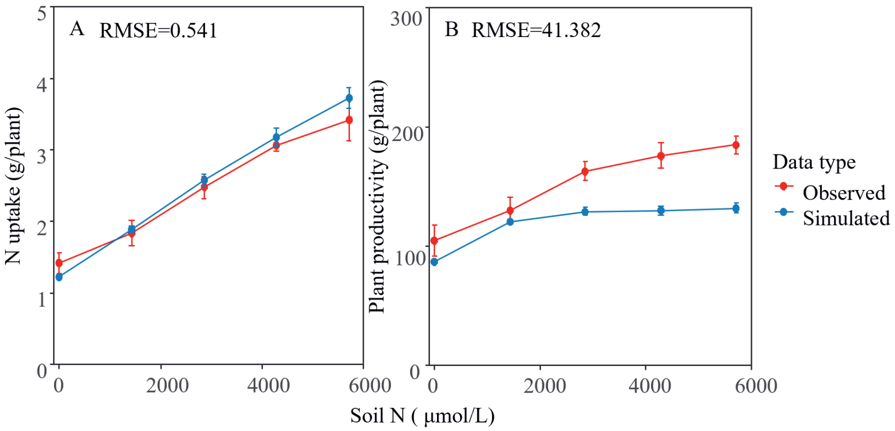

Figure 3.2: Comparison of plant N uptake (Panel A) and plant productivity (Panel B) between observed field data and model simulations for crop stands with plastic responses enabled. Dots of N uptake and plant productivity are the average values over the four years (2010 to 2013). Error bars are the standard errors across the four years.

Effect of the plasticity on root number and root to leaf weight ratio

Simulated root-to-leaf dry weight ratios (Fig.3.3A to D) were greater towards silking under low N both with plastic responses on or off. The simulated root-to-leaf dry weight ratio ranged from 0.202 to 0.282 at the silking stage (94 DAS) under low N, across the plasticity scenarios. When both plastic responses were enabled or only the nRL response was enabled, a small decrease in the root-to-leaf weight ratio was observed when N conditions changed from moderate N (2857 $\mu mol/L$) to high N (5714 $\mu mol/L$) conditions. Under low N, the simulated root-to-leaf dry weight ratio started to increase around 45 DAS due to accelerated leaf loss upon senescence caused by a N shortages.

The simulated root numbers over time increased with increasing N application (Fig.3.3E to H) when both plastic responses were enabled or when only the ER response was enabled. The root to leaf allocation ratio did not impact root number. Under high N conditions the potential number of roots is reached no later than 95 days after sowing or earlier if ER is not dependent on shoot N (Fig.3.3). Under low and medium N conditions this maximum root number is reached when ER is disabled, but not or later when enabled alone or in combination with nRL (Fig.3.3). Under all N conditions, enabling the ER response reduced the rate of root emergence.

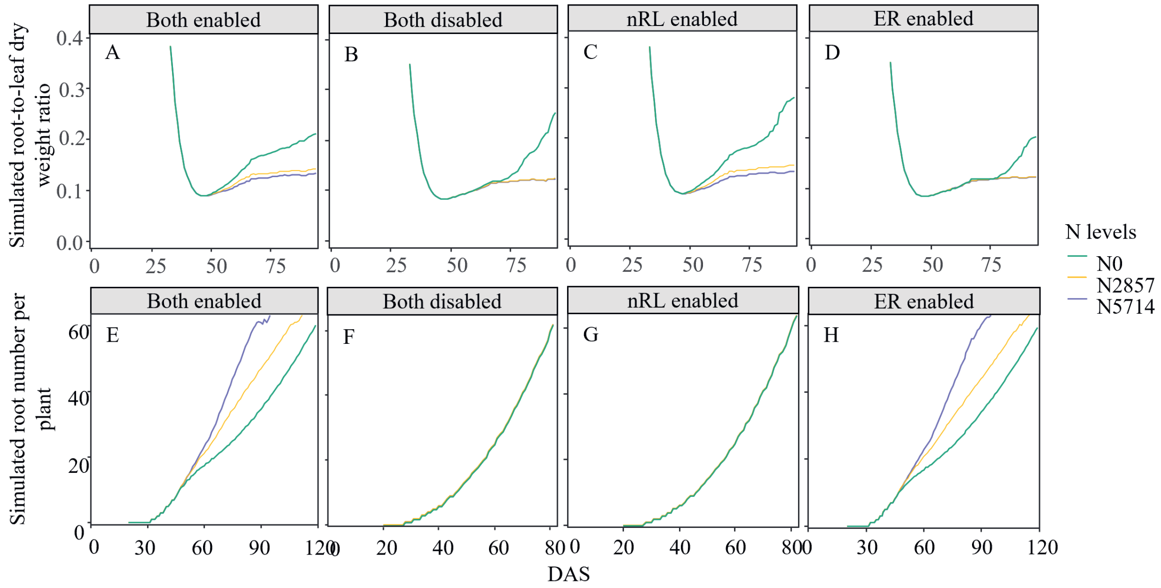

Figure 3.3: Effect of plastic responses on the simulated root-to-leaf dry weight ratio from days after sowing (DAS) until silking (i.e. 94th day after sowing in the model setting) and simulated axial root number until across different N levels. Both enabled (A, E) represents plastic responses in root-to-leaf ratio and root emergence rate are enabled. Both disabled (B, F) represents plastic responses in root-to-leaf ratio and root emergence rate are both disabled. *nRL* enabled (C, G) represents the plastic response in the root-to-leaf ratio is enabled while the plastic response in root emergence rate is disabled. *ER* enabled (D, H) represents plastic response in root emergence rate is enabled while the plastic response in the root-to-leaf ratio is disabled. Colors within each panel represent different N treatments. N5714 represents the high N condition (5714 $\mu mol/L$). N2857 represents the intermediate N condition (2857 $\mu mol/L$). N0 represents the low N condition (0 $\mu mol/L$). *ER* represents the plastic response in axial root emergence rate and *nRL* represents the plastic response in the root-to-leaf biomass partitioning coefficient.

Effect of plastic responses on N uptake, plant productivity and physiological efficiency

Both plastic responses used in this study enhanced modelled nitrogen uptake across all the N levels, and a larger increase was observed when both responses were enabled (Fig. 3.4A). Individually, ER and nRL responses showed a similar level of benefits in terms of N uptake across all N conditions, while the combined effect was slightly less than the sum of both individual effects. Across all N levels and irrespective of whether the two plastic responses were enabled or disabled, most N was taken up from the top 30 cm of the soil profile. Uptake ranged from 0.643 to 3.192 g/plant equal to 73.9% and 86.9% of total N uptake (Fig. 3.5A, 3.5B, 3.5C). In the 15-30 cm soil layer and across all N levels, the absence of plasticity resulted in reduced N uptake compared to when both or individual responses were enabled. The plants with disabled plastic responses had a lower RLD in the 15-30 cm layer across all the N treatments compared to when the plastic responses were enabled (Fig. 3.5D, 3.5E, 3.5F). There was a decrease in $rRLD$ (eq. 3.6) under both high N and low N in the 0-15 cm soil layer while there were increases in $rRLD$ under both high N and low N in the 15-60 cm soil layers when the ER response was enabled compared to when both plastic responses were disabled (Fig. 3.6A). Enabling the ER response reduced root number per plant at any given time and increased the N uptake across the 0-60 cm soil layers under both high N and low N conditions compared to disabling both plasticity responses (Fig. 3.6B).

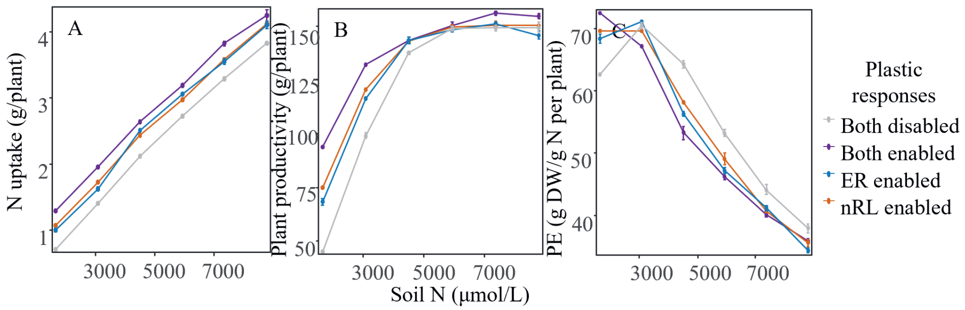

Figure 3.4: Effect of plastic responses on N uptake, plant productivity and physiological efficiency (PE) with plasticity in neither (grey), both (purple) or either nRL or ER (orange and blue respectively). ER represents the plastic response in axial root emergence rate and nRL represents the plastic response in the root-to-leaf biomass partitioning. Values are mean ± SE (n=3).

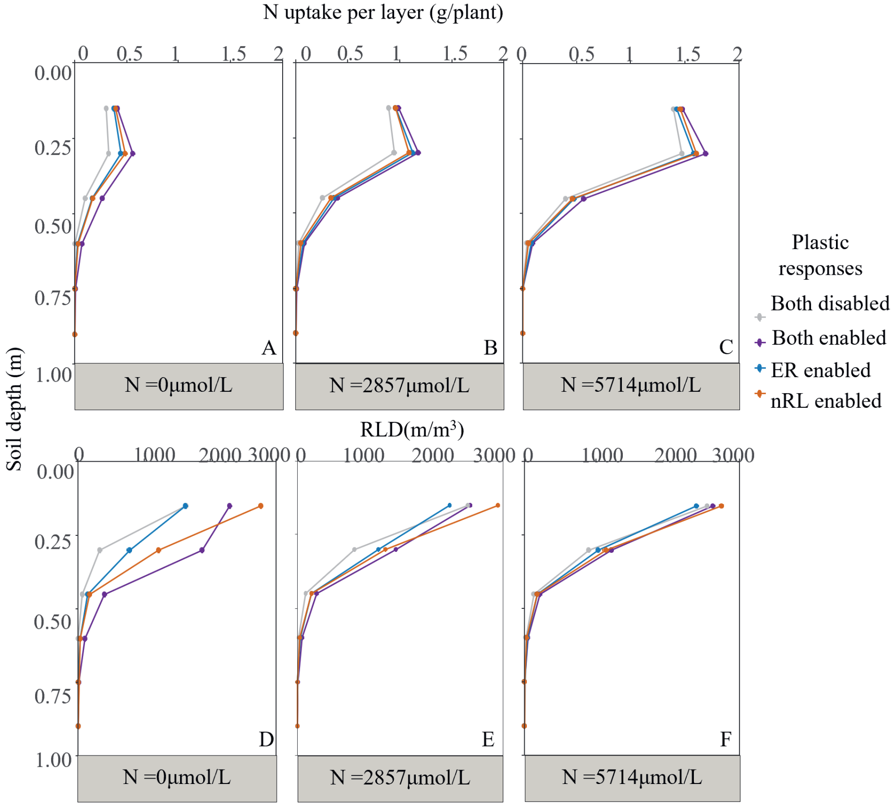

Figure 3.5: Root N uptake per soil layer and root length density per soil layer with plasticity in neither (grey), both (purple) or either *nRL* or *ER* (orange and blue respectively). A, B and C represent N uptake per soil layer with three different nitrogen levels (0, 2857 and 5714 $\mu mol/L$). D, E and F represent root length density (RLD) per soil layer with three different nitrogen levels (0, 2857 and 5714 $\mu mol/L$). *ER* represents the plastic response in axial root emergence rate and *nRL* represents the plastic response in the root-to-leaf biomass partitioning. Values represent all N uptake/RLD within each soil layer. Values are mean ± SE (n=3).

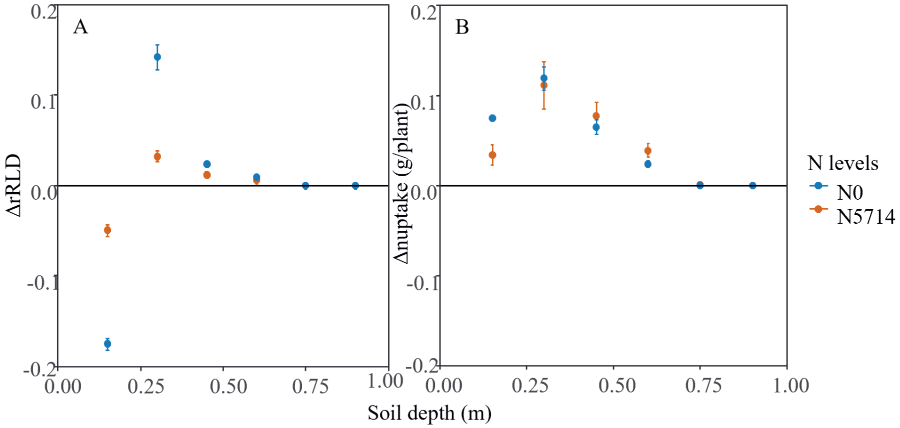

Figure 3.6: Differences in relative root length density (rRLD) and N uptake (Nuptake) per soil layer between when the ER response was enabled and when both responses were disabled. Panel A represents differences in rRLD under N5714 (high N in orange, 5714 $\mu mol/L$) and N0 (low N in blue, 0 $\mu mol/L$) conditions. Panel B represents differencse in Nuptake under N5714 (5714 $\mu mol/L$) and N0 (0 $\mu mol/L$) conditions. The error bars are the standard error calculated for the difference in rRLD and Nuptake between when the ER response was enabled and when both responses were disabled. Values are mean ± SE (n=3).

Plastic responses identified in this study enhanced modelled plant productivity under low N conditions (Fig. 3.4B). However, under moderate and high N, plastic responses did not show very large benefits in terms of plant productivity despite the extra uptake of N, likely due to lack of shoot plastic responses to shading. ER and nRL responses individually led to a similar increase in plant productivity (ER enabled: 68.15 ± 2.68 g/plant and nRL enabled: 74.57 ± 0.72 g/plant) and under low N their combination gave the highest increase in plant productivity (93.53 ± 0.17 g/plant) compared to disabling both plasticity. Similar trends were found in total aboveground dry biomass across N levels at the physiological maturity stage. In comparison to both plasticity's disabled, ER and nRL responses individually increased the total aboveground dry biomass to similar levels under low N while their combination resulted in the greatest increase in aboveground dry biomass (Fig. 3.7B). When the nRL response was enabled, root biomass was greater across all N levels than when the nRL response was disabled. Enabling the ER response to shoot N concentration increased root biomass only under low N conditions while no clear biomass increase was observed at moderate to high N levels (Fig. 3.7A). Simulations with both responses disabled showed the highest physiological efficiency (PE) except at the lowest N level when PE was lowest when one or both responses were disabled (Fig. 3.4C). Under moderate to high N levels, the lowest PE was found when both responses were enabled (Fig. 3.4C). This combines the observed gradual reduction in positive effect on plant productivity (Fig. 3.4B) and stable positive effect on N uptake (Fig. 3.4A) as more N is provided.

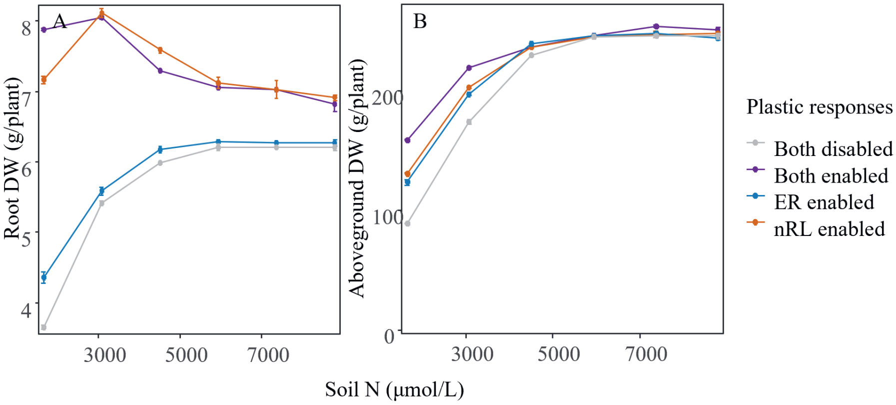

Figure 3.7: Root and aboveground dry weight across various nitrogen levels with plasticity in neither (grey), both (purple) or either nRL or ER (orange and blue respectively). ER represents the plastic response in axial root emergence rate and nRL represents the plastic response in the root-to-leaf biomass partitioning. Values are mean ± SE (n=3).

Effects of magnitudes of plastic responses on N uptake and plant productivity

Multiplying the ER response from 0 (disabled) to 1.4 times and increasing the nRL response from 0 (disabled) to 4 times the experimentally measured values was accompanied by a much larger change in N uptake under extremely limited N conditions than at intermediate or high N levels, where changes were comparable (Fig. 3.8A and D). Under intermediate to high N, changing the strength of the plastic responses did not impact plant productivity much, which indicates N was not limiting, therefore not much could be gained by responding more strongly. The plant productivity changes ranged from -5.8 % to +2.6 % compared with default plastic responses. However under low N, increasing the strength of the ER and nRL responses both resulted in variation in relative plant productivity (eq. 3.7) that followed the same trend and order of magnitude as the N uptake (Fig. 3.8B and E). Under moderate to high N levels, relative PE decreased with increasing magnitudes of the plastic responses. Under low N level, relative PE increased for ER response while relative PE increased until the default level but then remained almost unchanged at higher values for the nRL response (Fig. 3.8C and F).

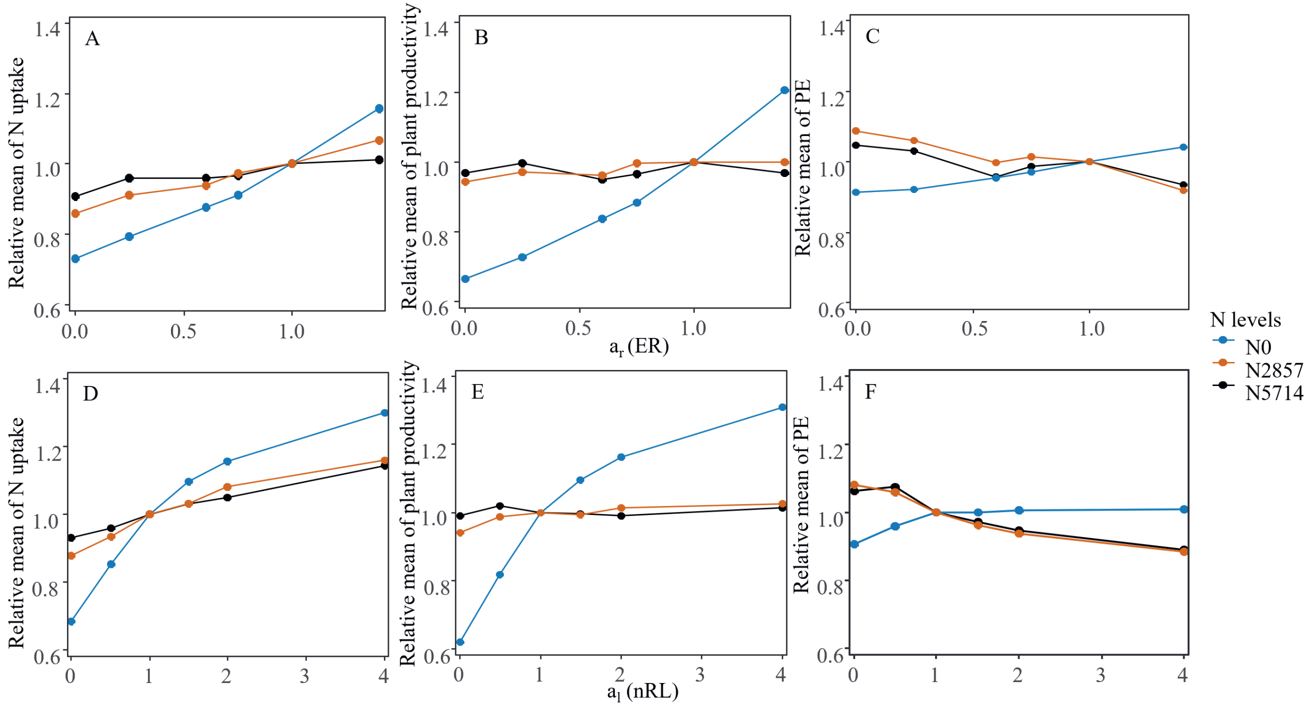

Figure 3.8: The effect of the magnitude of the plastic response across three different N application levels on N uptake, plant productivity and physiological efficiency (PE). The relative mean values is calculated with eq. 3.7. A, B and C represent the relative mean value of N uptake, plant productivity and PE across various magnitudes of parameter a_r of the plastic response in root emergence rate (ER, eq. 3.4). D, E and F represent the relative mean value of N uptake, yield and PE across various magnitudes of parameter a_l of the plastic response in root-to-leaf ratio (nRL, eq. 3.3). The blue color represents no extra N applied. The orange color represents the N application is 2857 $\mu mol/L$ and the black color represents the N application is 5714 $\mu mol/L$.

Discussion

In this study, we used a FSP model to quantify the relevance of plastic responses in root-to-leaf ratio (based on the functional equilibrium theory) and root emergence rate, on N uptake, plant productivity and physiological efficiency (PE, kg grain per kg N taken up) in the context of a crop canopy. In our model, nRL and ER were considered as two independent plastic responses. nRL is a determinant of carbon allocation to root biomass and ER is a determinant of axial root number (Fig. 3.3). Even though the maize cultivar used to parameterize the plastic responses was not the same as the ones used to parameterize the other plant traits, our model reproduced field experiment data of N uptake over a range of N levels well, under the light and temperature of the experimental years (Fig. 3.2A). We obtained a reasonable estimation of plant productivity under low N, but underestimated productivity at higher N. This underestimation was not due to rigidity of the model itself, as simulation of single plants (i.e. no competition) gave considerably higher plant productivity (Fig. B.8). Likely, our model lacks plastic responses to competition for light typical for dense canopies at high N: under non-limiting soil resources, maize plants are easily suffering from shading, and this is where plastic responses in shoot architecture and photosynthesis become important (Sharwood et al., 2014). Our model is designed as a tool to analyse how root plasticity influences maize N uptake and use, and not a crop model to predict yields given certain conditions. The fit to the N uptake data thus supports the conclusion that our model is a suitable tool to explore potential effects of plasticity on nitrogen capture.

Effect of plasticity on resource capture under low N conditions

The plastic responses to plant N status we derived from the hydroponic experiment affected the simulated root system architecture and increased simulated N uptake. At the beginning of the growing season, a large proportion of N still remained in the top 30 cm of the soil profile where also most roots were present at that time point and therefore most N was taken up from these rather than other soil layers. With plasticity enabled, root length density increased in the 15 to 30 cm soil layer, which was the layer where at later stages most nitrogen was taken up, especially under low N conditions (Fig. 3.5D). Consequently, an increase in total N uptake was observed with either or both plastic responses enabled (Fig. 3.4A), in line with observations reported in the literature (Saengwilai et al., 2014b; Guo & York, 2019; Schneider et al., 2021). A substantial carbon allocation shift in favour of the roots has been observed in plants facing nitrogen deficiency (Fig. 3.1, Lambers, 1983; Shipley & Meziane, 2002; Poorter et al., 2012). Our modeling study indicates that the combination of an increase in root biomass and an adjustment in vertical distribution of the roots, can be generated by two plastic responses, resulting in an increase in simulated nitrogen uptake across all N levels tested. The main effect of plasticity was an increase in root length density in the 15 cm to 30 cm soil layer where most N uptake took place. The effects of the plastic responses in nRL and ER on N uptake and grain yield are more additive under low N than at higher N levels.

Effect of plasticity on resource capture under high N conditions

Under high N, plastic responses also played a role in increasing N uptake (Fig. 3.4A). Plants did not invest in more root biomass (Fig. 3.7A), but did adjust root distribution (Fig. 3.6A), when the ER response was enabled. The higher percentage of RLD allocated to the 30 cm to 60 cm layer played a role in the greater N uptake under high N (Fig. 3.6A and B) while there was no clear increase in total root biomass (Fig. 3.7A). Shoot N status determines the number of axial roots that emerged per plant based on the ER response implemented in the model. Therefore, variation in shoot N status per plant within a plot leads to an increase in plant-to-plant variation in root number at a given moment in time. A reduced root number allows plant to invest root biomass in root extension leading to deeper rooting. Different axial root numbers per plant within a plot result in developing spatial niches of resource capture by root systems. Niche complementary of resource capture by root systems is one of the reasons that multi-species stands may have higher resource use efficiency than single-species stands (Zhang et al., 2017; Homulle et al., 2021). Our study indicates that similar complementary resource capture may occur in a mono-species stand of plants that have plastic responses under high N. Thus, even though these two plastic responses are considered to play a role under nitrogen stress conditions, there is no trade-off for resource capture under non-stressed conditions and it could even improve crop N uptake.

Should breeding include active plasticity as trait?

The focus of breeding for yield under high N seems to have resulted in cultivars achieving a high yield but at a high N demand (Ciampitti & Lemaire, 2022). In our study, the relevance of plastic responses for yield could not be verified well, as the increased productivity with increased uptake observed in field experiments was not reproduced by the model at moderate to high plant N levels. With a strong breeding pressure purely focused on the highest yield, the ideotype of plants in a crop setting would be one of identical plants that hardly compete with each other (Donald, 1968). Due to the current need for more sustainable agricultural practices with lower inputs, breeding should target not only high yield but also higher resource use efficiency (Chen et al., 2021). In order to move from high to medium-input agricultural systems, cultivars with high N uptake capacity under limited N input are needed (Ciampitti & Lemaire, 2022). When effectively capturing more N under limited or at least less luxurious N conditions, plants will automatically compete more intensely with each other (Casper & Jackson, 1997). Under these conditions, active plasticity such as the plastic responses quantified in this study can play a role. Rather than breeding for fixed trait values across various N levels, breeding for plasticity may create more productive genotypes that are adapted to a range of environments (Lobet et al., 2019).

Quantitative trait loci have been reported for plastic responses in roots based on observed genotype-by-environment interaction, though for other root traits than studied here such as root angle in response to water stress (Li et al., 2016; Kadam et al., 2017; Schneider et al., 2020). Genetic variation also exists for the plastic responses tested in our study (e.g. McMichael & Quisenberry, 1991) and can therefore be considered when selecting for plant performance, especially under limited N availability. Interestingly, these plastic responses also improved N uptake at high N conditions, thus reducing N losses to the environment or the need for high N application. While N uptake was improved at all N levels, this was accompanied by different root length density distributions across soil profiles. Where combination of fixed traits could have led to the same improvement at any of the N levels, only the active plasticity allowed optimal distributions at all N levels. Our modeling study therefore provides support to breeding for root plasticity as it indicates which traits to look for in view of high resource-use efficiency, while yield improvements are not always associated to the presence of root plasticity in crops.

While breeding for active plasticity has an advantage as crops can adjust their phenotype to the environment, there can also be costs that limit the adaptive value of plasticity for plant productivity (DeWitt, 1998; Schneider & Lynch, 2020; Van Kleunen & Fischer, 2005).Costs of plasticity are usually challenging to detect and quantify experimentally. More insight into the costs of plasticity could improve predictions from simulation models like ours. The FSP modeling approach could be used to study potential processes and pathways leading to costs of plasticity. This can then be used to design experiments to verify model generated insights (Bongers et al., 2018). Additionally, in breeding, there may be trade-offs when considering plastic traits: 1) Genetic linkage may mean selection for plasticity genes also selects for genes that reduce fitness. 2) Plasticity-related genes may have negative pleiotropic effects on other traits. 3) Epistasis may cause the regulatory loci producing the plastic response to cover the expression of other genes, which may reduce performance (Van Kleunen & Fischer, 2005; Schneider, 2022).

Besides the costs and limitations of plasticity in plant productivity, a discrepancy between the effects of plasticity on plant versus crop productivity may also exist, which may reduce benefits of selecting for plastic responses into current genotypes (Pedró et al., 2012; Sadras & Richards, 2014; Sadras, 2019). Overlooking plant-to-plant interactions can lead to unrealistic estimations on crop productivity when architectural traits are different at a low density compared to a typical crop density (Raper Jr & Barber, 1970; Sadras & Richards, 2014). Modeling could help postulate hypotheses on the contribution of the underlying processes in crop stands by including and enabling or disabling plasticity at plant level, as we showed in this study.

FSP modeling in plasticity studies

Our model can disentangle the variation in productivity and N uptake in plants at different developmental stages caused by active plasticity related to N limitation, from the effects of the seasonal course in light and temperature on plant phenotype (i.e. passive plasticity). These effects interact, and are hard to separate using statistical methods on field data. In addition, since the model also represents the architecture of individual plants, the spatial arrangement of plant organs and the consequences for neighbour competition for resources is mimicked. Therefore, FSP modeling provides great opportunities to explore plasticity effects at the plant level with the potential to scale up to the crop level. Previous studies used this type of models to explore the effect of plasticity on light interception in intercropping systems (Zhu et al., 2015; Li et al., 2021). Bongers et al. (2018, 2019) have used FSP modeling with shoot structures to explore the effects of shade avoidance response on natural selection at different densities over generations of Arabidopsis. Root models have been used to study the effect of plasticity on nutrient capture from different types of nutrient-rich patches in soils (Henke et al., 2014) or soil N and phosphorus uptake (Perkins & Lynch, 2021). In this study, we implemented root responses into a whole-plant model to explore the effect of dynamic plasticity in relation to modelled internal N status on plant performance, in the context of a crop canopy. This constitutes a new step in the use of FSP modeling by combining plasticity and whole-plant simulation in a competitive environment.

For future research, genetic variation among cultivars of the two plastic responses we considered can be studied to quantify gains in N uptake and yield. To further understand the effect of plasticity, multiple plastic responses for other traits such as root angle (York et al., 2015), lateral branching (Postma et al., 2014) and shade avoidance could be addressed simultaneously to explore their interactions, trade-offs, synergies and potential contribution to N-uptake and plant performance.

Conclusions

We used a whole-plant FSP model that contains plastic responses in root biomass allocation ratio and root emergence rate to nitrogen concentrations, in order to study the effects of plasticity on NUE and its components. Our results indicate that plants that alter biomass allocation and root system architecture can indeed improve N uptake across a large range of N conditions and that this improves plant productivity under low N. Plastically altering root biomass partitioning and root emergence rate in reaction to plant N status is an important strategy to improve N uptake and seems to work across N levels. These plastic responses led to an improved root distribution within the soil profile which constitute an important strategy to improve N uptake, also under high N. Both the observed plasticity in root:shoot allocation ratio of carbon and in root emergence rate showed a positive effect on simulated plant productivity under low N conditions. Including nitrogen uptake and plasticity in root traits as breeding targets could thus have potential for enhancing crop performance under limited N inputs. Further studies on cultivars with genetic variations of ER and nRL responses to plant N status would be needed to consolidate these findings.

Chapter 4

Phenotypic plasticity can mitigate the reduction in grain nitrogen associated to stay-green maize

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Abstract

Stay-green is an important plant characteristic in maize to increase grain yield by prolonging leaf life. However, a longer leaf life span can reduce nitrogen (N) remobilization from leaves to the grain, as leaves senesce later, leading to lower grain quality. Plastic responses to plant N status that increase N uptake potentially compensate for this negative effect on grain N in stay-green varieties. To explore to which extent such plastic responses interact with stay-green characteristics and their effect on grain N, we applied a functional-structural plant (FSP) modeling approach. We first used field observations to evaluate model performance for a range of cultivars differing in leaf senescence rate. Then we ran the simulations at a range of N levels with and without plastic responses, in stay-green and non-stay-green plants (by disabling and enabling leaf senescence, respectively). We analysed the relative contribution of plasticity to N uptake, grain yield, and grain N concentration. Grain yield in stay-green plants was higher under high N, while under low N, grain yield was only higher in stay-green plants when the plants were plastic. Plastic responses reduced the negative effect of stay-green on grain N concentration by improving N uptake especially under low N. Using maize cultivars that plastically respond to plant N status and improve N uptake may therefore mitigate the negative effects of stay-green on grain quality.

Introduction

In maize, post-silking photosynthesis can contribute around 80% to grain yield accumulation (Zhu et al., 2010; Chen et al., 2015a). Therefore, extending the time the crop can maintain a high rate of photosynthesis is one of the breeding strategies to increase maize yield. Functional stay-green characteristics in which leaf life-span is prolonged due to delayed senescence, extends the period with high photosynthetic rate, improving grain yield (Thomas & Howarth, 2000; Thomas & Ougham, 2014). Stay-green traits have been gradually bred into current maize cultivars, especially cultivars released from the 1960s to the early 2000s in China (Chen et al., 2015a, 2021). However, this extra photosynthetic capacity also requires more nitrogen to be retained in the leaves. About 22–46 % of grain N is reallocated from leaves, and 50 to 70 % of leaf N can be remobilized to the grain (Chen et al., 2014b, 2016a). Grain N content determines grain quality in terms of protein concentration. Therefore, in stay-green varieties there is a key trade-off between maintaining leaf photosynthesis for higher yield and ensuring sufficient N allocation to grains for high grain quality. Interestingly, in field experiments, a decrease in grain N concentration has not always been observed in stay-green cultivars (Chen et al., 2013). The underlying mechanisms that avoid any negative effect on grain N in stay-green cultivars are difficult to study and to quantify experimentally.

To compensate for the negative effect on grain N of staying green, the amount of N available within the plants should be increased. This could be achieved by increasing N uptake through changes in root system architecture. Deeper roots are important for resource uptake, such as nitrogen and water in deep soil layers, especially under suboptimal conditions (Rogers & Benfey, 2015). Deeper roots under low N or drought can increase grain yield (Uga et al., 2013; Battisti & Sentelhas, 2017). Several root system traits such as small root angle or low nodal root number can contribute to deep rooting, and thereby to enhanced N uptake (Rogers & Benfey, 2015; Saengwilai et al., 2014b). The root system is highly plastic, as it can shape its development, architecture and physiology to the environment it grows in (Schneider & Lynch, 2020). Some plastic responses such as changing root angle in response to low N make roots grow more steeply which can result in deeper rooting (Trachsel et al., 2013). In a previous study (Chapter 3), we showed that plastic responses such as root-to-leaf biomass allocation and axial root emergence rate to plant N can improve N uptake across N conditions and improve yield under low N conditions. Therefore, in this study, we hypothesised that plastic responses to plant N can mitigate the negative effects of leaf life span on N remobilization to the grains through enhancing N uptake.

To test this hypothesis, a modeling approach called functional-structural plant (FSP) modeling, has been applied (Vos et al., 2010). This type of model can simulate the development and growth of plants including both above- and belowground parts in a virtual 3D canopy, that compete with each other for resources. FSP models have been instrumental in exploring novel plastic responses (Bongers et al., 2018, 2019) and test for novel biological mechanisms(Prusinkiewicz et al., 2009). We applied FSP modeling to quantify the trade-off between yield and grain N in stay-green maize, and to assess the importance of plastic responses in root system traits to plant N in reducing this trade-off.

Methods

First, we analysed data on green leaf area at physiological maturity for six maize cultivars from a study by Chen et al. (2013), and we chose the two most contrasting cultivars in terms of green leaf area duration to identify the relative differences of N uptake, grain yield and grain N concentration. In the model (Chapter 3), we represented stay-green by disabling leaf senescence and with that disabling leaf senescence related nitrogen remobilization. We compared simulated and field data to qualitatively assess model performance for N uptake, yield and grain N concentration. After that, we used the model to assess to which extent plasticity in root traits in reaction to plant N status interacts with leaf senescence, to contribute to N remobilization from leaves to grain. To achieve this goal, simulations were run with and without the plastic responses to plant N enabled, with both stay-green (no leaf senescence) and default leaf senescence settings. The simulations were run at five N application levels ranging from 120 kg/ha to 240 kg/ha.

Model description

An existing maize FSP model driven by light and soil nitrogen was used (Chapter 2 and 3). The model had been developed in the GroIMP modeling platform (Hemmerling et al., 2008). It mechanistically simulates on a daily time step shoot and root growth and development based on carbon and nitrogen sink-source relationships between organs, light interception, temperature and nitrogen uptake.

In the model, the soil is composed of soil cells of 0.15*0.15*0.15 m until 0.9 m deep which is beyond the maximum depth of the root system of the cultivars on which our study is based. Simplified soil processes for leaching, mineralization and N application have been implemented in the model (Chapter 2). High-affinity and low-affinity transport systems are considered as the mechanisms to simulate N uptake by root segments(Siddiqi et al., 1990).

Temperature is defined as a function of day of the year, and incoming radiation is defined as a function of latitude, day of the year and transmissivity of the atmosphere during the growth season (Spitters, 1986). To simulate field light conditions, 24 directional light sources and 72 diffuse light sources were placed into a hemisphere (Evers et al., 2010). Light capture is simulated by the reverse Monte-Carlo ray tracing algorithm in GroIMP (Hemmerling et al., 2008). To minimize the border effect, the simulation plot was set to 3 rows of 3 plants each, with 0.6 m between rows and 0.15 m between plants, in this way all plants always had neighboring plants other than themselves allowing for full plant-to-plant competition for light and N resources (Fig. B.5).

Organ carbon sink strength is defined as a sigmoid function of organ potential dry mass and growth duration (Yin et al., 2003). Leaf level photosynthesis is simulated through a light-photosynthesis response curve (Archontoulis & Miguez, 2015). The parameter that represents leaf photosynthetic capacity in this relation depends on photosynthetic N through a logistic model (Sinclair, 1989; Vos et al., 2005). After carbon is assimilated, simulated organ growth is based on sink strength of each organ and available carbon.

N sink strength of each photosynthetic organ and available N within the plant determine the distribution of photosynthetic N among photosynthetic organs. N sink strength is determined by the light-interception gradient in the canopy and a parameter for maximum specific leaf N of fully-lit leaves at the top of the canopy (Hikosaka, 2003). There is structural N for each organ to support plant growth, which is expressed as fractions of organ biomass; structural N cannot be remobilized. The N remaining after assigning the photosynthetic N and the amount of N to support organ growth, is stored in an N pool. N stored in the pool and photosynthetic nitrogen are available for reallocation to leaves, stems and grains the next time step (day). Once the plant enters the reproductive stage, part of the available nitrogen is allocated with priority to the grains (Masclaux-Daubresse et al., 2008, 2010). Under low nitrogen, the grain nitrogen influx rate was less than the rate under sufficient nitrogen (Ning et al., 2017; Fernandez et al., 2021). Therefore, to account for balancing the plant N flux between photosynthetically active leaf tissue and grain protein accumulation, the fraction of nitrogen allocated to the grains was considered to be a function of leaf nitrogen concentration from the previous time step (see Chapter 2, Grain nitrogen section).

Leaf senescence in the model is determined by the maximum leaf life span, specific leaf nitrogen and leaf light interception. A Gaussian function of leaf rank is used to represent maximum leaf life span (Lizaso et al., 2003). Both a critical level of specific leaf nitrogen and of leaf light capture are set as a threshold for leaf senescence. The actual moment an individual leaf starts senescing is determined by whichever of these three conditions occurs first. Upon leaf senescence, the non-structural N in the leaf becomes available for allocation in the next step, which represents N remobilization.

Two root plastic responses to plant N status are included in our model: the root-to-leaf biomass partitioning coefficient (nRL, dimensionless) and the root emergence rate (ER, in number of roots per °Cd). The nRL response is expressed as a negative exponential function of leaf N concentration based on functional equilibrium theory (Chapter 3; Brouwer, 1962; Lambers, 1983; Shipley & Meziane, 2002). The ER response is defined as a linear function of shoot N concentration (both introduced in Fig. 3.1).

Field experiment

For comparison we used data from a field experiment conducted in Siping (43°17'N, 124°26'E), Jilin province, China, in 2010 (Chen et al., 2013). A split-plot design was used with 4 replications where N treatments were the main plots and genotypes were the subplots. The maize cultivars tested were Zhengdan958 (ZD958), Xianyu335 (XY335), Zhongdan2 (ZD2), Danyu13 (DY13), Yedan13 (YD13) and Nongda108 (ND108) which have all been developed between the 1970s and the 2000s in China. Two N levels were applied: 240kg/ha (HN) and 0kg/ha (noN). The N available to the plants before N application had not been recorded (Chen et al., 2013). Maize seeds were hand-sown at a density of 6 plants/m^2, at a row distance of 60cm and a within-row plant distance of 28 cm. The experiment was rain-fed.

The green leaf area was destructively measured for three plants of each cultivar within the high N treatment and for each replicate, every 10 days from silking to physiological maturity (six times). Grains were collected for three plants of each cultivar within each N treatment and replicate, at physiological maturity and oven dried at 70°C until the dry weight was constant. Grain weight was then measured and grain N concentration was determined by the semi-micro Kjeldahl method (Nelson & Sommers, 1973).

To calculate the relative difference (eq. 4.1) between stay-green and early senescence cultivars in N uptake, grain yield and grain N concentration, we selected the two most contrasting cultivars based on their green leaf area at physiological maturity.

Model simulations

For testing the capacity of the model to simulate stay-green performance, simulations with 10 replications were first run for two N application levels (0 and 240 kg/ha) with initial soil N at 70 kg/ha and at a density of 6 plants/m^2. Simulations were done with leaf senescence turned either off or on representing a theoretical extreme case of stay-green and early senescence plants, respectively. We used the relative difference (eq. 4.1) between no leaf senescence and early senescence in N uptake, grain yield and grain N concentration to compare with field observations. Equation 1 was used to express relative differences in N uptake, grain yield, grain N, RLD, leaf area and SLN (all represented by X in eq. 4.1).

$$Relative\Delta\bar{X} = \frac{\bar{X}_i - \bar{X}_j}{\bar{X}_j} \tag{4.1}$$

i represents either functional stay-green characteristics or with plastic response to plant N and j represents either early leaf senescence characteristics or without plastic response to plant N.

Next, to explore the relation between stay-green, plasticity and plant performance, simulations with 10 replications were run under five N application levels (120, 150, 180, 210 and 240 kg/ha) with initial soil N at 70 kg/ha and at a density of 11 plants/m^2. The simulations were done for four scenarios representing all combinations of plants with leaf senescence disabled (representing stay-green) and enabled (representing early leaf senescence) and with either or both plastic responses in root/leaf partitioning and root emergence rate enabled or disabled. We compared the N uptake, grain yield, grain N concentration and relative differences (eq. 4.1) of N uptake, grain yield and grain N concentrations between plant types (no leaf senescing vs early senescing or plastic vs non-plastic).

Finally, to analyze how plasticity affects the relation between stay-green characteristics and plant N uptake, we compared the relative difference in root length density(RLD, m/m^3) per soil layer for stay-green plants between with and without plasticity under high N (240 kg/ha) and low N (120 kg/ha). To interpret the patterns in plant production, functional leaf area duration, a good indicator for grain production, was derived from simulation outputs across various N conditions by adding up plant leaf area per day from silking to physiological maturity. To further explore the consequences of plasticity and stay-green on leaf area, the vertical distribution of leaf area was quantified, which is important for the efficiency of light interception. The relative differences in individual leaf area obtained two weeks before physiological maturity for plants with plasticity enabled were compared between early leaf senescence and stay-green plants. Leaf N determines the photosynthetic capacity of the leaf area and therefore, influences the efficiency of light use. Thus in addition, in order to understand differences in radiation use efficiency between plants with stay-green and early leaf senescence, we compared relative differences in SLN

(δSLN) between plants with and without plasticity enabled at maturity and silking under high N (240 kg/ha) and low N (120kg/ha).

All statistical analyses were done using R version 4.0.2 (R Core Team, 2021). The 'ggplot2' package (Wickham, 2009) was used to produce figures. Means and standard errors are shown in all figures.

Results

Selection and evaluation of contrasting cultivars

At physiological maturity, DY13 had the lowest remaining green leaf area while ND108 had the largest green leaf area. (Fig. 4.1). We selected these two cultivars for further analysis. The stay-green cultivar ND108 had higher N uptake and grain yield, but slightly lower grain N concentrations under both high and no N (Table. 4.1). In simulations, the total leaf area for the functional stay-green cultivar and the early senescence cultivar differed considerably, as intended (Fig. 4.2). The simulations showed similar increases in relative N-uptake and grain yield, and a similar relative decrease in grain N concentration for stay-green plants as the field data for both studied N levels (Table. 4.1; Fig. 4.3).

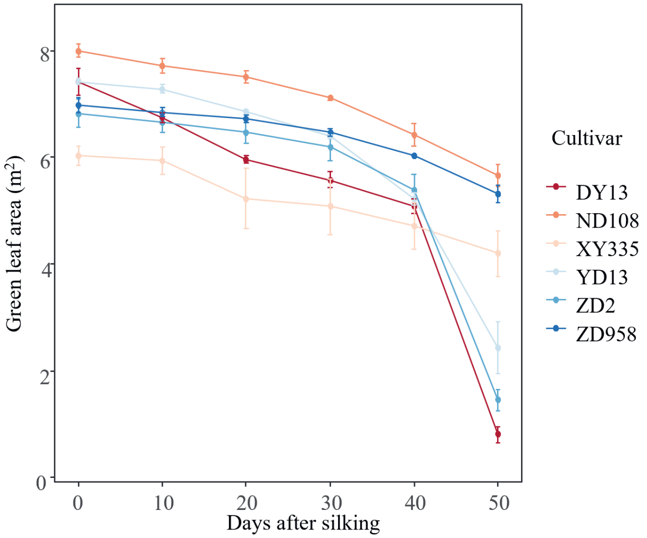

Figure 4.1: Green leaf area for plants of seven cultivars grown at 240 kg N/ ha from the reported field experiment in 2010 by Chen et al. (2013). Symbols and error bars represent means ± SE (n=4).

Table 4.1: Field observations on N uptake, grain yield, grain N concentration and relative differences between the two maize cultivars under high N (240kg/ha) and no N (0 kg/ha).

N levels	Traits	Early senescence cultivar (DY13)	Stay-green cultivar (ND108)	Relative differences
High N	N uptake (g/plant) Grain yield (g/plant) Grain N (%)	2.95 ± 0.097 125 ± 4.46 1.14 ± 0.015	3.52 ± 0.18 154 ± 4.9 1.10 ± 0.028	0.20 ± 0.069 0.24 ± 0.055 -0.037 ± 0.027
no N	N uptake (g/plant) Grain yield (g/plant) Grain N (%)	1.75 ± 0.23 108 ± 13.0 0.83 ± 0.044	2.18 ± 0.12 127 ± 3.85 0.82 ± 0.031	0.32 ± 0.19 0.24 ± 0.16 -0.016 ± 0.058

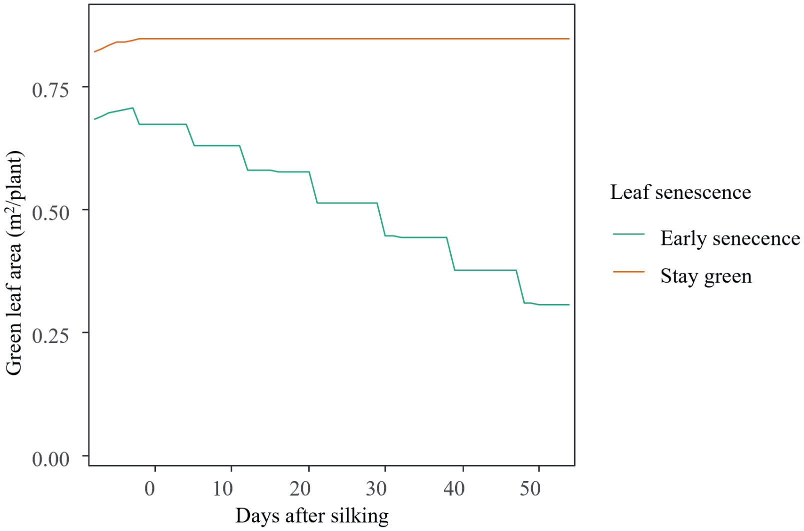

Figure 4.2: Simulated green leaf area per plant for plants grown at 240 kg N/ ha, for plants with early leaf senescence (green lines and symbols) and without leaf senescence (orange lines and symbols). Lines and error bars represent means ± SE (n=10).

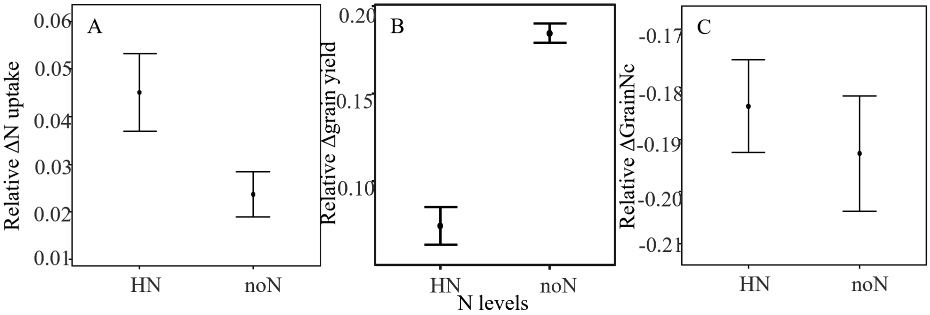

Figure 4.3: Relative gain in N uptake (A), grain yield (B) and grain N concentration (C) of being stay-green vs having early leaf senescence from simulations at two N conditions (HN: 240 kg/ha and noN: 0 kg/ha). Relative gains were calculated using eq. 4.1. Points and error bars represent means ± SE (n=10).

Plastic response to plant N status, stay-green and interactions

Our simulations showed that plants with plastic responses resulted in higher N uptake than plants without plasticity (Fig. 4.4A). Stay-green plants had higher N uptake than plants with early leaf senescence across all N levels. The relative improvement in N uptake due to stay-green was larger with plastic responses enabled, at all N application levels except for the highest N level (Fig. 4.5A).

Highest yield was achieved by the stay-green plants that were allowed to respond plastically to plant N, across all N levels. For N applications from 180 to 240 kg/ha, plants having no leaf senescence and not being plastic outperformed plants with early leaf senescence but being plastic (Fig. 4.4B). At lower N levels, the relative gain in yield due to no leaf senescence profited from plastic responses, while as N levels increase the yield gain due to plasticity with no leaf senescence becomes smaller (Fig. 4.5B).

The lowest grain N concentrations were obtained by stay-green plants that did not respond plastically to plant N status, across all N levels (Fig. 4.4C). Having either no leaf senescence or being plastic resulted in similar grain N concentrations, except under high N (Fig. 4.4C). Being plastic resulted in a reduction in the grain N penalty associated to stay-green, at all but the highest N level (Fig. 4.5C).

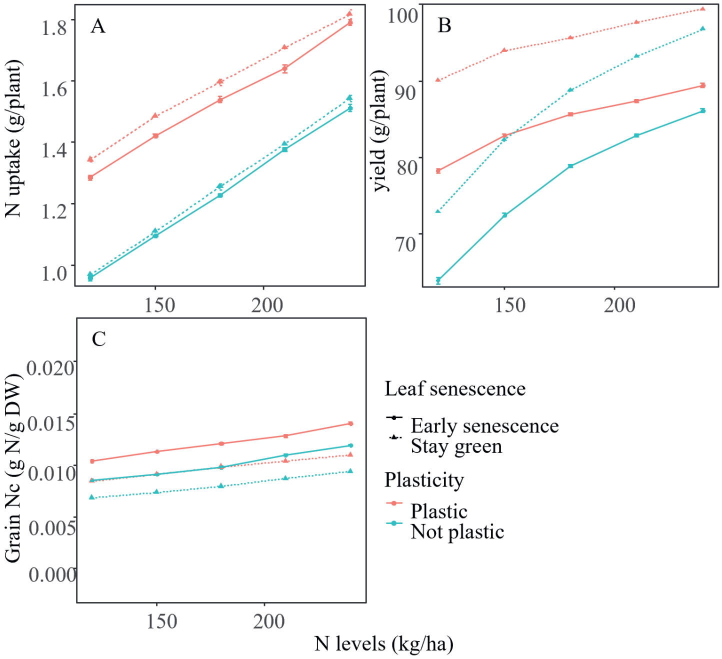

Figure 4.4: N uptake (A), grain yield (B) and grain N concentration (C) as a function of applied N from simulations of plants grown at high planting density (11 $plants/m^2$). The blue lines represent simulations without plastic responses to plant N (Not plastic) and the red lines represent simulations with plastic responses to plant N (Plastic). The solid lines represent simulations with early leaf senescence and the dotted lines represent simulations with no leaf senescence (stay green). Symbols and error bars represent means ± SE (n=10).

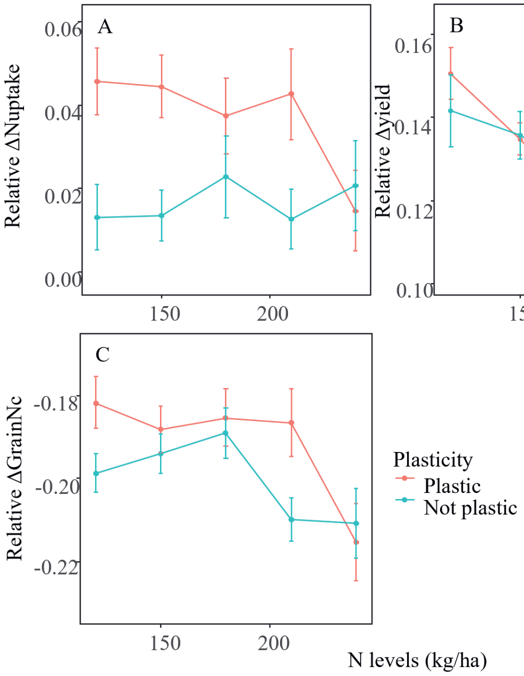

Figure 4.5: Simulated relative gains in N uptake (A), grain yield (B) and grain N concentration (C) as a function of applied N of being stay-green vs having early leaf senescence under high planting density (11 plants/m^2). The blue lines represent simulations without plastic responses to plant N (Not plastic) and the red lines represent simulations with plastic responses to plant N (Plastic). Symbols and error bars represent means ± SE (n=10).

Root vertical distribution, functional leaf area duration and specific leaf N

Both with and without plasticity, stay-green plants had higher RLD in all soil layers both at high and low N (values above 0.0 in Fig. 4.6). In the soil layer between 15 and 30 cm, RLD increased more with plasticity than without. Presence of plasticity increased functional leaf area duration under low N in plants with senescing leaves while it slightly reduced functional leaf area duration under high N in stay-green plants (Fig. 4.7). For lower leaf ranks, plasticity resulted in less leaf area in no leaf senescence plants, under both high and low N (Fig. 4.8A, B). Under low N, plasticity resulted in an increase in leaf area for higher leaf ranks regardless of leaf senescence type (Fig. 4.8B). Under high N, plasticity resulted in a decrease or no change in leaf area for higher leaf ranks (Fig. 4.8A). Under high N, the relative gains in SLN of enabling plasticity were comparable between early leaf senescence and no leaf senescence at both silking and maturity (Fig. 4.9). Under low N at maturity, the improvement of relative gain in SLN by plasticity was larger for plants without than with leaf senescence (Fig. 4.9B). The improvement was smaller at silking (Fig. 4.9A).

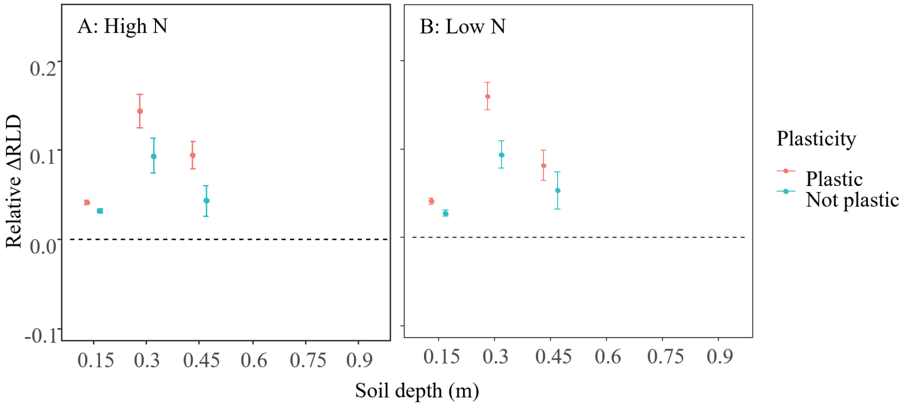

Figure 4.6: Simulated relative gains in root length density (RLD, m/m^3) of being stay-green vs having early leaf senescence under high N (HN, 240 kg/ha, Panel A) and low N (LN, 120 *kg/ha*, Panel B) conditions and for plants with or without plastic response to plant N status. The X-axis represents the different soil layers. In this figure, information was only provided for soil layers from 0 to 0.45 m depth due to the importance of N uptake from these layers. Full RLD information is provided in Table B.1. Symbols and error bars represent means ± SE (n=10).

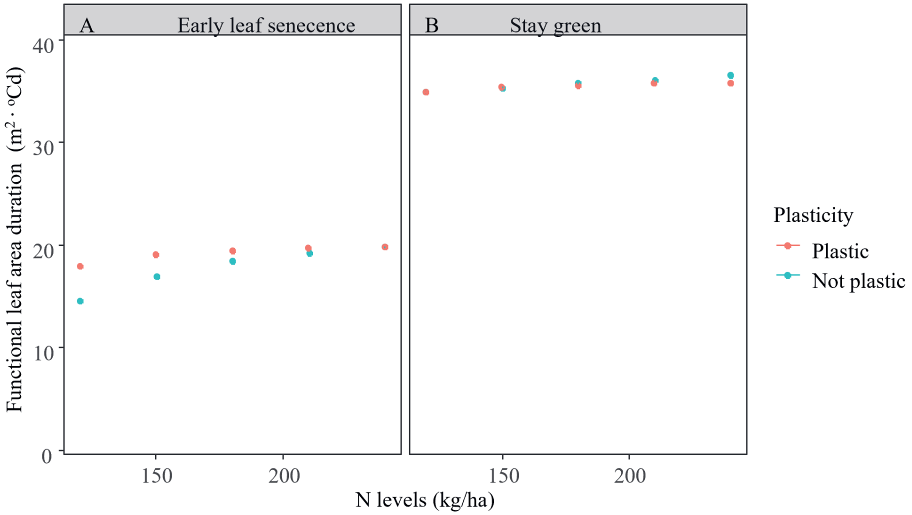

Figure 4.7: Functional leaf area duration from silking (92 days after sowing) to physiological maturity (154 days after sowing) for plants with early leaf senescence or without leaf senescence (Stay green, B), and for simulations without (not plastic) or with plastic responses to plant N (plastic). Symbols and error bars represent means ± SE (n=10).

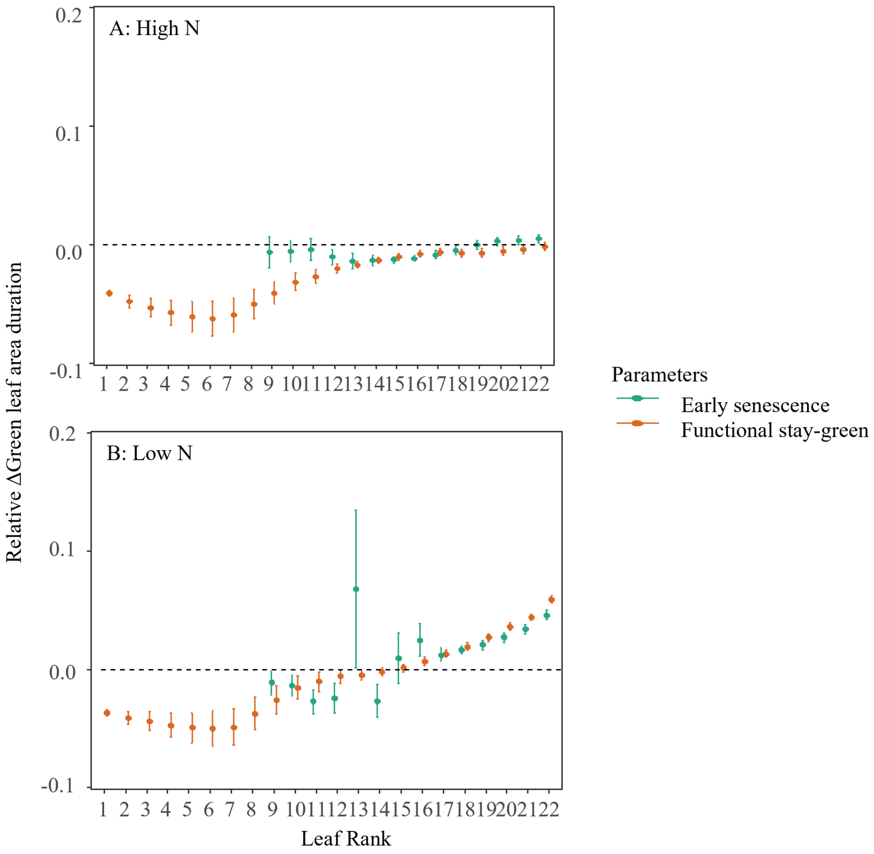

Figure 4.8: Relative gains of being plastic to plant N vs not plastic and for plants with early senescence or without senescence (stay green) grown under either high N (240 kg/ha, Panel A) or low N (120 kg/ha, Panel B) and for leaves of different ranks. Symbols and error bars represent means ± SE (n=10).

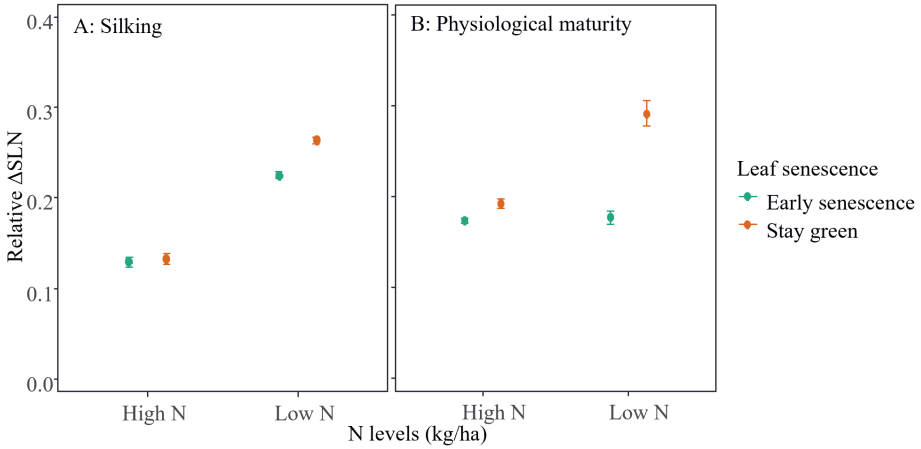

Figure 4.9: Relative gains in specific leaf nitrogen (SLN) when plants are plastic to plant N vs not plastic and for plants with early senescence or without senescence (stay green) grown at either silking (Panel A) or physiological maturity (Panel B) and under high N (240 kg/ha) and low N (120 kg/ha). Symbols and error bars represent means ± SE (n=10).

Discussion

Even though trade-offs between grain N concentration and grain yield have been demonstrated for stay-green cultivars (Kosgey et al., 2013; Ciampitti et al., 2013), Chen et al. (2013) found only minor decreases in grain N concentration in their cultivar panel, while there were clear differences in leaf longevity (Fig. 4.1; Table 4.1). The magnitude of the differences between real cultivars and simulated cultivars in N uptake, yield and grain N (Table 4.1; Fig. 4.3) shows that cultivar differences other than in leaf senescence must have been playing a role. Our model can represent virtual knock-out mutants, whereas the real cultivars vary in many traits simultaneously, of which leaf senescence is only one. This shows the strength of a modeling approach like ours to analyse the interaction effect between plastic response to plant N and stay-green characteristics on grain quantity and quality.

N uptake

The consequences for N uptake of the plastic responses to plant N we studied were larger in stay-green plants than in those with early leaf senescence (Fig. 4.4A; Fig. 4.5A). Our simulation results indicate that deeper rooting may occur due to interactions between stay-green and plastic responses to plant N. With stay-green, the effect of plastic responses on N uptake was slightly enhanced (Fig. 4.5A) due to increased relative differences in root length density (RLD) (Fig. 4.6). Previously we observed that the 15 to 30 cm soil layer is the major region for N uptake in the model (Chapter 3) and the increased RLD in this soil layer can result in more N uptake. In line with our results, deeper roots enhanced N uptake for stay-green barley cultivars as reported based on field and greenhouse observations, which may compensate reduction in grain N concentration for stay-green cultivars (Williams et al., 2022; Thomas & Ougham, 2014). While in our study the enhanced N uptake was enhanced by plasticity in root traits in reaction to plant N status, delayed root senescence and improved post-silking N uptake have been proposed as reasons for improving N uptake (Mi et al., 2003; Ciampitti et al., 2013). Root senescence is a phenomenon currently not included in our model, but a higher post-silking N uptake was also observed in our simulations; much stronger so with plasticity enabled (Fig. C.1).

Grain yield and grain N concentration

Under high N, stay-green plants had more functional leaf area duration to intercept light and assimilate carbon, which resulted in higher simulated grain production (Fig. 4.4B; Fig. 4.7; see also Borrell & Hammer, 2000; Liu et al., 2009). Plastic responses to plant N showed minor effects on individual leaf area specially for higher leaf ranks (Fig. 4.8A). A reduction in functional leaf area duration caused by leaf senescence can be the major reason for reduced yield under high N. In our simulations at high N, the yield gain due to plasticity when leaves did not senesce is smaller than at low N (Fig. 4.5B), due to decreased functional area of leaves at lower leaf ranks (Fig. 4.8A). In line with our results, old maize cultivars, which were assumed to have stronger plasticity compared with modern maize cultivars, were observed to have smaller leaf area below the ear at silking under non-limiting N (Ma et al., 2014; Zhai et al., 2017; Perez et al., 2019). In addition, the relative differences in SLN in the presence of plasticity between early leaf senescence and stay-green were comparable (Fig. 4.9A, C) indicating a negligible effect on leaf N distribution.

When N was limiting, we showed that being able to improve N uptake through plasticity was highly relevant for yield, irrespective of being stay-green or not (Fig. 4.4B; Chapter 3). Plastic responses had a stronger effect on the increase in N uptake for stay-green plants than for plants with early leaf senescence (Fig. 4.5A). Therefore, SLN increased more due to plasticity in stay-green plants than in plants with early leaf senescence through increasing N uptake (Fig. 4.9). SLN closely links to leaf photosynthetic capacity (Vos et al., 2005) and therefore high SLN supports grain carbon accumulation.

Stay-green is negatively correlated with N remobilization to grain (Kosgey et al., 2013; Ciampitti et al., 2013; Shao et al., 2021). Grain N concentration does not always reduce significantly under low N in stay-green cultivars compared to early-senescence cultivars. Increasing post-silking N uptake can mitigate grain N reduction (Mi et al., 2003; Chibane et al., 2021). Combining this with our simulation results, we conclude plasticity could be one of the reasons to explain smaller differences in grain N under low N between stay-green cultivars and early-senescence cultivars (Fig. 4.4C; Fig. 4.5C). Under high N, the effect of plastic responses on grain N concentration in stay-green cultivars became smaller than the effects of leaf senescence mainly because the rate of N remobilization to the grains became the limiting factor. In other words, N remobilization is more important compared with N uptake when plant N is not limiting (Fig. 4.5C, cf. Fradgley et al., 2021).

Exploring the role of plasticity and interaction with plant traits through FSP modeling

Plant eco-physiological mechanisms such as plastic responses to environmental cues may interact with each other, and cannot always easily be disentangled and quantified experimentally. While virtual experiments, like the ones we used, are always a simplification and come with uncertainties, a modeling approach like the one used in this study can be instrumental in the analysis of the consequences of plant responses and their interactions with plant traits for plant functioning. We see further applications of this approach to improve understanding of the relevance of plasticity in stay-green cultivars. For instance, additional plasticity such as avoiding shade by reorienting leaves (Maddonni et al., 2002) can be directly implemented in the current model to further analyze the consequences for target outputs in stay-green cultivars. Furthermore, our model can be used to analyze the genetic variation of plasticity creating multiple phenotypes and thereby provide quantitative suggestions to breeding programs.

We showed that the effect of the interaction between plastic responses and stay-green for N uptake, grain yield, and grain N concentration can be directly analyzed. Nevertheless, the current model overestimates the reduction in grain N concentration and underestimates increases in N uptake, even with plastic responses enabled. However, unlike knock-out mutants, cultivars differ in a number of traits, which complicates tracing back differences in functioning and productivity to individual mechanisms like plasticity. The model we used mimics the knock-out mutants for one or more processes to study the potential effects and interactions between processes of interest; something much harder to achieve in real plants.

Enhancing post-silking N uptake may mitigate the reduction in grain N for stay-green cultivars (Mi et al., 2003; Ciampitti et al., 2013). Stay-green cultivars have prolonged active N uptake, due to a longer photosynthetic period and a longer period for carbon transportation to the roots, leading to higher post-silking N uptake (Williams et al., 2022; Borrell & Hammer, 2000). The stronger increases in N uptake in the stay-green cultivar (ND108) than in the early leaf senescence cultivar (DY13) compensated for the reduction in grain N concentration comparing with simulations where the increase of N uptake was minor (Fig. 4.3A). Our model currently does not take linkage between available carbon assimilates and active uptake into account. Further expansion of the model could include this linkage and quantify the consequences of the interactions between stay green and root active uptake for plant functioning and productivity.

Conclusion

Plastic responses to plant N can increase N uptake for stay-green maize varieties compared to early senescing cultivars. Stay-green is the major reason for grain yield improvements under high N, while stay-green plants plastically responding to N can contribute to extra gain in grain yield under low N. Plastic responses to plant N in stay-green plants do contribute to mitigating the expected reduction in grain N concentration at low N application, but have only a minor effect when N application is high. This study has shown that plasticity is one of the likely mechanisms that reduce the trade-off between grain yield and grain N concentration in stay-green cultivars.

Chapter 5

Maize root system phenotypes for high yield and nitrogen uptake

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Abstract

Identifying maize genotypes with a high capacity for nitrogen uptake without yield penalty is relevant to maize breeding for sustainable production systems with limited inputs. Identifying the root system phenotype to achieve this is complex in field or greenhouse experiments, due to high variation in root phenotypes within a single genotype. Therefore, we applied a functional-structural plant (FSP) modeling approach. We first identified root phenotypes that give either high yield or high N uptake at a wide range of soil nitrogen levels. Data from a field experiment with six maize cultivars released in China between the 1970s and the 2000s was used to evaluate the model results. Subsequently, we performed a virtual experiment to identify root phenotypes that could combine both high N uptake and high yield at a high plant density common for maize, and across a wide range of nitrogen levels. Our results demonstrated trade-offs in root phenotypes for high yield and for high N uptake, between root sink strength for carbon, and root-to-leaf biomass partitioning. Using cluster analysis we identified two root phenotypes that give both high yield and high N-uptake. We show low root biomass and high root length density at 15 cm to 45 cm depth allowed maize to combine high yield and high N uptake. Our study demonstrates that cluster analysis of FSP modeling results can be used to identify improved root system phenotypes across a wide range of environments, which can be useful information when defining targets for breeding.

Introduction

Reducing N fertilization is an urgent requirement to make future agriculture more sustainable. Simultaneously such reduced input will require crop genotypes that maximally maintain productivity e.g. through enhanced uptake of soil N. For decades maize breeding programs have been selecting high-yielding genotypes under high N input. This has resulted in genotypes that produce high yield but also have high N demand (Ciampitti & Lemaire, 2022). Reduced N fertilizer application can lead to N-stress for current genotypes and reduce their grain yield. Therefore, we need genotypes that are efficient in taking up the limited N that is available. However, there may be a price. Trade-offs could exist between high N uptake capacity and high yield of maize cultivars since resource investment in one trait may come at the expense of other traits (Lundgren & Des Marais, 2020; Bloom et al., 1985). Little is known about such trade-offs between high N uptake capacity and high grain yield of maize. Experimentally determining this is difficult given the complexity of the genetic background of current maize cultivars and the resources required for root system analyses. Plant models covering both root and shoot processes related to carbon and nitrogen economy can help to make a first step prior to targeted experimentation (Chapter 2 and 3).

Planting maize at a high density (10 plants per m^2 or more) is an important management strategy to obtain high crop yield (Hou et al., 2020; Xu et al., 2017). In order to support the higher biomass production per unit area at high densities, higher N fertilizer input may be required (Yan et al., 2017). However, grain yield at high density can be maintained while reducing N application by 15% compared to the application required at lower density (Du et al., 2021). Biomass per plant decreases with increasing planting density (Qian et al., 2016) and plants at high planting density grew more roots in the top soil layer (0-18 cm) (Shao et al., 2018). However, with increasing competition for soil nutrients and water at higher planting density, roots were forced to grow deeper and plants reduced the number of axial roots (Shao et al., 2018). What plant strategy in fact optimizes N uptake remains unclear. In other words, there is a need to identify beneficial root phenotypes to increase the use of soil N at high planting density.

Characteristics of individual roots and root systems, which exhibit active plastic responses to growth conditions, are highly relevant to plant N uptake capacity (Schneider & Lynch, 2020). The root phenotype is shaped by the growth environment, often due to both passive plasticity resulting from resource availability, and active plasticity resulting from responses to specific signals (Van Kleunen & Fischer, 2005; Schneider, 2022). The root system architecture in the field is thus the result of a combination of genetically determined root traits as well as plasticity in those traits in relation to environmental conditions and plant resource status. Within a single genotype fewer roots are grown under N stress while rooting depth increases (Saengwilai et al., 2014b; Yang et al., 2019). Allocation of a larger fraction of plant biomass to the roots is also an important response to N limitation in the plant. A steeper root angle is beneficial to enhance N uptake under N-limiting conditions as it can increase N capture from deeper soill layers, as shown *in silico* and in the field (Schneider et al., 2022; Trachsel et al., 2013). The root system phenotype to optimize water and N acquisition has been proposed as ”steep, cheap and deep” (Lynch, 2013) for plants grown in isolation, but this was determined for isolated plants, not considering plant-to-plant competition as encountered in a crop stand.

For a maize breeding program, high grain or biomass production has always been the primary target, while the need to develop sustainable systems has also brought resource use efficiency into the picture. However, there is a lack of insight into what root system phenotype is beneficial to achieve high N uptake while still maintaining yields and what traits are responsible for such high N uptake. In this study, we will apply a plant modeling approach to identify and quantify the maize root trait combinations and root system phenotype(s) that give both high yield and N uptake of maize plants grown at high density and across a range of N environments.

Methods

General approach

To explore what root and root system phenotypes are beneficial for high yield and high N uptake at the same time, we used a parameterized and tested maize functional-structural plant model to conduct virtual experiments (Chapter 2, 3). FSP models can be used to analyse plant traits and related processes relevant to yield and N uptake. They simulate plant performance, their growth, physiological functioning and above and below-ground 3D architecture when growing in a crop stand and competing among each other for resources (Vos et al., 2010). FSP models provide the opportunity to not only combine functional and architectural plant traits, but also to take plant-to-plant interactions and plastic responses to changing environments and plant status into consideration. It allows to identify and quantify the trait combinations beneficial for system performance, where the system can be defined as either the individual plant or a stand of multiple plants competing.

For seven key root traits, 300 combinations of input-parameter values were randomly selected from uniform distributions. These combinations were used as model input parameter values to run the simulations at five N levels (Table 5.1). Two analyses were made based on the simulation outputs. The first analysis was to determine whether the trait values leading to either high yield or high N uptake were the same or not across all N levels. Data from a field experiment with six cultivars released between the 1970s to the 2000s were compared with the model results to trends in root system traits over this period of breeding. Based on the results of this first analysis, the second analysis was done to find out what root and root system phenotypes give high N uptake in combination with high yield.

Table 5.1: List of target plant traits with definitions, range values and units

Parameters	Description	Units	Min	Max	References
$wmaxRoot$	Potential root dry weight which is one of the model parameters to determine root sink strength	g/root	7.5	25	
MP	Maximum potential root number	per plant	10	75	(Lynch, 2013)
$Dinit$	Average first order root diameter	m	0.001	0.0016	(Chen et al., 2014a)
RTD	Root tissue density	g/dm^3	80	120	(Chen et al., 2014a)
$rootAngle$	initial axial root angle between stem and root	^{o}	10	80	(Lynch, 2013)
a_l	coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen		0	16.482	Chapter 3
a_r	coefficient of root emergence rate in response to shoot nitrogen		0	0.1316	Chapter 3

Model description

An existing maize FSP model driven by light and soil nitrogen was used here (Chapter 2, 3). This model has been developed in the GroIMP platform (Hemmerling et al., 2008). This 3D maize model can mechanistically simulate shoot and root growth and development based on organ level carbon and nitrogen sink-source relationships, light interception and nitrogen uptake. Production of new plant organs (leaves, internodes, roots) follow classical rewriting rules and the organs are represented in 3D using simple geometrical shapes (Vos et al., 2010).

In the model, the soil is composed of soil cells of 0.6*0.15*0.15 m until a depth of 1.5 m. Within a single soil cell, the three sources of N are: leaching from the soil cell above (except for the top soil cells), mineralization of organic soil N, and N application both only for the top 0.3 m soil layer. N uptake by roots and leaching to soil cells below causes N depletion within a cell. To simulate N uptake from the soil, Michaelis-Menten kinetics are used for the high-affinity transport system and a linear relation for the low-affinity transport system (Siddiqi et al., 1990).

Averaged weather data is used to represent temperature as function of day of the year and incoming radiation as the function of latitude, day of the year and transmissivity of the atmosphere during the growth season (Spitters, 1986; Evers et al., 2010). To simulate field light conditions, 24 directional light sources and 72 diffuse light sources are placed into a hemisphere (Evers et al., 2010). Light capture is simulated by the reverse Monte-Carlo ray tracing algorithm in GroIMP (Hemmerling et al., 2008). A light-photosynthesis response curve following an asymptotic exponential relationship is used to simulate photosynthesis at the leaf level as a function of leaf light absorption (Archontoulis & Miguez, 2015). After carbon is assimilated, simulated plant growth is based on available carbon to each organ and organ sink strength which is defined as a sigmoid function of potential organ dry mass and organ growth duration (Yin et al., 2003).

A modified logistic model is used to link carbon assimilation and photosynthetic N (Sinclair, 1989; Vos et al., 2005). Distribution of photosynthetic N among photosynthetic organs is the result of organ N sink strength and available N within the plant. Organ N sink strength is determined by the fraction of light intercepted by the organ (Hikosaka, 2003), organ age, and a parameter for leaf N of fully-lit leaves. Structural N in the stem, root, leaf and grain are expressed as fractions of organ biomass, and do not reallocate to other organs. The N remaining after assigning the photosynthetic N and the amount of N to support organ growth, is stored in an N pool. N stored in the pool and photosynthetic N are available for reallocation to leaves, stems and grains every time step.

We included two active plastic responses to plant N status: the root-to-leaf biomass partitioning coefficient (nRL) and the root emergence rate (ER, in number of roots per ^{o}Cd). The nRL response is defined as a negative exponential function of leaf N concentration. The ER response is defined as a linear function of shoot N concentration. Both relationships have been derived from experimental data (Chapter 3).

Simulation experiments

To identify which plant traits comprise phenotypes that give high N uptake or high yield, we ran simulations for a wide range of values of root traits. The simulation plot was set to 3 rows of 3 plants each, with 0.6 m between rows and 0.15 m between plants within the row. Because border effects were minimized using a cloning approach built into GroIMP, all nine plants had neighboring plants other than themselves allowing for full plant-to-plant competition for light and N resources (Fig. B.5). The average temperature and incoming radiation were derived from weather data during the growth season in 2010 at Siping (43°17'N, 124°26'E), Jilin province, China. Plant parameters except for target root traits were parameterized for maize cultivar ZhengDan958 from a field experiment done in 2011 at Siping, Jilin province, China (Chen et al., 2014a).

We randomly selected 300 root trait value combinations from uniform distributions of a set range of values for 7 individual root traits: $wmaxRoot$, MP, $Dinit$, RTD, $rootAngle$, a_l and a_r (Table 5.1). Correlations were used to demonstrate the independence of the values of these seven root traits (Fig. D.1). These 300 trait value combinations were the input parameters fed into the model. Simulations were run at five N levels at 0, 60, 120, 180 and 240 kg/ha with initial available soil N of 70 kg/ha.

In the first analysis, we determined if differences exist in optimal trait value combinations for only high yield or only high N uptake. To this end, the average trait values of all 7 traits from the 100 simulations giving the highest grain yield and from the 100 simulations giving the highest N uptake at each N level were selected separately. N uptake (g/m^2) and yield (t/ha) for the two separate simulation sets were then analysed for all five N levels. To further analyse the cause of difference in N uptake and yield for the two simulation sets, root dry weight (g/plant) was treated as one of the target model outputs. In addition, the average values from all 300 simulations of $wmaxRoot$, MP, $Dinit$, RTD, $rootAngle$, a_l and a_r were included for comparison.

In the second analysis we defined the root phenotypes that were associated to both high N uptake and high yield across N levels. The simulations (i.e. the combinations of 7 trait values) were first divided into either top or bottom half based on yield and N uptake output for each N level. Then, simulations common to all five N levels were selected. This yielded simulation input sets that across all N levels provided high yield and high N uptake (HYHN), high yield and low N uptake (HYLN), low yield and high N uptake (LYHN) or low yield and low N uptake (LYLN). The primary interest was the HYHN group, and the other groups were used to evaluate the trait differences related to the absence of high N uptake, of high yield, or of both. To finally identify what the beneficial root phenotypes were, values and interactions among $wmaxRoot$, MP, $Dinit$, RTD, $rootAngle$, a_l and a_r in these four simulation input sets were further analysed. Besides these input parameters, yield and N uptake output, additional relevant model output (root length density (RLD, m/m^3) and root system dry weight) at all five N levels in these four simulation input sets were determined.

Field experiment

Data was used from a field experiment conducted in Siping (43°17'N, 124°26'E), Jilin province, China, in 2011 reported by Chen et al. (2014a). In brief, a split-plot design was used with 5 replications where N treatments were the main plots and genotypes were the subplots. The tested maize cultivars were Zhengdan958 (ZD958), Xianyu335 (XY335), Zhongdan2 (ZD2), Danyu13 (DY13), Yedan13 (YD13) and Nongda108 (ND108) which were developed at different moments between the 1970s and the 2000s in China (Table 5.2). Two N levels were applied: 240kg/ha (HN) and 0kg/ha (LN). Maize seeds were hand-sown at a density of approximately 6 plants/m^2 and a between row and within row plant distance of 60 and 28 cm, respectively. The experiment was rain-fed.

Table 5.2: List of maize cultivars information (Chen et al., 2014a).

Hybrids	Parental combination	Breeding Institution	Year of release
ZD2	Mo17 × Zi330	Chinese Academy of Agricultural Science, Beijing	1973
DY13	Mo17 × E28	Academy of Agricultural Sciences in Dandong, Liaoning Province	1979
YD13	Ye478 × Dan340	Academy of Agricultural Sciences in Laizhou, Shandong province	1989
ND108	178 × Huang C	China Agricultural University, Beijing	1991
ZD958	Zheng58 × Chang7-2	Henan Academy of Agricultural Science, Henan province	1996
XY335	PH6WC × PH4CV	Tieling Seed-Pioneer Co., Ltd., Jilin province	2000

At grain filling stage, roots of the six cultivars grown at HN and LN in 2011 were excavated by sampling a 60 cm wide by 28 cm long by 60 cm deep soil block that was divided into three 20cm layers. Per layer, roots were washed with a 0.4mm sieve and axial roots and lateral roots were separately scanned (Epson1600, India). WinRhizo (version Pro 5.0, Canada) was used to analysis root traits (such as root diameters and root length). After scanning, root samples were dried at 70°C and weighed.

Average root diameter ($Dinit$, cm), average root tissue density (RTD, g/dm^3) and root dry weight ($rootDW$, g/plant) were directly derived from the measurements on plants grown under high N. To compare these derived measurements to model results, the same variables were calculated from the model output of the simulation sets that gave either high yield or high N uptake, since the exact trait values we analysed in the model were not available in the experimental data set.

Statistical analyses

All statistical analyses were done using R version 4.0.2 (R Core Team, 2021). Correlation values presented in the figures are Pearson correlation coefficient (Benesty et al., 2009). The "PerformanceAnalytics" package and "corrplot" package were used to produce correlation figures (Peterson et al., 2018; Wei et al., 2017). The "ggVennDiagram" package was used to visualize the selection for high yield or high N uptake simulations through Venn diagrams (Gao et al., 2021a). To identify clusters of root trait combinations, the hierarchical clustering analysis was used. Hierarchical cluster analysis is a method to define clusters by comparing dissimilarity between each pair of observations (Murtagh & Contreras, 2012). In this study, the maximum values of dissimilarity were considered as the distance between the two clusters. The "ggplot2" package was used to produce bar graphs and line graphs (Wickham, 2009). Values presented in the figures are means ± SEs.

Results

Target outputs and inputs by selecting high yield or high N uptake across N levels

Under high N, simulations that showed high yield (”Top 30% yield)”) exhibited lower N uptake values (Fig. 5.1A). Under medium to low N, simulations showing high yield had N uptake values similar to the overall mean values (Fig. 5.1A). Simulations showing high N uptake yielded lower yields under high N than the overall yield. Under medium to low N conditions, simulations showing high N uptake had similar yields to overall mean yield (Fig. 5.1B). Simulations resulting in high yield had the smallest per plant root biomass across all N conditions while simulations for high N uptake had the largest root biomass across all N conditions, indicating root biomass is more important for N uptake than for productivity (Fig. 5.1C).

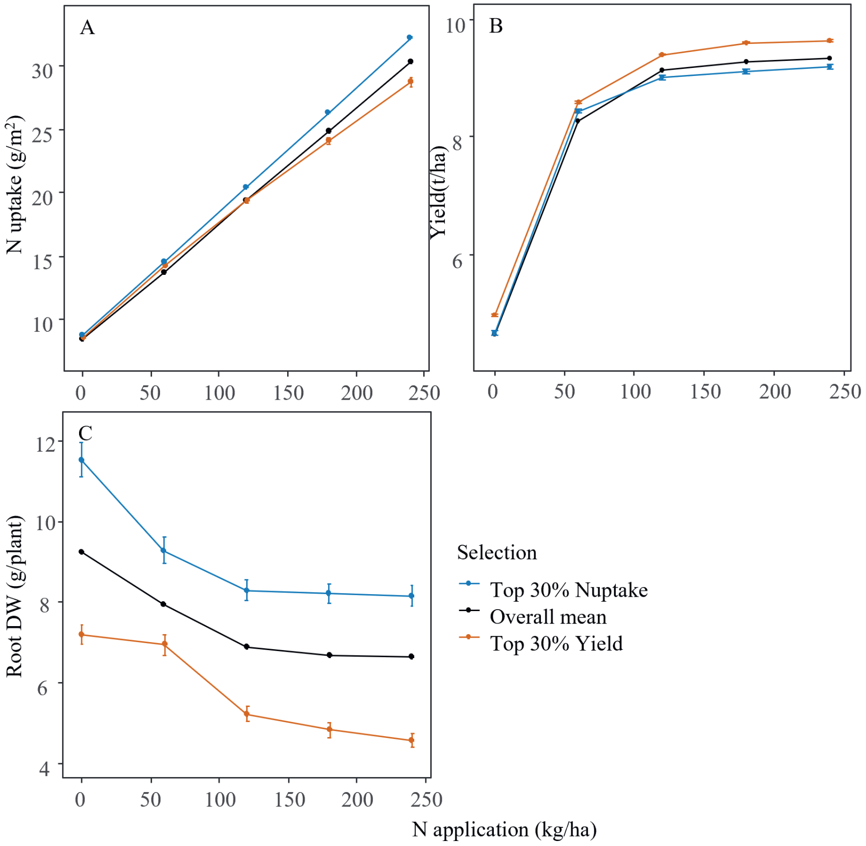

Figure 5.1: Trade-off in N uptake (g/m^2), yield (t/ha) and root dry weight (g/plant) across all nitrogen (N) treatments when selecting for high yield or high N uptake in the system. Blue lines and symbols represent average of 100 simulations with the highest N uptake across N treatments. Black lines and symbols represent averages of all 300 simulations without any selections. Orange lines and symbols represent averages of 100 simulations with the highest yield across N treatments. Symbols and error bars represent mean ± SEs (n=100).

Zooming in on the individual traits, high yield was achieved with low values for: 1) potential root dry weight values ($wmaxRoot$) especially when N levels were high, 2) the coefficients of root-to-leaf biomass partitioning in response to leaf N (a_l), and 3) the coefficient of root emergence rate in response to plant N (a_r) especially at N application equal to 120 kg/ha (Fig. 5.2A, B, D). Besides, high yields were also reached with low RTD, $Dinit$ and maximum root number (MP) at low N while values for these three parameters increased with increasing N levels to achieve high yield (Fig. 5.2C, E, F). High N uptake was achieved with high $wmaxRoot$, a_l and a_r values and low RTD and $Dinit$ values (Fig. 5.2A-F). Root angle values did not differ between high yield or high N uptake (Fig. 5.2G). The parameter values for high N uptake were more constant among N levels than parameter values for high yield.

Larger average root diameter (*Dinit*), and smaller root dry weight were found to be required for high yield than for high N uptake, while the required root tissue density (RTD) for high yield and N uptake was comparable (Fig. 5.3). For cultivars released in the 1970s or 1980s, slightly larger root dry weights were found when compared with the cultivars released after the 1990, except for the tested maize cultivar developed in 1973 (Fig. 5.3A). Our simulated root dry weight for high yield was similar with cultivars released after 1990s (Fig. 5.3A), consistent with the focus on yield, rather than N uptake, in the selection process for the more recent cultivars. No clear trend was found for RTD for the six cultivars but the magnitude of RTD was the same with simulated high yield or high N uptake parameter values (Fig. 5.3B). For the cultivars developed between the 1970s and the 2000s, experimentally obtained average root diameters show an increasing trend with year of release of the cultivar which is in a line with our simulation results that in order to pursue high yield, a larger initial root diameters are preferred (Fig. 5.3C).

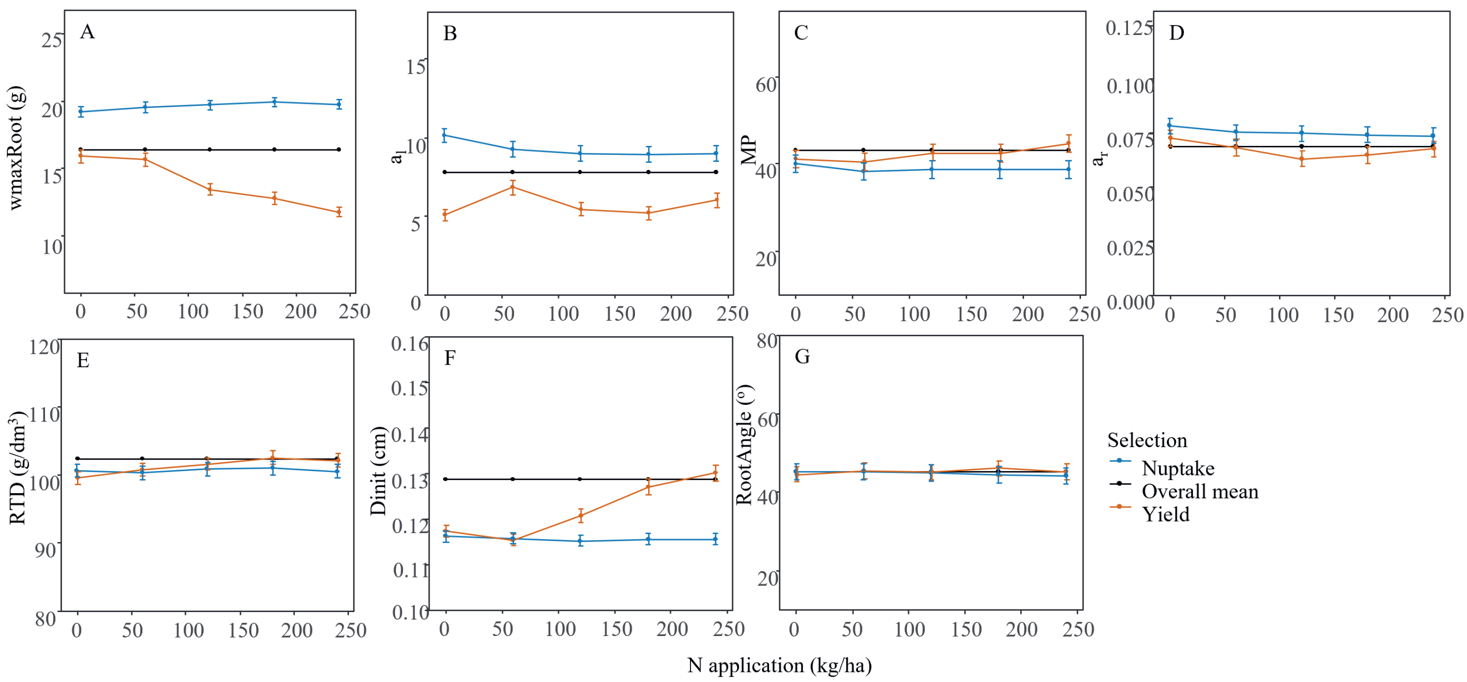

Figure 5.2: The input parameter values when selecting for high yield or high N uptake plants across five nitrogen (N) levels. Panel A: $wmaxRoot$ (g/plant), representing potential root dry weight which determines root sink strength. Panel B: a_l, representing the coefficient of root-to-leaf biomass partitioning in response to leaf nitrogen. Panel C: MP, representing maximum potential root numbers. Panel D: a_r, representing coefficient of root emergence rate in response to shoot nitrogen. Panel E: RTD, representing root tissue density (g/dm^3). Panel F: $Dinit$ (m), representing average first order root diameter. Panel G: rootAngle (^{o}), representing the initial axial root angle between stem and root. Blue lines and symbols represent the average of 100 simulations with the highest N uptake across N treatments. Black lines and symbols represent averages of all 300 simulations without any selections. Orange lines and symbols represent the average of 100 simulations with the highest yield across N treatments. Symbols and errror bars represent mean ± SEs (n=100).

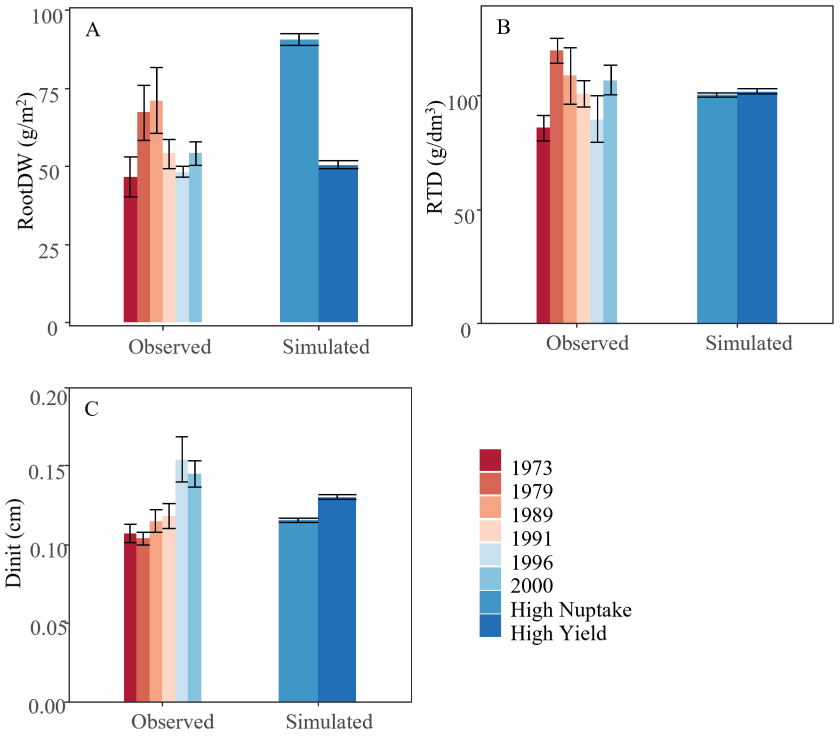

Figure 5.3: Observed root traits for maize released in different years compared with average root trait values of the high N uptake set and high yield set under high N condition (N= 240 kg/ha). The observed values were derived from a 2011 field experiment under high N condition reported by Chen et al. (2014a). The panel A represents the root dry weight per m^2. Panel B represents root tissue density (g/dm^3). Panel C represent average initial root diameter (cm). Colors for observed values represent cultivars released in different years and colors for simulated values represent simulation sets for either top 30% N uptake or top 30 % yield. Error bars indicate ± SE (n_{obs}=5, n_{sim}=100).

Root traits associated to both high yield and high N uptake

We identified 19 % and 21 % of trait combinations as common across all N levels when selecting for high (Fig. 5.4A) or low (Fig. 5.4B) yield, respectively, while 83 % and 84 % of trait combinations were identified as common when selecting for high (Fig. 5.4C) or low (Fig. 5.4D) N uptake. Further trait combinations were found for a subset of the N levels tested. To explore the simulations of yield and N uptake, there were 19 to 25 common trait combinations when selecting either the top or the bottom 50% simulations of yield and N uptake across all N levels (Fig. 5.4E-H).

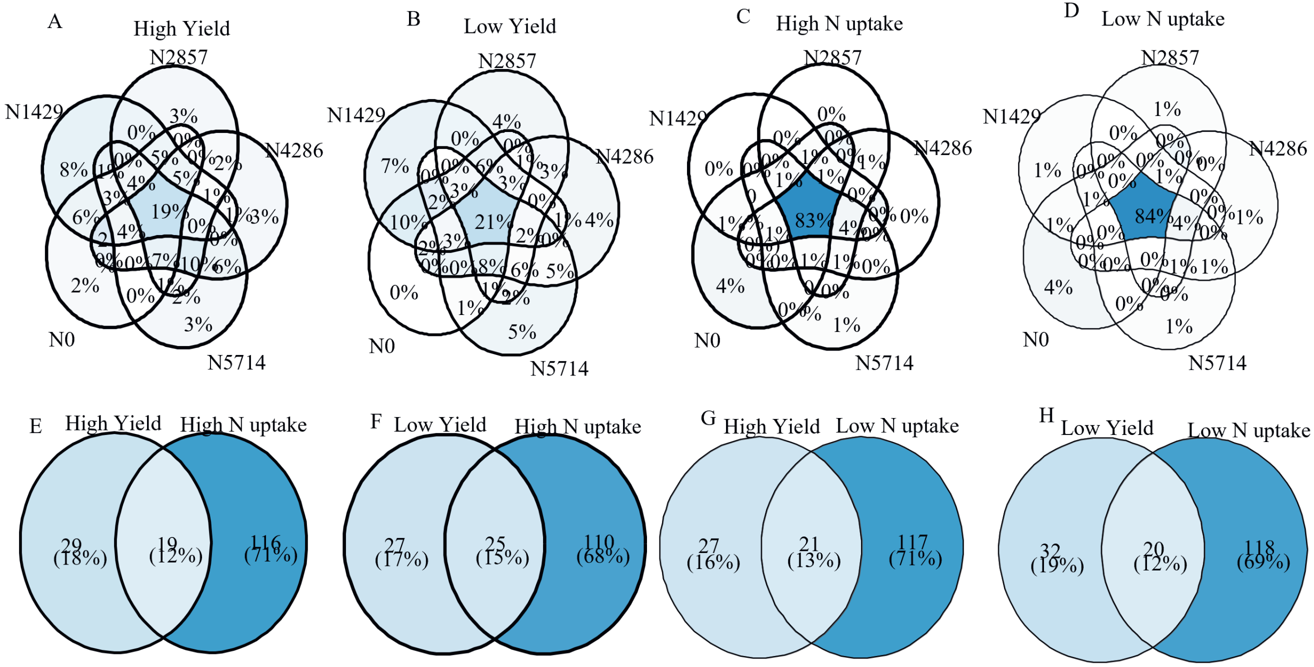

Figure 5.4: Venn diagram of common simulations with various selection criteria. Panel A to D represent the common simulations of four groups for either top or bottom 50 % of yield and N uptake regardless of N treatments. Panel E to H represent common simulations groups of high yield with high N uptake (HYHN), low yield with high N uptake(LYHN), high yield with low N uptake (HYLN) and low yield with low N uptake (LYLN). Darker blue represent the higher percentages.

Compared with the low yield and high N uptake (LYHN) group, the HYHN group resulted in significantly (P-value= <0.0001) lower simulated root dry mass per plant and absolute RLD, especially from 0 to 0.45 m depth (Fig. D. 2A, B; Fig. 5.5A, B). Compared with the HYLN group, the HYHN group resulted in slightly higher root dry mass and RLD especially from 0.15 to 0.45 m depth (Fig. D.2B, D; Fig. 5.5B). Compared with LYLN, the HYHN group showed significantly (P-value=<0.0001) lower root dry mass but higher RLD from 0.15 to 0.45 m depth under high N. Under low N, a lower RLD in the 0 to 15 cm soil layer was found in the HYHN group than in the LYLN group (Fig. D.2B, C; Fig. 5.5A).

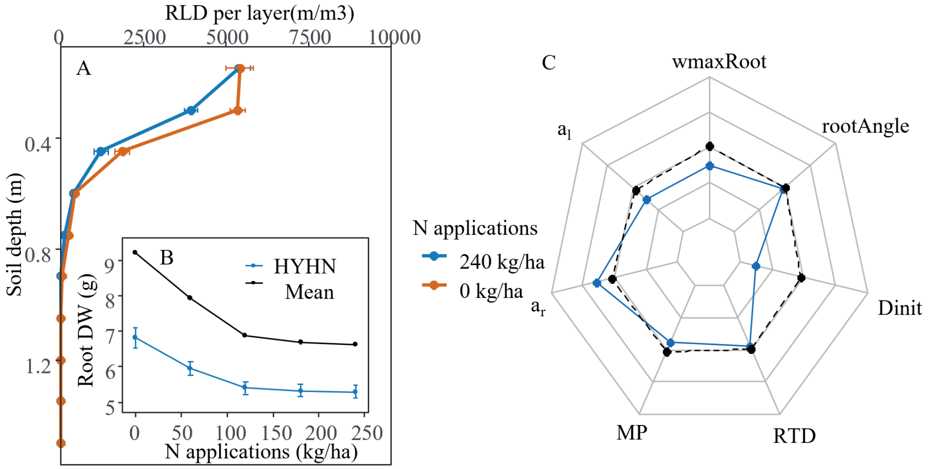

Figure 5.5: The root length density (RLD, m/m^3, Panel A), root dry weight (root DW, g/plant, Panel B) and input parameter values (Panel C) for the high N uptake and high yield category. In panel A, blue line and symbols represent the RLD of selected common simulations for the highest N treatment (N=240 kg/ha) and orange lines and symbols represent the RLD of selected common simulations for the lowest N treatment (N= 0 kg/ha). In panel B, blue line and symbols represent the average root dry weight of selected common simulations for high yield with high N uptake across all N treatments. The black line and symbols represent the overall mean of all 300 simulations across all N treatments. In Panel C, the *wmaxRoot* (g/plant) represents potential root dry weight which determines root sink strength. The a_l represents the coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen. The MP represents maximum potential root numbers. The a_r represents coefficient of root emergence rate in response to shoot nitrogen. The RTD represents root tissue density (g/dm^3). The *Dinit* (m) represents average first order root diameter. The rootAngle (^{o}) represents initial axial root angle between stem and root. Blue color represents input parameter combinations of selected common simulations for high yield with high N uptake. Black color represents average of the input parameter of all 300 simulations without any selections. Symbols and error bars represent means ± SEs (n=19).

The comparison between the four groups shows how the differences in output predictions for root dry mass and RLD between the four groups were related to differences among the four groups in five of the seven input root traits. Comparing the HYHN and LYHN groups their difference in yield at similar high N uptake was related to smaller $wmaxRoot$, $Dinit$ and a_l values for the HYHN group than the LYHN group (Fig. D.3 A, B; Fig. 5.5C). The comparison between the HYHN and HYLN groups, differing in N uptake while producing similar high biomass, showed the HYHN had slightly higher $wmaxRoot$ and a_r values and lower $Dinit$ values than the HYLN group (Fig. D.3 B, D; Fig. 5.5C). The difference between HYHN and its full opposite LYLN was related to $wmaxRoot$, $Dinit$, a_l and RTD values and higher a_r value for the HYHN than the LYLN group (Fig. D. 3 B, C; Fig. 5.5C). Only the root angle and the maximum number of root primordia (MP) did not differ among the four groups.

In the agronomically most interesting group (HYHN), only $wmaxRoot$ and a_l were strongly negatively correlated (p ¡ 0.001; correlation coefficient: -0.80; Fig. 5.6) due to the obvious link between biomass partitioning to the root and root potential growth rate. The other trait correlations were not significant. Two groups of trait combinations were identified through a complete hierarchical cluster analysis. Out of the 300 tested phenotypes, root trait combination type 1 contained 13 phenotypes and root trait combination type 2 contained 6 phenotypes (Fig. 5.6B). Both root trait combination types had higher N uptake and yield than the overall means of all 300 simulations across all N levels and had similar averages with the top 100 simulations either for N uptake or for yield (Fig. 5.7). Root trait combination type 1 was more beneficial for grain yield than type 2 under moderate to high N levels (Fig. 5.7B). The two correlated root traits (see above) a_l and $wmaxRoot$ were different between the two root trait combination types. Type 1 had higher a_l and lower $wmaxRoot$ than type 2 (Fig. 5.8D, G).

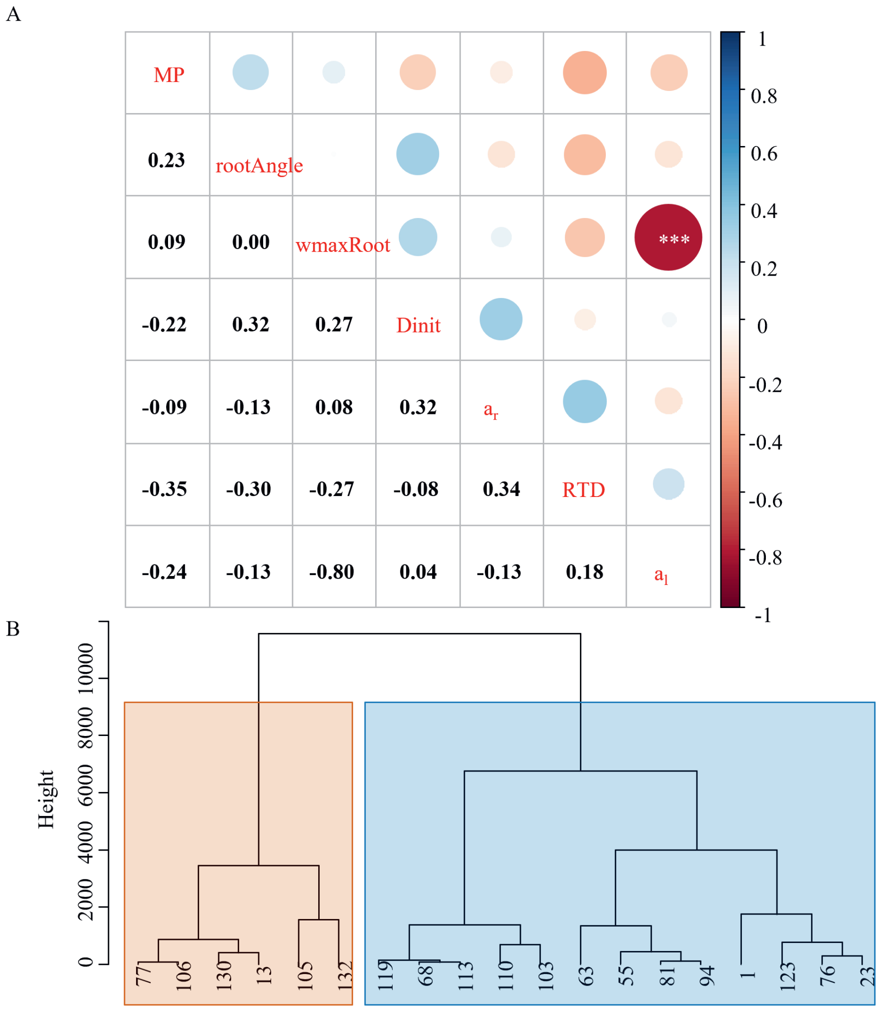

Figure 5.6: Common simulations for both high yield and high N uptake across all nitrogen levels. Panel A shows the correlations among the seven plant traits based on the common simulations for high yield and high N uptake regardless of N treatments. Where *wmaxRoot* (g/plant) represents potential root dry weight which determines root sink strength; a_l represents the coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen; MP represents maximum potential root numbers; a_r represents coefficient of root emergence rate in response to shoot nitrogen; RTD represents root tissue density (g/dm^3); *Dinit* (m) represents average first order root diameter; rootAngle (^{o}) represents initial axial root angle between stem and root. *** present in panel A significance at $\leq$0.001 of the correlation. Red color in panel A represents negatively correlated and blue color represents positively correlated. Panel B represents hierarchical clustering analysis result. Blue in panel B represents type 1 with n = 13 and orange represents type 2 with n= 6.

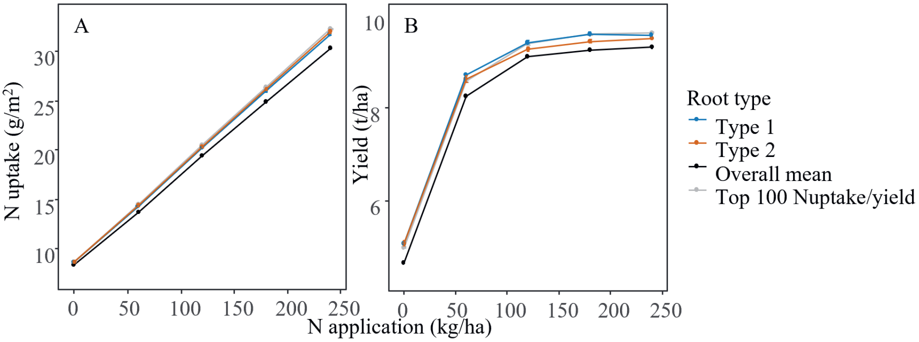

Figure 5.7: Comparison of N uptake and yield between the two root trait combination types across N levels. Blue lines and symbols represent type 1 and orange lines and symbols represent type 2. Black lines and symbols represent overall means for either N uptake per unit area or grain yield across all levels. Grey lines and symbols in panel A is N uptake for top 100 N uptake simulations and in panel B is yield for top 100 yield simulations across all N levels. Symbols and errror bars represent mean ± SEs (n=100, n_{type1}=13 and n_{type2}=6), when error bars are not visible, these fall within symbols.

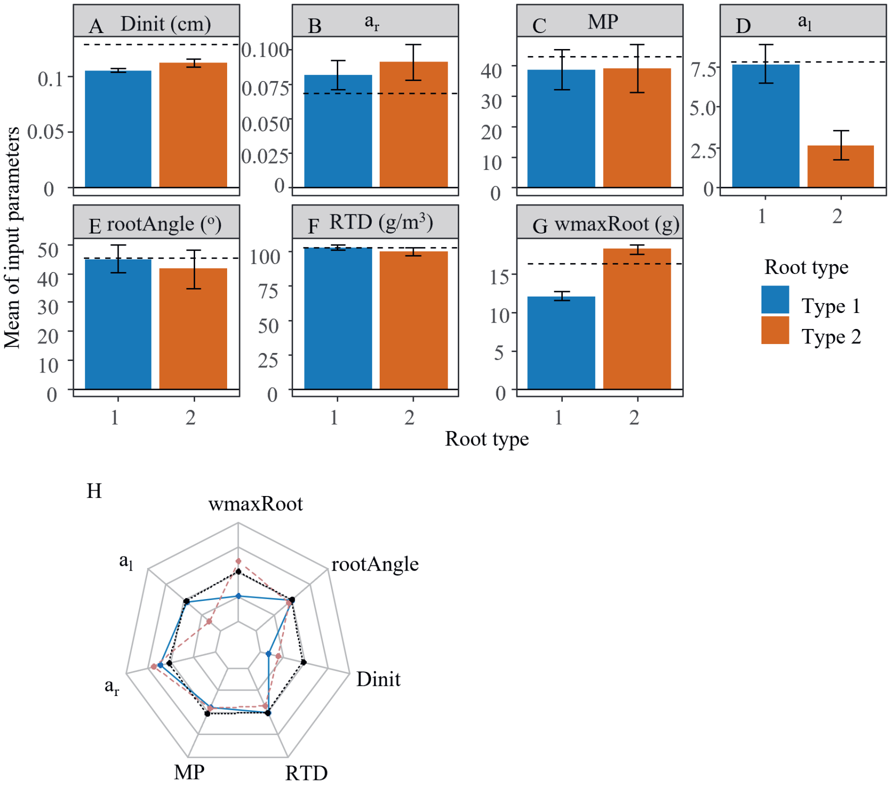

Figure 5.8: Root traits combinations for the two distinguished types. Panel A to G represent comparisons between individual root traits for the two trait combination types. The *wmaxRoot* (g/plant) represents potential root dry weight which determines root sink strength in Panel A. The a_l represents the coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen in Panel B. The MP represents maximum potential root numbers in Panel C. The a_r represents coefficient of root emergence rate in response to shoot nitrogen in Panel D. The RTD represents root tissue density (g/dm^3) in Panel E. The *Dinit* (m) represents average first order root diameter in Panel F. The rootAngle (^{o}) represents initial axial root angle between stem and root in Panel G. Blue represents type 1 and orange represents type 2. The black dotted lines represent overall means of all 300 simulations without any selection. Values are presented as mean ± SEs (n1=13 and n2=6). Panel H represents comparisons between the root traits for the two root trait combination types. Blue line and symbols represent root traits values for type 1 and orange line and symbols represent root traits values for type 2. The black line and symbols represent the overall means of all 300 simulations without any selection.

There was no difference in the root dry mass between the two root trait combination types under low N, while under higher N levels, type 2 had a higher root dry mass than type 1 (Fig. 5.9C). Generally there was no difference in RLD between the two root trait combination types except for the soil layer between 15 cm to 30 cm under high N. For this soil layer, type 1 showed slightly smaller RLD value at the high level of N (Fig. 5.9A, B).

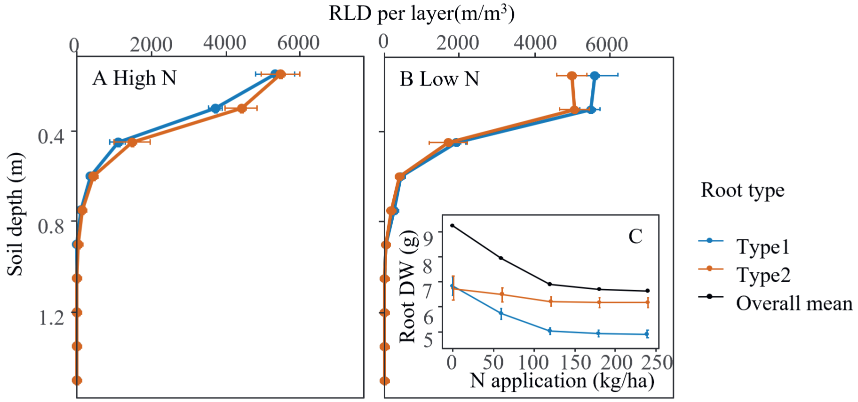

Figure 5.9: Comparison of root dry weight (Root DW, g/plant) and root length density (RLD, m/m^3) per soil layer between the two root trait combination types and nitrogen (N) levels. Blue lines and symbols represent type 1 and orange lines and symbols represent type 2. Panel A presents high N condition (240 kg/ha) and Panel B represent low N condition (0 kg/ha). Panel C represent root dry weight across wide range of N treatments. Symbols and error bars represent mean ± SEs (n_{type1}=13 and n_{type2}=6)

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Discussion

This study aimed to identify maize root system phenotypes beneficial for high N uptake while maintaining yield. In this study we applied a whole-plant FSP model that considers feedback between plant structure and function, and spatio-temporal effects of competition for light and soil N among neighbouring plants. Despite the trade-offs observed, as some traits supporting high N uptake were found to be accompanied by low yields (e.g. Fig. D.3A), and *vice versa* (e.g. Fig. D.3D), the modeling provided possible trait combinations supporting both and thus potential synergies for N-uptake and yield (Fig. 5.1C; Fig. 5.4E; 5.5A, B, Fig. D.3C).

Comparable FSP models have been used to explore effects of individual traits or combinations of a few traits on resource capture (Postma & Lynch, 2011; Li et al., 2021; Saengwilai et al., 2021). However, due to the complexity of plant phenotypes, interactions with neighbouring plants, and local environmental conditions such as light and soil N, only a few studies attempted to find improved phenotypes, typically simulating single plants or sets of identical plants and varying a limited number of parameters (Rangarajan et al., 2022; Renton & Poot, 2014; Dunbabin et al., 2003; Ho et al., 2004). Here we made an important step by identifying phenotypes in the context of competition with neighbouring plants representing a crop stand. This led to unique individuals differing from one another in organ size and biomass, as real plants do. We optimized seven root traits, of which two plasticity traits related to plant nitrogen status, thus including feedback between carbon and nitrogen allocation on the one hand and photosynthesis and nitrogen uptake on the other.

Trade-offs between high N uptake and high yield

Our results showed that trait values relevant to N uptake and grain yield under low N conditions were comparable, while they started to diverge for higher N application levels (Fig. 5.1A, C). When N was not limiting, it became beneficial for plants to shift carbon investment from root to shoot growth, resulting in a lower root biomass for the 30% highest yielding phenotypes (Fig. 5.1B), corroborating experimental findings (Shao et al., 2018). More root biomass means enhanced capacity for N uptake. Both the 30% highest N uptake phenotypes across all N conditions and the 30% highest yield phenotypes under N limiting conditions had larger root biomass compared with the 30% highest yield phenotypes under non-limiting conditions (Fig. 5.1B). Higher N uptake is associated to larger root systems, especially under low N conditions (Wang et al., 2005; Mu et al., 2015). Our results also revealed that grain yield relied on plant N uptake capacity more under low than high N (Fig. 5.1B).

Between the 1970s and the 1990s maize breeding goals have evolved from maximizing ear size per plant to high yield at high density (Chen et al., 2014a; Zhao et al., 2010). The root biomass per unit of area for the cultivars released in China during this process first increased and then reduced (Fig. 5.3B) indicating the two breeding goals indirectly selected root biomass in two opposite directions. Consequently by the 1990s a relatively low root biomass was selected for at high plant density and non-limiting N (Shao et al., 2018) in line with our results (Fig. 5.3A). Both simulations and observations showed a large root diameter is beneficial for high yield when N is non-limiting, as discussed by Schneider et al. (2021). None of the maize cultivars from the experiment were identical in all traits to the high yielding phenotypes identified in our simulations (Fig. 5.3). This suggests there may be potential linkages among the investigated traits but also potential to breed the observed beneficial trait combinations into a single genotype, since the genetic variation clearly does exist (Fig. 5.3).

Due to differences between trait values when selecting for high yield or high N uptake alone, it would be important to select genotypes with both characteristics at the same time when breeding for use in more sustainable maize cropping systems (York et al., 2022). High N uptake root phenotypes across all N conditions also provide opportunities to maintain N uptake while reducing N application in the system.

Root phenotype for high yield and high N uptake

Combining both high yield and high N uptake was reached by restricting total root biomass per plant (Fig. D.2B vs A and D) while maintaining RLD through thinner roots (a lower initial root diameter $Dinit$) and lower root emergence rate in reaction to a drop in plant nitrogen status (lower a_r) (Fig. D.3). A lower root number can reduce RLD in the top soil layer (0-15 cm) but maintain RLD in deeper soil layers to source extra N when N is limiting, in line with our findings (Saengwilai et al., 2014b; York et al., 2015; Yang et al., 2019; Chen et al., 2014a). Both larger and smaller root diameters can be linked to low N (Yang et al., 2019; Gao et al., 2015; Schneider et al., 2021). However, the strong interaction between root number and root characteristics such as root tissue density (RTD) can change the carbon distribution within the root system (Schneider et al., 2021). From experimental observations alone, it is hard to conclude whether thicker roots are beneficial for N use efficient maize. Combined with our simulation results in Fig. D.3A and B, we conclude that a smaller root diameter can be beneficial to enhance N uptake and will only be beneficial for yield when limited by N uptake.

We found root angle not to be relevant for yield or N uptake, in contrast to other studies (York et al., 2015; Ren et al., 2022). Root angle only had a small effect on either N uptake or yield in comparison to the stronger effects of traits such as root sink strength ($wmaxroot$). When the other traits with stronger effects would not have been varied a root angle effect might have shown, but in combination with the other traits tested, root angle turned out to be of minor importance.

Combining high yield and high N uptake as selection criteria led to lower root dry weight per plant (Fig. 5.5B). This means that predictions by the functional equilibrium theory were observed within our virtual genotypes as a consequences of the plastic responses implemented (chapter 3), but across genotypes investment in more root biomass came with a yield penalty (Fig. D.2 A and C vs B and D). The higher root dry weight investment at all N levels in low yield - high N uptake phenotypes (Fig. D.2A and D.3A) was reached with a stronger increase in root allocation at low plant N status (higher a_l) and a larger root sink strength ($wmaxRoot$) compared to high yielding high N uptake phenotypes (Figs D.2B and D.3B). This led to the high-yield high-N-uptake phenotypes having lower plant root biomass, a lower RLD across the full soil profile and no increase in RLD at 15-30 cm soil depth compared to the top layer in comparison to the low-yield high-N-uptake phenotypes. Plants reduce the carbon allocation to roots to optimize grain yield under high plant density due to stronger competition for light (Gao et al., 2021b; Shao et al., 2018). At the same time, more competition among roots for nutrients than competition among shoots for light occurs when nutrients are limiting (Wang et al., 2014; Kiær et al., 2013). Plants tend to invest more carbon to form roots to enhance nutrient uptake and optimize yield. The trade-off in root biomass between high N and medium to low N fertilizer application emphasizes the importance of the plastic response of carbon allocation between root and shoot.

FSP modeling for trait selection

Breeding for crop ideotypes has been first introduced in the late 1960s when an ideotype was defined as the idealised plant under certain environmental conditions, with a specific combination of traits (growth, developmental and morphology), based on plant and crop physiological knowledge (Donald, 1968). Since then, ideotype breeding has been used in some breeding programs, especially in rice (Peng et al., 2008). Most current work focuses on identifying the ideotype of a crop species for certain environmental stress factors (such as drought) or crop design (such as intercropping systems) (Breitler et al., 2022; Reddy, 2021; Eker et al., 2022; Bourke et al., 2021). In addition to a large amount of empirical data from field and greenhouse experiments, crop or plant modeling approaches have been used to identify an ideotype of different crop species (Kaloki et al., 2019; Ravasi et al., 2020). However, the majority of models used to identify ideotypes ignored most morphological traits and interactions among neighbouring plants within a crop stand (Kaloki et al., 2019; Ravasi et al., 2020). This is where an FSP modeling approach can come in (as in e.g. Sarlikioti et al., 2011), especially when it includes both aboveground and belowground developmental, physiological and morphological characteristics. Such a model can help identifying ideotypes for different and changing environments and management choices, which may increase efficiency in the selection process (Rangarajan et al., 2022; Bourke et al., 2021; Wang et al., 2019a). However, to better estimate the range of values of relevant root traits, we still require better understanding of root functioning and the relationships between the various root functions. For instance, thinner roots that improve yield and N uptake may not be optimal for plant anchorage (Liu et al., 2012; Hostetler et al., 2022). If plant anchorage or penetration strength are important for agronomic performance in specific environments and would be included in the modeling, such extra boundary conditions on root diameter requirements could lead to different combinations of optimal trait values. While adding steps to enrich the system aspects considered will always pose new questions on limitations, our study illustrates how this type of model can be applied to identify root ideotypes for more sustainable agricultural systems.

Chapter 6

General discussion

Crop production potential has been treated as the primary breeding target for a long period. During the past few decades, ignoring agro-ecological sustainability has resulted in increasing negative impacts of our food systems on the environment (Lammerts van Bueren et al., 2018). Rather than targeting only on grain yield potential, combining both improved productivity per unit area and reduced negative impact on the environment becomes a necessity for future agriculture. To produce more from the same area of land while reducing negative impacts on the environment, increasing yield potential, improving soil quality, and optimizing field management could be three major agronomic strategies (Struik & Kuyper, 2017; Cassman, 1999). Many approaches can contribute to these three major strategies to achieve the goal.

In the thesis, I aimed to quantify the effects of relevant maize traits and mechanisms on nitrogen use efficiency to further support maize breeding programs toward sustainable development in China. However, N use efficiency is a complex trait that depends on soil characteristics, plant physiological and architectural traits including developmental root and shoot traits, and plasticity in those traits in relation to plant N status. Rather than focusing on nitrogen use efficiency for the system, I looked into the relative contribution of crop traits and plasticity in those crop traits to N uptake and physiological efficiency of internal N use at both plant and crop levels to identify future breeding targets. To address the objectives raised in the general introduction, I extended a whole-plant functional-structural maize model by implementing both plant carbon and nitrogen sink-source dynamics driven by plant N and light (Chapter 2). Then, I used this model to quantify and assess the contribution of plant traits (Chapter 2) and active plasticity in root traits (Chapter 3) to N uptake and internal N use. I explored how plasticity in root traits interacts with stay-green maize cultivars (Chapter 4) and what the beneficial root trait combinations (Chapter 5) are to maintain high yield and high N uptake when taking plasticity to plant N status and plant-to-plant interaction into consideration.

Using the model developed in Chapter 2 and Chapter 3, I found:

1. The plastic responses to plant N quantified experimentally can improve N uptake across all N levels by adjusting the vertical distribution of the root length density. However, the current model only can assess the plastic responses to plant N on plant productivity under low N due to a lack of plasticity in light capture (Chapter 3).

2. The plastic responses to plant N can reduce the negative effect on grain N concentration in stay-green cultivars under low N through increasing N uptake (Chapter 4).

3. A root system phenotype with low root biomass and high root length density at

15cm to 45cm soil can provide high N uptake and high yield at the same time. It is possible to achieve this beneficial root system phenotype through multiple combinations of root traits (Chapter 5).

In the following sections, I will first discuss the potential roles of FSP modeling in 1) identifying the effect of plasticity and in 2) phenotyping, especially focusing on the trade-offs between plant traits. Then, I will discuss the contribution of improved root system phenotypes to nitrogen use efficiency. Furthermore, I will discuss the potential applications and challenges of this modeling approach to provide insight in options to improve maize nitrogen use efficiency.

Including plasticity in FSP modeling

In this thesis, I distinguished active and passive plasticity to quantify the role of plasticity in maize functioning through FSP modeling. Active plasticity is associated with changes in allocation patterns of e.g. carbon or nitrogen leading to an active search for extra resources triggered by an environmental or an internal cue (Nicotra et al., 2010). Passive plasticity, on the other hand, refers to hanges in resource allocation due to resource limitations and the associated reduction in resources assigned to plant organs leading to smaller organs (Nicotra et al., 2010). Passive plasticity is an emergent property of the model used in this study, due to sink-source dynamics where sink strength and source co-determine the actual growth rate of all organs, and hence their size. Passive plasticity, in other words, did not require the implementation of any extra mechanisms beyond resource competition among organs. In contrast, active plasticity needs the incorporation of relationships between a signal and the related response. In this thesis, active plasticity and its relevance in nitrogen uptake and use were studied in Chapters 3 and 4. Plasticity is an interesting trait in breeding, providing the potential to fit diverse environments (Nicotra et al., 2010). Plasticity could be beneficial, detrimental, or neutral to yield in specific environments. To have an initial overview of the adaptive value of specific plasticity before starting a large trial of experimental work to breed for plasticity, I suggest a workflow starting with FSP modeling to first evaluate the potential effect of plasticity *in silico* and then designing *in vivo* experiment. In the following subsections, I will discuss the different ways of including plasticity in FSP modeling and the use of FSP models to explore the effect of plasticity on plant N uptake and use.

Modeling plasticity

In this thesis, I established a continuous plastic response curve between target traits (i.e. root to leaf ratio and root emergence rate) and plant N status through a manipulative greenhouse experiment. After quantifying these plastic responses from observations and implementing the response curves into the FSP model, the latter was used to identify the effect of plasticity on target plant performance. This approach has been used in FSP models to simulate other types of active plasticity (e.g. root polarity and shade avoidance) (Henke et al., 2014; Bongers et al., 2018). To quantify response curves, multiple data points for the signal (e.g. internal N status or light spectral composition) are necessary to prevent oversimplification and promote understanding of biological meaning (Arnold et al., 2019; Schneider, 2022). In addition, using response curves in plant models provides the opportunity to study the effect of active plasticity on interactions among plants. For instance, the plastic response in root emergence rate to shoot N status can result in more N uptake under a non-limiting N situation, since the variation of shoot N within a plot leads to different root emergence rates of neighboring plants and therefore to differences in the average root system architecture (Fig. 3.6).

There are still limitations to using response curves in plant models. It is difficult to establish a universal form of a relationship to quantify plasticity due to possible differences among genotypes or species. This leads to having to evaluate the form of relationships every time when studying a new species or a new genotype (Valladares et al., 2006; Schneider, 2022). In addition, obtaining good data to establish continuous response curves from multiple environments is more laborious than using fixed values of individual plant traits from a couple of different environments to explore the effects of plasticity on plant performance and functioning. To obtain effective data to establish a continuous response curve, a targeted gradual increase in N application, based on the expected growth, may be necessary during experiments (Chapter 3).

Besides the methods we used, different methods exist to quantify plasticity, each with its benefits and limitations, suggesting that there is no one-size-fits-all approach. One common approach to studying the plastic effect using FSP models is to use different parameter values representing different phenotypes resulting from plastic responses to environmental conditions or management strategies (Zhu et al., 2015; Li et al., 2021). This approach is useful to quantify the contribution of plasticity as a result of differences in the environment to light capture or assimilate production. However, the effect of plant-to-plant interactions can be underestimated since each plant in the simulated canopy has the same parameter values for each simulated condition. However, in reality, different plants within a canopy react differently, so would require a different parameter values, due to plastic responses to local resource availability which is co-shaped by the neighboring plants. Furthermore, plasticity cannot be viewed as a simple binary switch, with only two possible states “on” or “off ”. Plasticity allows plants to delicately tailor their phenotype to the conditions they experience locally, and therefore a model analysis on the effects of plastic responses in a canopy needs response curves that allow for a range of trait values rather than only two.

Another approach to studying the effect of plasticity on plant functioning through FSP models is to implement a mechanistic submodel of the plastic response at a more detailed scale. For example, branch production by plants is a plastic response driven by hormonal activity and environmental signals such as light quality (Domagalska & Leyser, 2011), with fewer branches being produced when competition between plants is high. Branch production can be mechanistically simulated with a hormonal submodel (Prusinkiewicz et al., 2009). Using this approach, hormonal activity can be linked to environmental signals for competition (Evers et al., 2011), allowing for the simulation of branching responses to competition based on hormonal dynamics. This type of approach should only be used when considering the underlying physiology is required to address the research question. Parameterizing physiological submodels with experimental data is usually also more difficult than parameterizing response curves. To investigate the impact of a particular plastic response on plant N utilization and N uptake considering plant-to-plant interactions (as discussed in Chapter 3), using a response curve is a suitable and effective method that provides an optimal balance between complexity and efficacy.

Using an FSP model to understand plasticity

In this thesis, I utilized quantified plastic responses in an FSP model to investigate their impact on plant N use and N uptake. An FSP model provides some unique opportunities to explore such effects of plasticity. The explicitly coded plant architecture (e.g. shoot and root geometry) in an FSP model affects resource capture above and below ground. Plasticity can result in changes in this plant architecture. For example, when enabling a plastic response in root number to plant N status, the model simulated a deeper root system under low N than under high N (Fig. 3.5). In line with our finding, fewer roots have been found under low N which resulted in deeper roots in both field and greenhouse experiments (Saengwilai et al., 2014b; Guo & York, 2019). These examples from empirical and *in silico* results mutually confirmed each other and reflected how plasticity in root number affects root system architecture and resource capture.

In addition, this FSP model can help to explore the role of plasticity in plant-to-plant interactions, which are complex in real life. This study also contributes to understanding what the contribution of plasticity in root-to-leaf ratio and root emergence rate are to interaction among plants, even within the same genotype, and in improving N uptake (Chapter 3). A few studies have started to use FSP modeling to investigate plant population stability and overyielding (Louarn et al., 2020; Bongers et al., 2018). However, this is still in its early stages due to lack of knowledge on major plant mechanisms that affect interactions among plants and observational data to validate model outcomes. The FSP model developed and applied in this study can be a promising tool to further explore the effects by implementing potential plant physiological processes one by one with different species as neighboring plants.

When applying the FSP model to identify the effect of plasticity on plant functioning, there are a couple of points that require attention:

1) Since in reality, several plastic responses are interacting with each other, including only one or two plastic responses can lead to simulated data deviating from observed data for certain conditions. In Chapter 3, Fig 2, the model underestimated the yield at high N levels while the N uptake was be estimated reasonably well. The results suggest that the current model lacks plasticity in the shoot to avoid shading by neighbors, particularly relevant under favorable N conditions when the leaf area is large and therefore competition for light is large (Ruberti et al., 2012). Capturing enough aspects of plasticity is challenging within one study, also because of the related computer capacity requirements, and may reduce the value of FSP modeling in prediction. Including both too many or too few plastic responses easily leads to lower reliability of the model results and reduces its accuracy for prediction. Although, this type of model can still provide valuable new insights in understanding plant behavior. Deviations between model predictions and empirical data may lead to discovering processes that were not considered to be playing a role before. This is connected to the purpose of an FSP model like the one in this study: understanding plant behavior and seeing how traits affect growth and development in plant stands. Nevertheless, a clear identification of the purpose of the study and a careful balance between effectiveness and accuracy remains necessary when applying FSP modeling to explore the effects of plasticity.

2) Without appropriate interpretation, the model can easily ignore the linkage between plasticity and other plant traits due to the lack of underlying mechanisms in the model. For example, our results (Fig. 2.6 and Fig. 3.4) indicated a lower root number under low N stress can enhance N uptake and increase the yield. However, root number is negatively linked with plant lodging which can result in extra yield loss (Hostetler et al., 2022). In the current model, plant lodging has not been taken into consideration. Even though our modeling results of plasticity in root number showed this plasticity is beneficial for N

uptake and yield under low N, a field evaluation is still needed. The underestimation of the linkage in modeling can lead to wrongly assessing the value of plant plasticity in plant performance (such as yield) in reality. Therefore, combining results from experiments, and literature is necessary to interpret modeling outcomes on plasticity.

Trade-offs in plant functioning and the role of FSP modeling in identifying associated traits

Considering the whole plant, i.e. root and shoot, is required to assess phenotype effects on N uptake and internal plant N use (York et al., 2022; Lynch et al., 2023). Rather than assessing plant traits individually, assessing combinations of plant traits can reveal trade-offs among individual traits when aiming to achieve optimal plant performance. In the following subsections, I further discuss trade-offs and the role of FSP modeling in assessing trade-offs.

Trade-offs in trait optimization

Optimizing production with limited amounts of resources introduces trade-offs that plants will have to cope with. An example is the trade-off between high yield and high grain protein concentration (Kosgey et al., 2013; Ciampitti et al., 2013). As shown in Chapter 4, if leaves do not undergo senescence, grain N concentration will decrease by around 20 % (Fig. 4.3C). Such a decrease in grain N concentration significantly reduces the protein quality of grains. At the same time, there is around 5 to 20 % improvement in stay-green grain yield (Fig. 4.3B). In reality, such theoretical cases do not exist. Even though stay-green is considered a beneficial trait to enhance grain yield, an optimal value of the degree of stay-green (i.e. increase in leaf life span) exists at which grain protein concentration is maintained while yield is still improved (Chen et al., 2021).

In breeding for high yield, individual plant traits are always indirectly optimized so that other plant functions (e.g. resistance to biotic stress), which may not be the primary target, are not affected (Ackerly et al., 2000; Sadras & Richards, 2014). This type of indirect selection of plant traits reduces the effectiveness of artificially designed breeding processes, such as inserting genes to enhance crop performance (Sadras & Richards, 2014). For example, when enhancing herbicide resistance by using a single-gene *Amaranthus hybridus* mutant, the photosynthesis rate was reduced, and therefore biomass production was reduced compared to the wild type (Arntz et al., 1998). Rather than considering using a single or a few genes to largely improve crop productivity, a better understanding of plant physiological mechanisms using systematic thinking can avoid unexpected trade-offs and therefore increase the effectiveness of breeding for multiple targets.

The requirement of plants to capture multiple resources to support growth can also cause trade-offs in plant functioning. Optimal root systems differ depending on the conditions (Schneider & Lynch, 2020; Lynch et al., 2023). In this thesis, I focused only on root system architecture beneficial for the mobile soil nutrient, N. To achieve high N uptake, long roots, especially in deeper soil layers are beneficial (Fig. D.2A and D.2D). However, when considering immobile soil nutrients, such as P, more foraging in top soil is supposed to be more beneficial, indicating a different root system phenotype is required for P-uptake efficiency (Lynch, 2019). Fewer axial roots are preferred for high N uptake while more axial roots are preferred for high P uptake (Schneider & Lynch, 2020). The differences in the preferred axial root number for maximizing N and P capture indicates there cannot be a single phenotype that is ideal for both N and P capture. When including the uptake of both resources as a target in root system phenotype selection, trade-offs in the effects of root traits on resource capture exist and therefore optimization is needed and possible using FSP modeling.

Single-criterion selection is inadequate for crop breeding due to such trade-offs (Lynch et al., 2023). In reality, breeders look for a phenotype that can fit well in multiple conditions. Rather than considering maximizing one trait value, optimization of multiple traits across environments would be preferable. To achieve this goal, multi-location and multi-year experiments are usually required allowing the selection of beneficial genotypes in breeding programs (e.g. Farfan et al., 2013). Such experiments are costly for large breeding panels. New technologies, such as genotyping, are widely used to speed up the progress by linking phenotypes to genotypes while selecting improved cultivars (Poland & Rife, 2012). Besides, the FSP modeling approach used in this thesis has the potential to further expand and accelerate the pace of breeding. As the examples mentioned above indicate, trade-offs between traits or environmental factors can be observed quantitatively through underlying plant physiology, plant architecture, and environmental settings in the model. Applying this modeling approach can therefore enable researchers to design real-life experiments with clearer targets.

Using FSP modeling to explore further trade-offs

Besides the trade-offs mentioned above, there are other examples to further explore trade-offs through FSP modeling. Firstly, the output of the model is the result of trade-offs due to underlying mechanisms standard include in the model. In this model, sink-source dynamics of C and N are the primary mechanisms that support the growth of individual plant organs. The total available C of a plant can only be used once. For example, increasing C allocation to roots reduces C investment to leaves and results in lower leaf area. Less leaf area in turn reduces the light interception and thereby has the potential to reduce the amount of assimilated carbon. In model simulations, I found lower grain yield was associated with genotypes with high N uptake capacity (i.e. larger root biomass) (Fig. 5.1). Secondly, since a model is a simplified system and cannot contain all relevant factors, critically and carefully setting up the input parameters can also be a way to consider trade-offs. For instance, there is a trade-off between plant lodging and the number of axial roots the plant produces. Too few roots can result in a high chance of lodging (Hostetler et al., 2022; Liu et al., 2012) while genotypes with fewer axial roots can root deeper and take up more N or water from deeper soil layers compared with genotypes with a higher root number (Guo & York, 2019; Schneider et al., 2021). Lodging thus determines the reasonable range for root number, even though lodging as a mechanism is not a part of the model. Besides, unlike real-life experiments, virtual experiments can be used to analyze the effect of unrealistic situations. As in the example above, the model could quantify the contribution of root number to N uptake even outside of the boundaries set by lodging risk. This would allow to compute the gain in N uptake a plant would show if lodging risk could be reduced in another way than having more roots.

Root system phenotype contribution to nitrogen use efficiency

As proposed in the General Introduction, we planned to focus on exploring novel phenotypes that can increase grain yield under reduced N application in this thesis. After running the simulations with wide ranges of parameters and dividing phenotypes into four different groups in Chapter 5, I found the root system phenotype with both high N uptake and high yield can lead to increasing N uptake efficiency (g plant N/g N applied) and therefore increasing N use efficiency (t yield/ kg N applied) when N is limiting (Fig. 6.1). The increased N uptake efficiency is due to higher root length density at 15 to 45 cm of the soil depth but not in the top layer of the soil (i.e. 0-15 cm) (Fig. D.2A and D.2D). However the absolute value of N uptake efficiency from simulations was overestimated. The overestimation is due to more complicated soil processes in relation to N in reality than in the current model version. In future studies, to better quantitatively assess the N uptake efficiency, rather than the simplified soil N processes that only included effects of N leaching and mineralization, more mechanisms such as volatilization could be included, or the water balance and related leaching process could be more closely followed.

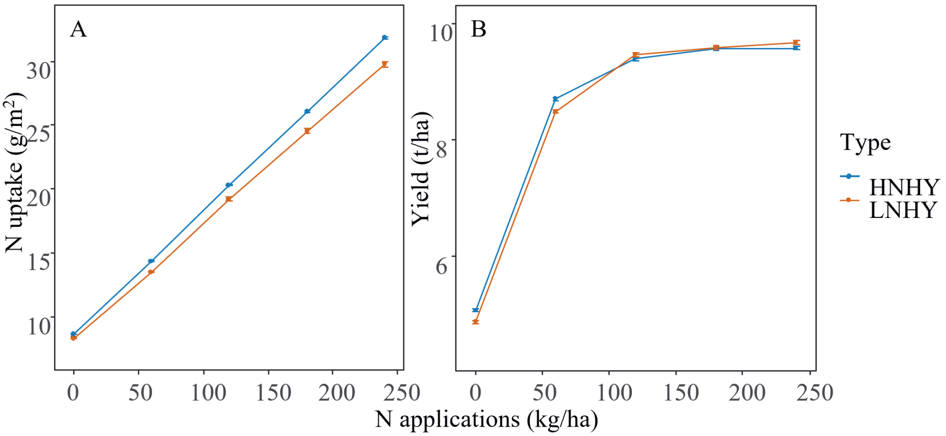

Figure 6.1: Comparisons on N uptake and grain yield between a root system phenotype with high N uptake and high yield (HNHY, blue lines and symbols) and a root system phenotype with low N uptake and high yield (LNHY, orange lines and symbols). The simulation data is from the modeling study in Chapter 5.

Future applications of FSP modeling

Combining genomic studies with FSP modeling

Since nitrogen use efficiency and yield are complex traits, identification of a limited number of regulatory components is not enough to understand the variation of yield or nitrogen use efficiency (Hirel et al., 2001). Genomic prediction models are widely used in the quantitative genetics field to predict the contribution of individual alleles to complex traits such as grain yield (Crossa et al., 2014). However, current methods of genomic selection are mainly based on statistical methods (e.g. deep learning) and do not include any eco-physiological mechanisms (Montesinos-López et al., 2018; Wang et al., 2023). Therefore, even when a genomic prediction model accurately predicts grain yield under current environments or with a current genetic population, it has a much lower accuracy of prediction when changing to new environments or a population with a different genetic background. Systematic approaches using tools such as crop growth models can theoretically provide opportunities to reveal how physiological processes are related in their effect on NUE and yield. Directly associating genomic prediction with crop growth model input could expand genomic studies beyond static traits and limited environments (Rincent et al., 2017; Jighly et al., 2023).

A few studies started to associate genomic prediction models with crop models to identify relevant quantitative traits loci (QTL) of model parameters across water gradients (Gu et al., 2014; Kadam et al., 2019). In most crop models, the structural characteristics of canopies or root systems have not been explicitly included. Therefore, linking an FSP model with QTLs, especially when canopy or root system traits co-determine crop performance, could add extra value to ranking the importance of QTLs on crop performance. Several attempts to link FSP modeling with genome-wide information have been made but the prediction ability needs to improve significantly (Migault et al., 2017; Letort et al., 2008; Xu et al., 2011, 2012b). Theoretically, by associating genomic prediction models to FSP model inputs, the effect can be assess of recombining QTLs of maize that contribute to processes of C-N uptake, assimilation, allocation, and remobilization throughout maize growth and the architecture of shoot and root on yield, grain quality, and nitrogen use. Ultimately, such a QTL-based FSP model could be used to assist future maize breeding for high nitrogen use efficiency under different environments, by identifying QTL combinations of maize that allow for high productivity and high nitrogen use efficiency under or even across different environments. However, some challenges are preventing the approach from being applicable at the current stage.

Firstly, FSP models are computationally demanding. Compared to crop models, FSP models simulate plant structure and localized environments at an individual organ scale, which requires considerable computational power. Linking genomic information to model parameters can further slow down the speed to achieve valid runs of simulations. Secondly, FSP models focus more on exploring the effect of mechanisms on plant functioning rather than making precise predictions while genomic prediction aims to be a predictive method. Linking genomic prediction models to mechanistic models can lead to increasing uncertainty around the final output (e.g. grain yield). Thirdly, FSP models have a large number of parameters. It requires a considerable amount of work to obtain detailed trait data from the field and link them with genetic information. To tackle these issues, clear research questions are needed beforehand to prioritize the importance of certain plant traits and decide how to simplify the current FSP model to reduce the demands for computational power while keeping the major mechanism in the model, such as using a cone to represent the root system rather than explicitly coding each root segment (Pagès et al., 2020). Furthermore, new approaches to link with genomic information may be required to replace the direct linkage between genomic prediction model and FSP models. Good collaboration among different research fields (modelers, quantitative geneticists, breeders) is required, as in the teams behind Gu et al. (2014); Kadam et al. (2019).

Exploring mechanisms from monoculture to mixtures

The current work mainly focused on applying FSP models to look for traits and mechanisms that lead to more efficient N-uptake in intensive monoculture maize systems, so N application can be reduced. To increase nitrogen uptake and use of maize, another approach could be to identify companion species that can complement maize for N-uptake, in so-called intercrop systems. Complementarity in N uptake in crop mixtures can lead to improved total nitrogen capture in the system due to a combination of species that source nitrogen in different ways (uptake from the soil vs. fixing from the air) at different locations in the soil (deep vs. shallow root systems) and/or at different moments during crop development (early vs. late) (Loreau & Hector, 2001). Complementarity can result in relaxing competition and increasing production (Vandermeer, 1992; Li et al., 2020a). Maize has been commonly intercropped with other cereals like wheat and with legumes, due to maize having a later growth peak (Li et al., 2001). Even though maize growth can be negatively influenced at the early growth stage due to shading by other earlier sown companion species, the growth of maize can recover after harvesting of the companion species (Li et al., 2001; Gou et al., 2016). Other than different developmental times in mixtures, spatial niche complementarity of the root system can result in the overyielding in the system (Postma & Lynch, 2012). In the ”three sisters” system (maize/beans/squash), maize roots can forage more in the topsoil while bean roots are distributed equally across all soil layers, and squash roots are located more based on the presence of the P distribution (Zhang et al., 2014). In addition, legumes can fix N from the air and therefore they do not compete for the same N resource (Jensen, 1996).

However, beneficial trait combinations in such crop species mixtures can be different from the monoculture system and dependent on the companion species (Nelson & Robichaux, 1997; Lavergne et al., 2021). Rather than directly going to the field to select suitable combinations, FSP modeling can be a useful tool to first explore the potential traits that may be beneficial in the system and thus propose species or genotypes that may likely provide mutual advantages when combined. There are a few attempts to apply FSP modeling to identify beneficial traits or mechanisms for over-yielding or high resource use in intercropping system. Zhu et al. (2015) developed and applied an FSP model to quantify the contribution of aboveground plasticity to the overall complementarity in maize/wheat system. Similarly, Li et al. (2021) used FSP modeling to quantify the contribution of leaf plasticity of soybeans on light capture in soybeans/maize intercropping systems. In addition to aboveground interactions, Postma & Lynch (2012) applied FSP modeling to identify that the spatial niche differences of the root systems can result in more yield by increasing N uptake in maize/bean and maize/bean/squash systems.

Louarn et al. (2020) applied FSP modeling to explore the overyielding and stability of a legume-based mixture. However, since over-yielding or enhanced resource use of different species mixtures is a complex phenomenon, the physiological mechanisms behind the phenomenon largely remain unclear and require future exploration. FSP modeling can be used as one of the first steps to disentangle the complex of mechanisms since traits influencing both above- and below-ground plant structures and major functionality can be included. Models like the one presented in this thesis could be expanded to cover multiple species and with further relevant mechanisms, such as plasticity to promote lateral root proliferation to soil nutrient patches and explore their importance in crop mixtures.

Conclusion

Designs for future agricultural systems often consider sustainability as a key target. Combining ecological principles with current breeding criteria can add new dimensions to the selection process of new cultivars. However, we still need to understand more about the role of trade-offs between traits to maximize plant growth while being efficient in resource use, as well as the role of phenotypic plasticity in the relationship between productivity and resource capture. This thesis aimed to address this gap by combining the understanding of crop physiological processes, ecological principles, and computational mechanistic modeling approaches. Specifically, I assessed the contributions of specific plastic responses, plant traits, and their interactions to plant N uptake and N use in intensive systems. Both *in silico* and *in vivo* studies are equally important in tackling different angles of the same questions and could strengthen each other. For example, in line with observations of lower root numbers for the same genotype under low N than under high N (Saengwilai et al., 2014b), our modeling study (Chapter 3) found that the plasticity in root number increased N uptake through adjusting the vertical distribution of root system under low N as the same biomass allocation to the root system was used by fewer roots allowing further soil exploration. In the future, combining *in silico* and *in vivo* approaches together can allow a better and more solid understanding of how individual physiological mechanisms influence both plant and crop performance.

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Appendix A

Table A. 1: List of management and environment parameter values used for model validation (2010). The parameter values were derived or fitted from experimental and local weather station data.

Parameter	Description	2010
startingdate	Sowing date	129
delay	Time lag between sowing and emergence	17
DNapp2	Second N application at V8	60
harvest date	Growing days after plant emergence	138
rowDistance	Distance between two rows (cm)	60
plantDistance	Distance between two plants (cm)	30
a	Year average temperature	9.121
b	Seasonal variation in daily average temperature	15.72
c	Day of the year when temperature is the yearly average temperature	104
Transmissivity	Percentage of incoming radiation that is transmitted through the atmosphere	0.3566

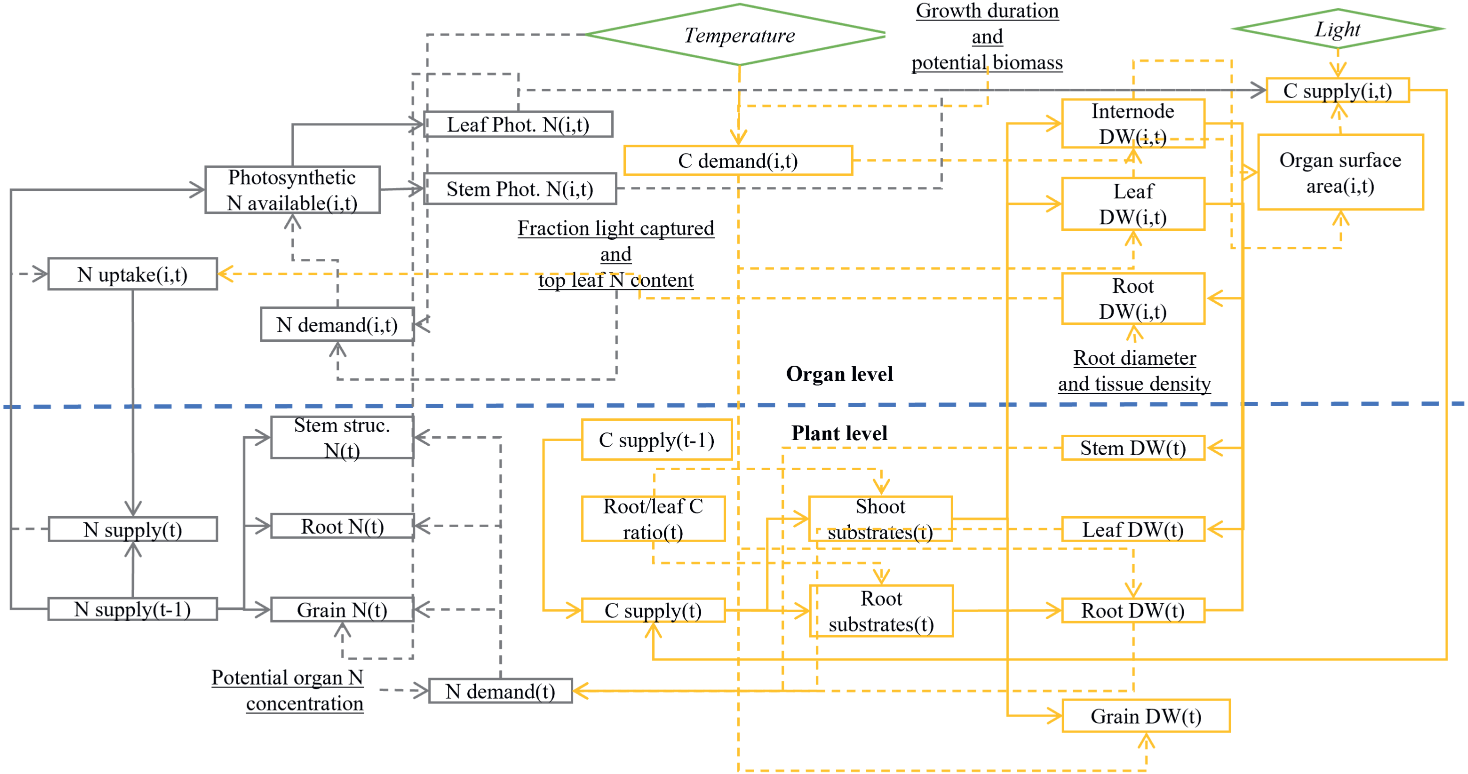

Figure A. 1: Flowchart of the nitrogen (N, grey) and carbon (C, yellow) flows and related processes in the model. Solid arrow lines indicate matter flows determining N and C accumulation at each simulation step (per day). Dotted arrow lines represent information flows. The rectangles represent state variables. The input parameters are underlined. The diamonds represent environmental inputs. The top half refers to organ level flows and states represented by individual leaf, internode and root segments. The bottom half refers to plant level flows and states calculated from the addition of individual leaves, internodes or root segments into whole plant organ types (leaves, stem or root). In addition, grain is treated as separate organ type (individual grains are not simulated so not represented).

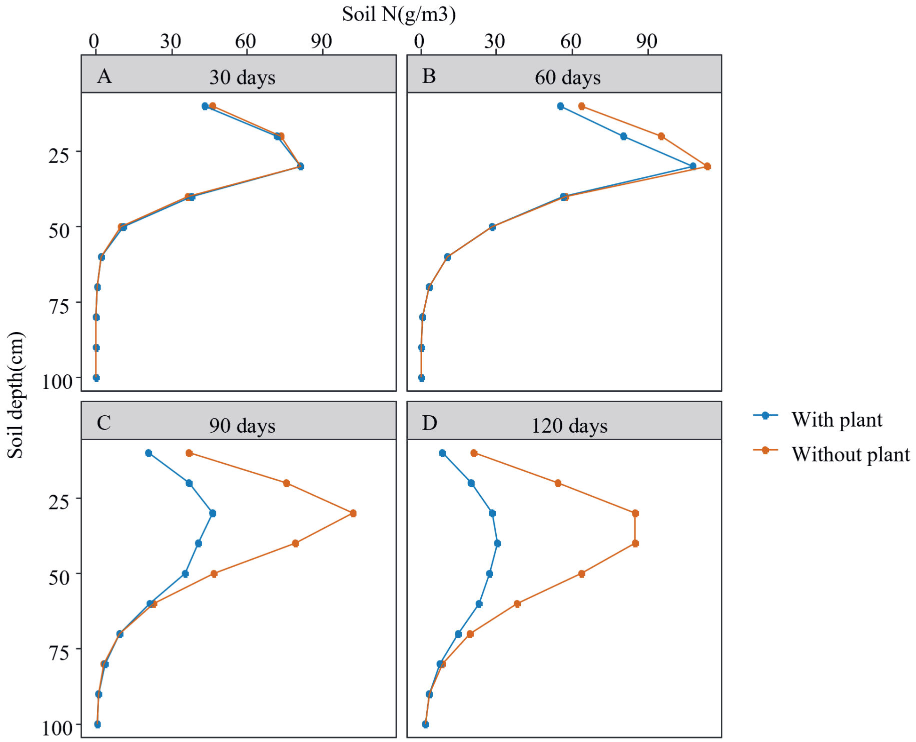

Figure A. 2: Simulated soil N dynamics during plant development under high N condition (5.714 μmol/m^3). Blue color represents soil N dynamics with a plant growing on. Orange color represents soil N dynamics without a plant growing on. Different panels represents different days after applied N fertilizer.

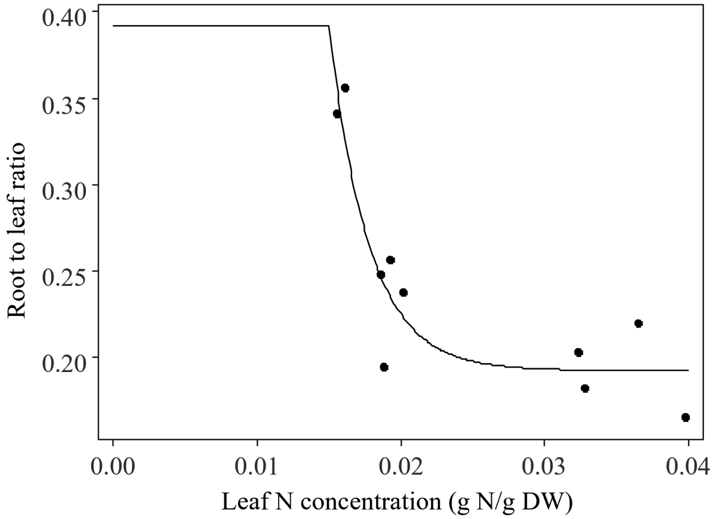

Figure A. 3: Root to leaf ratio in response to leaf N concentration. The dots represents experiment data for cultivars ZD958 and XY335 and the solid line represents the fitted relationship to implement into the model.

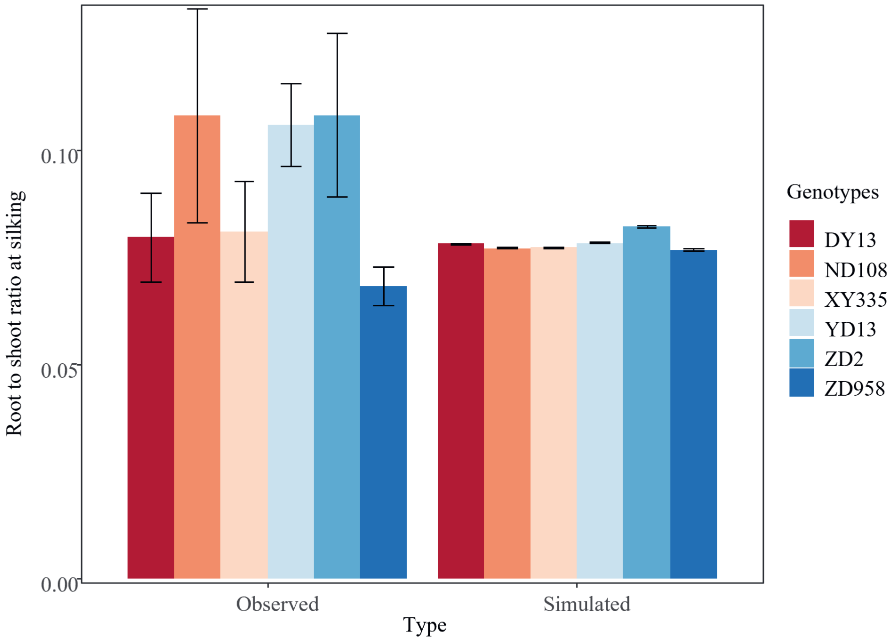

Figure A. 4: The evaluation of root to shoot ratio. The observed root to shoot ratios of each maize genotype were derived from the field experiment under high N condition (240 kg/ha and 5.714 μmol/m^3) in 2010 from Chen et al. (2013). The simulated root to shoot ratios were the output based on 2010 light and temperature data under high N condition. The Different colors represented different genotypes. the value were showed in Mean ± SE (n_{obs}=4 and $n_{sim} = 5$.)

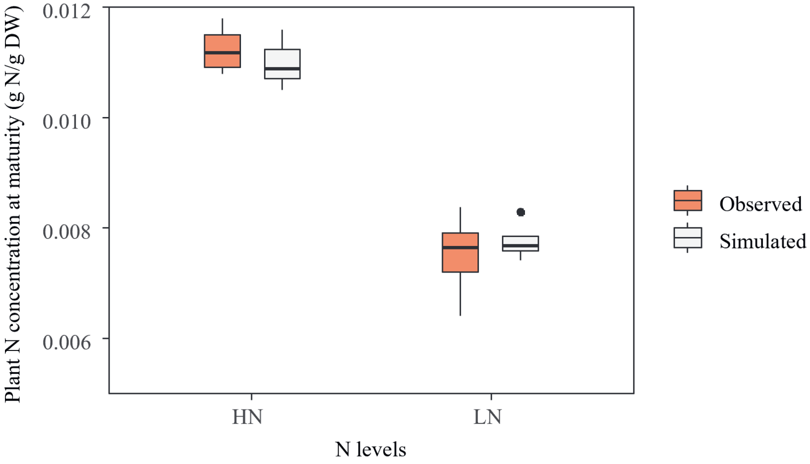

Figure A. 5: The boxplot of plant internal N concentration at physiological maturity. The default cultivar used here was ZD958 in order to illustrate plant N status under limiting and non-limiting N conditions.The orange bars represented the plant N concentration of ZD958 in 2010 from Chen et al. (2013). The white bars represented simulated plant N concentration of ZD958 based on the 2010 light and temperature data. HN represented high N level (5.714 μmol/m^3) and LN represented low N level (0 μmol/m^3). n for observed data is 4 and for simulation data is 5.

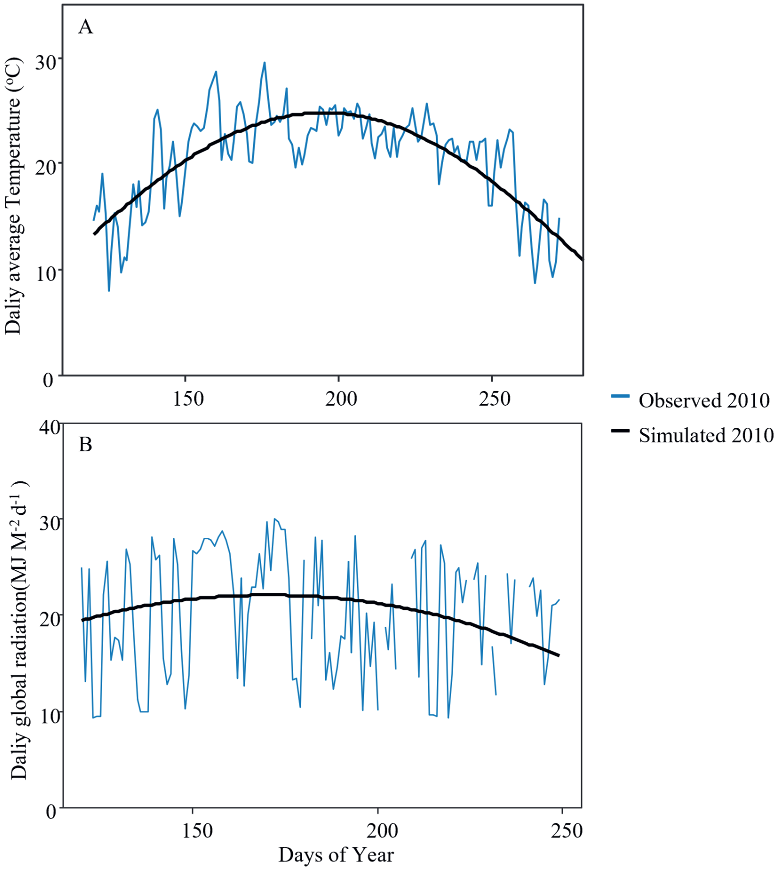

Figure A. 6: Daily average temperature (A) and daily global radiation (B) for the maize growing season in Lishu, Jilin in 2010 (Chen et al., 2013). Blue lines link actual measured data for 2010, where breaks represent days with missing values. Black lines represent simulated data for 2010.

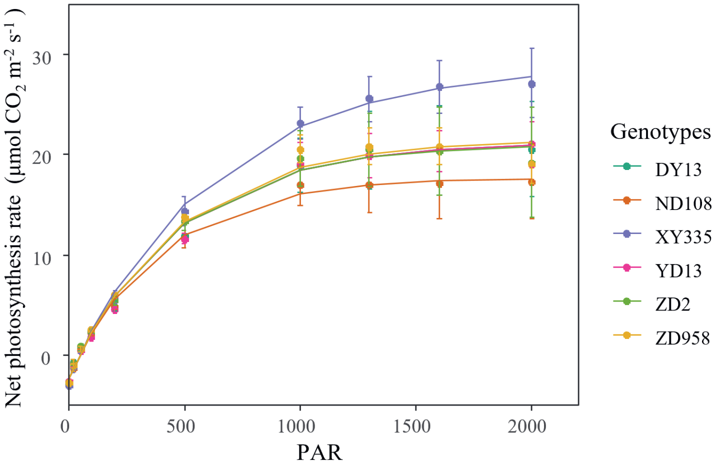

Figure A. 7: Leaf photosynthesis measurements from plants grown in the field under high nitrogen condition during the 2011 growing season for six maize cultivars (points) and fitted light response curves for non-limiting leaf nitrogen (eq. 23), where the maximum net photosynthesis rate λ was considered a cultivar specific parameter. Observed values were presented by mean ± SE (n=4). Based on the data from Chen et al. (2014a).

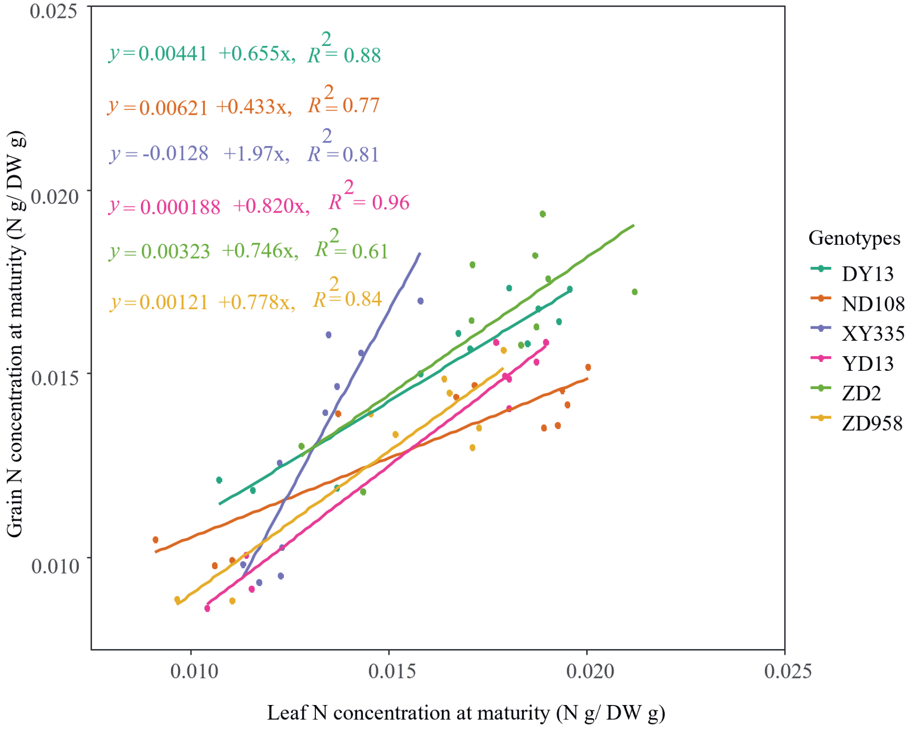

Figure A. 8: Relations between grain N concentration and leaf N concentration as fitted on observed data of six maize cultivars collected at physiological maturity during the 2011 growing season (original data from Chen et al. (2014a).

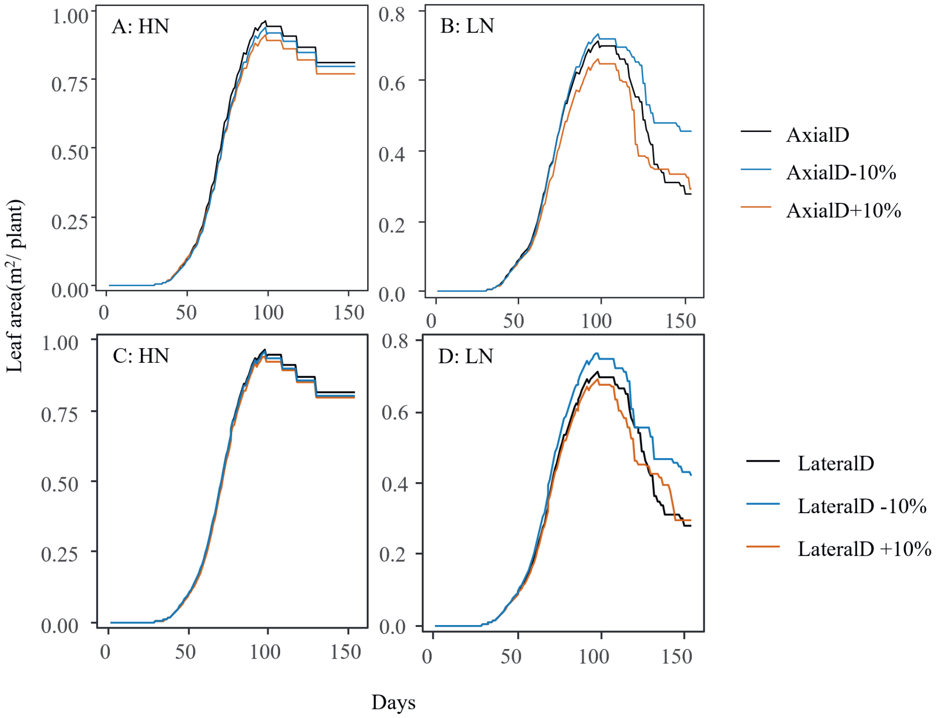

Figure A. 9: Dynamics in green leaf area per plant over the growing season when changing root diameter related traits: only axial root diameter (AxialD, A, B), or only lateral root diameter (LateralD, C, D) under high nitrogen condition (High N, A, C) and low nitrogen condition (low N, B, D). Value ranges of y axis for high N condition is from 0 to 1 m^2 and for low N condition is from 0 to 0.7 m^2. Blue and orange lines represent green leaf are per plant for trait values that are respectively 10% higher of lower than their default in black lines.

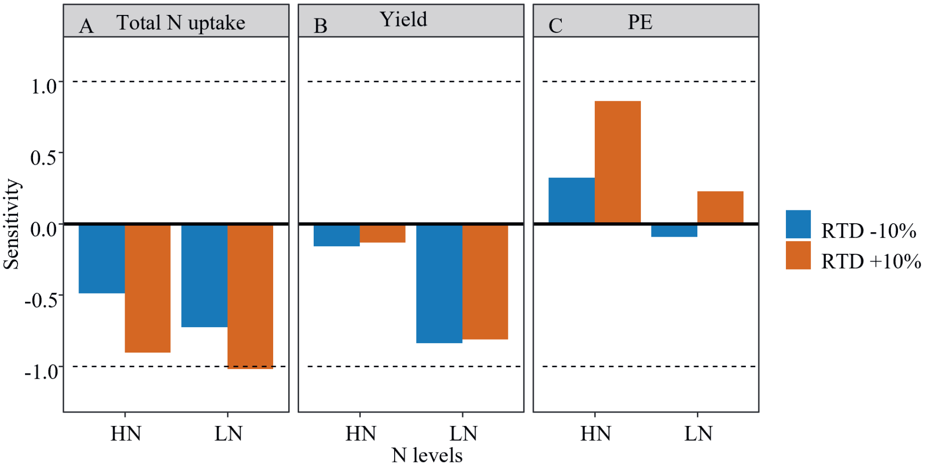

Figure A. 10: Sensitivity values of total N uptake (g/plant, A), yield (g/plant, B) and PE (g yield/g N, C) under high nitrogen condition (240kg/ha, high N) and low nitrogen condition (0kg/ha, low N) to changes in root tissue density. The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of an output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

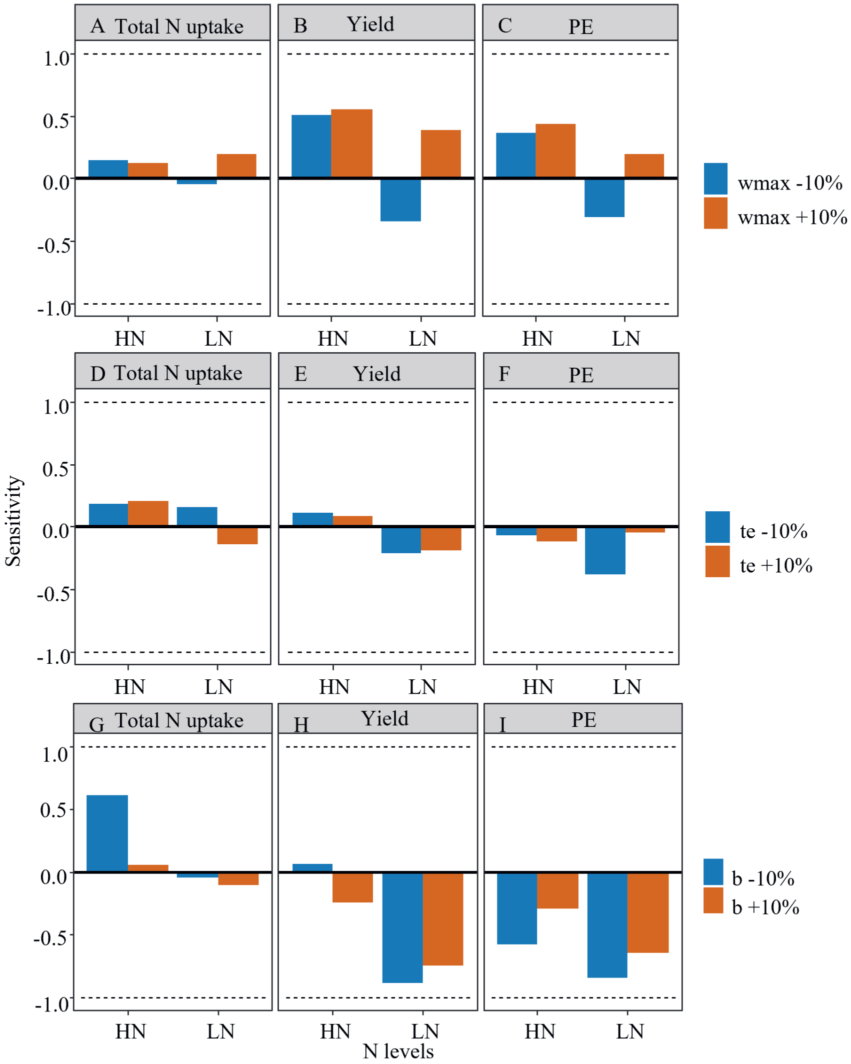

Figure A. 11: Sensitivity values of total N uptake (g/plant, A, D, G), yield (g/plant, B, E, H) and PE (g yield/g N, C, F, I) under high N condition (240kg/ha, high N) and low N condition (0kg/ha, low N) for nitrogen remobilization related traits: potential grain dry weight (wmax, A, B, C), grain growth duration (te, D, E, F), and grain N influx rate (b_g, G, H, I). The dashed lines represent a model sensitivity of 1 and -1. Parameter and output change in the same direction give a positive sensitivity value; parameter and output change in the opposite direction give a negative sensitivity value. Blue and orange bars represent sensitivity of the output parameter to respectively a 10% decrease or increase in a trait value compared to its default.

Table A. 2: List of cultivar specific parameter values for six Chinese maize cultivars derived from experimental data obtained during the 2011 growing season (Chen et al., 2013, 2014a).

Parameters	Description	ZD958	ZD2	DY13	YD13	ND108	XY335
final Phytomer	Leaf number	22	22	22	22	22	21
seedMass	Endosperm mass of the seed the plant grows from initially (mg)	295 ± 1.75	291 ± 4.8	214 ± 1.35	232 ± 4.9	272 ± 4.75	312 ± 4.85
a_g	Intercept of grain N concentration to leaf N concentration (g N /g DW)	0.00121 ± 0.00305	0.00323 ± 0.00357	0.0044 ± 0.00205	0.000188 ± 0.00282	0.0062 ± 0.00248	-0.0128 ± 0.0044
b_g	Slope of grain N concentration to leaf N concentration (g^{-1}DW)	0.778 ± 0.192	0.746 ± 0.206	0.655 ± 0.124	0.82 ± 0.171	0.529 ± 0.151	1.97 ± 0.321
fNstem	Stem structural N concentration (g N/g DW)	0.0038 ± 0.00017	0.0045 ± 0.00013	0.0049 ± 0.0005	0.0043 ± 0.00016	0.0038 ± 0.00017	0.0042 ± 0.00028
λ	Maximum photosynthesis under non-limiting leaf nitrogen (μmol/($m^2 \cdot s$))	24.7 ± 0.866	23.8 ± 0.863	24.1 ± 0.658	23.9 ± 0.868	21.2 ± 0.839	31.8 ± 0.979
initD	Initial root Diameter (m)	0.00154 ± 0.00013	0.00107 ± 0.000052	0.00104 ± 0.000034	0.00115 ± 0.000062	0.00118 ± 0.000069	0.00145 ± 0.000077
RTD	Root tissue density (g/dm^3)	90 ± 11.7	86 ± 6.57	120 ± 6.44	109 ± 14.4	101 ± 6.77	96 ± 7.57
RDM	Ratio in diameter of mother and daughter root	0.305 ± 0.032	0.396 ± 0.018	0.456 ± 0.017	0.388 ± 0.015	0.407 ± 0.026	0.318 ± 0.020

Table A. 3: List of soil N related parameters values used for model validation as derived from experiments in 2011 (Chen et al., 2013)

Parameter	Description	HN	MN	low N
Ninit	Initial soil nitrogen before N fertilizer application (μmol/m^3)	3	3	3
Nm	Soil N gradually released during the growing season by mineralization (μmol/m^3)	6	6	6
total Napp	Total N fertilizer applicated for whole growing season (μmol/m^3)	5.714	2.857	0

Table A. 4: AIC values and required number of parameters for models to relate rate of maize photosynthesis to radiation density from available data (Chen et al., 2013) allowing for more or less cultivar specific parameters. In bold the finally selected model based on the lowest AIC-value

Model	α	A_{max}	Rd	Number of parameters	AIC
Fit full	D*	D	D	18	169.57
Fit1	S	D	D	13	164.38
Fit2	D	D	S	13	160.08
Fit3	D	S	D	13	246.48
Fit4	**S**	**D**	**S**	**8**	**156.41**
Fit5	D	S	S	8	257.40
Fit6	S	S	D	8	242.95
Fit7	S	S	S	3	311.17

* D represents different values for each cultivar while S represents same value for all cultivars

Appendix B

Greenhouse experiment

Table B. 1: Hydroponic nutrition medium concentrations

Nutrients	Amount	Unit
NH_4N0_3	3	mM
KCl	3	mM
$CaCl_2 \cdot 2H_20$	1.5	mM
$MgSO_4 \cdot 7H_20$	2	mM
KH_2PO_4	1.5	mM
H_3BO_3	46.3	μM
$MnCl_2 \cdot 2H_2O$	2.28	μM
$ZnSO_4 \cdot 7H_2O$	0.76	μM
$CuSO_4 \cdot 5H_2O$	0.6	μM
$(NH_4)_6Mo_7O_24 \cdot 4H_2O$	0.23	μM
$Fe(EDDHMA)$	1.15	mL/L
$FeSO_4 \cdot 7H_2O$ *	20	μM

* $FeSO_4$ was added gradually during the experiment

Modelling approach on plastic responses of root to leaf ratio

Leaf nitrogen concentrations were assumed to affect fraction of carbon allocation between root and leaf ($RLratio$) and modeled as following:

$$RLratio = a \times e^{bleafN} + c \tag{B.1}$$

a,b and c represent plasticity coeffcients.

Plant developmental stages influence plant size which also could affect the allocation patterns if allocation would be a function of plant size. To identify whether response of the root:leaf allocation ratio to leaf nitrogen is development stage dependent, nested models (mle2) were used. In the nested model, normal distribution was used as stochastic model and eqn. B.1 was used as deterministic model. The AIC results are shown in Table S2. The model without nesting development stages showed the lowest AIC value. Therefore, the model that only accounts for leaf N concentration is to be considered best.

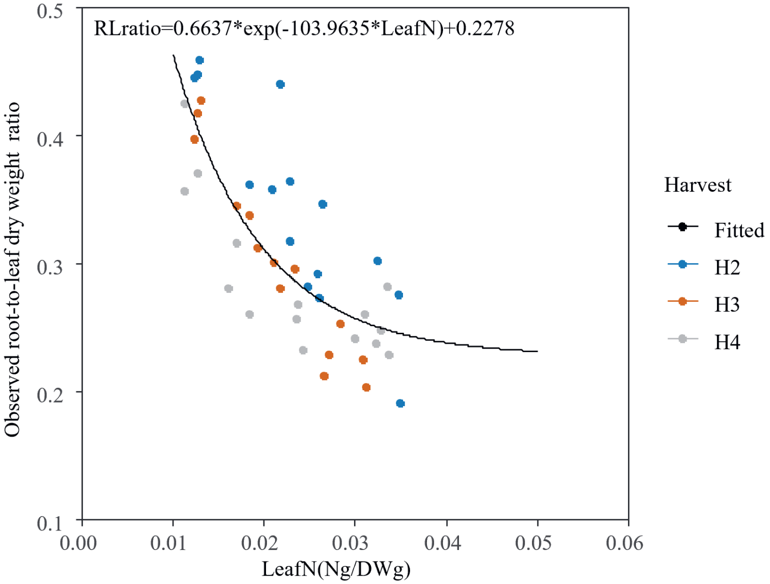

Figure B. 1: Root to leaf ratio (*RLratio*) in response to leaf N concentration. Second, third and forth harvests were included to fit the linear exponential equation. The *RLratio* is 0.6637*exp(-103.9635*$LeafN$)+0.2278 by using maximum likelihood method to fit the model. In the model fitting procedure, the negative exponential curve was treated as the determinate component and a normal distribution was treated as the stochastic component.

Table B. 2: Model selections based on AIC criteria for root-to-leaf ratio in response to leaf N and harvest time

Model[1]	a	b	c	Parameter number	AIC
Fit1	M[2]	M	M	9	-43.333
Fit2	M	M	S	7	-43.208
Fit3	S	M	S	7	-47.183
Fit4	M	S	M	7	-42.848
Fit5	M	S	S	5	-51.109
Fit6	S	M	S	5	-50.934
Fit7	S	S	M	5	-50.961
Fit8	**S**	**S**	**S**	**3**	**-54.049**

[1] Different models were fitted with eq. B.1

[2] M represents different values for each harvest while S represents same value for all harvest

Modelling approach on plastic responses of root emergence rate

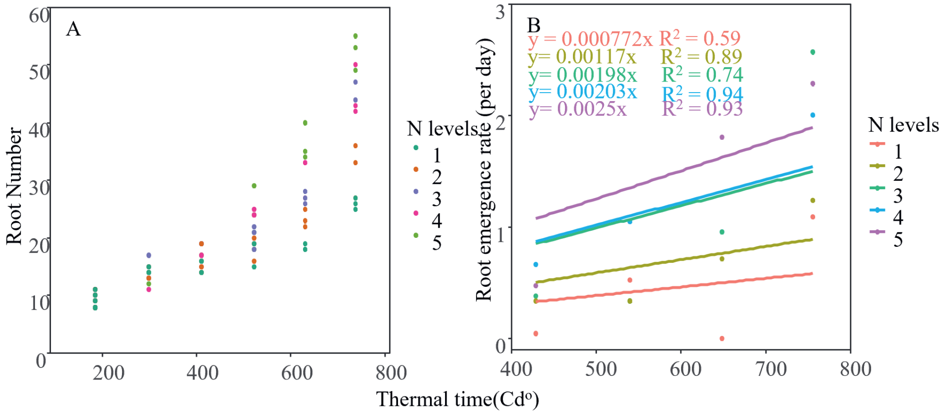

Figure B. 2: Root number increased over time under five nitrogen treatments. Panel A represents root number over thermal time. Panel B represents root emergence rate accelerated with thermal time. The different colors represent different nitrogen levels. 1 represents the lowest nitrogen treatment and 5 represents the highest nitrogen treatment.

Weather patterns

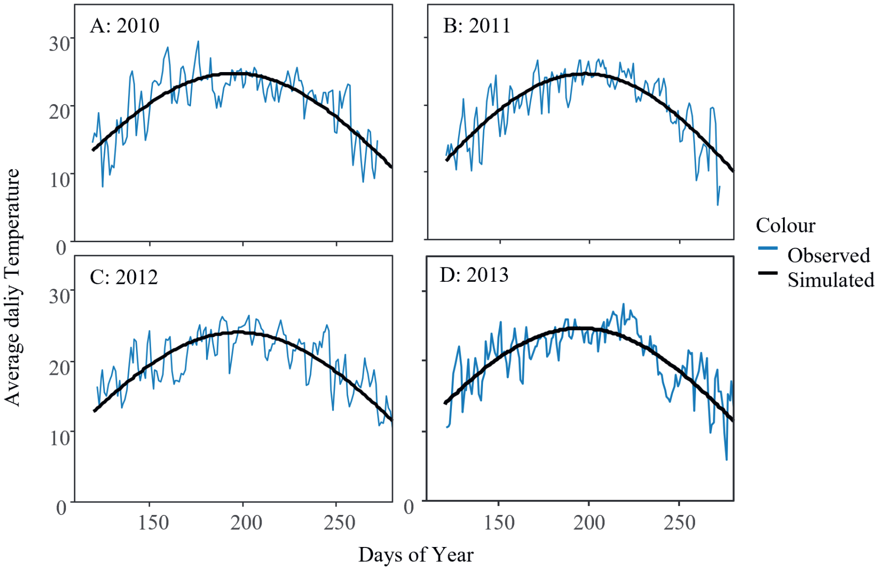

Figure B. 3: Observed and simulated average daily temperature over growth season across the four years. The blue color lines represent observed daily temperature and the black lines represent simulated daily temperature.

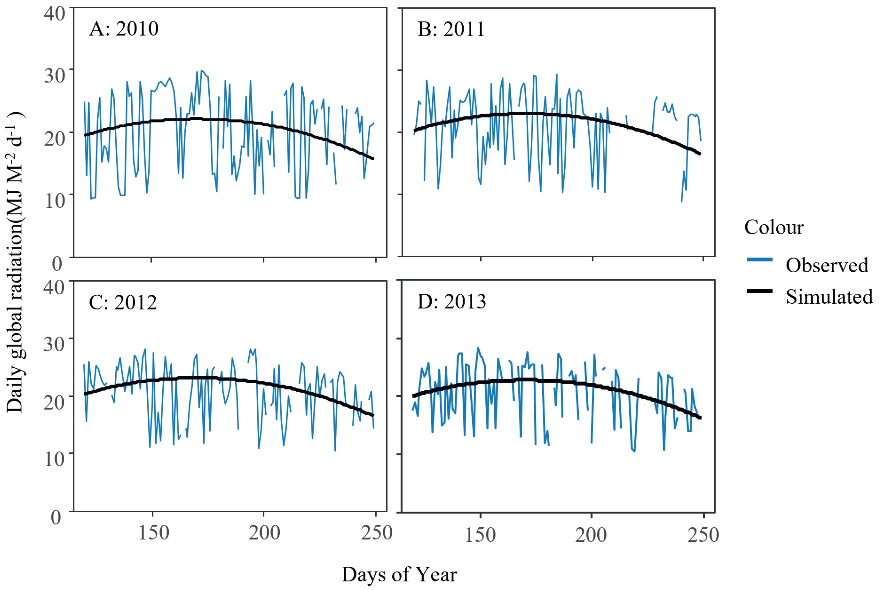

Figure B. 4: Observed and simulated daily global incoming radiation over growth season across the four years. The blue color lines represent observed daily global incoming radiation and the black lines represent simulated daily global incoming radiation.

Simulation experiment layout

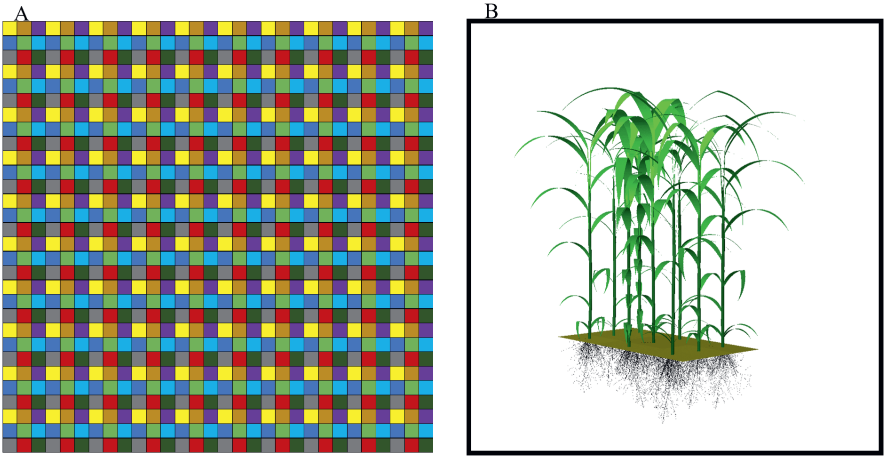

Figure B. 5: Layout of the virtual experiments. In panel A, each color represented the same maize plants. In the simulation experiment, nine different plants were simulated in a 3 by 3 plot setting and cloned 100 times. In this setting, a maize plant is always surrounded by different plants. Panel B is the 3D graph to demonstrate the maize plant growth in a 3 by 3 plot setting.

Simulated range of leaf N concentration and plant N concentration

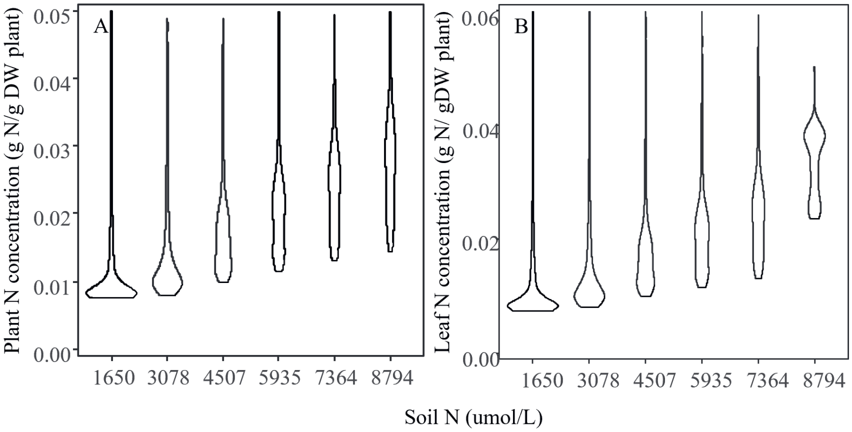

Figure B. 6: Violin plot of simulated plant N concentration and leaf N concentration range across various N levels. Width represents the frequency of the plant/leaf N concentration values during whole growing season.

Magnitudes of plastic response

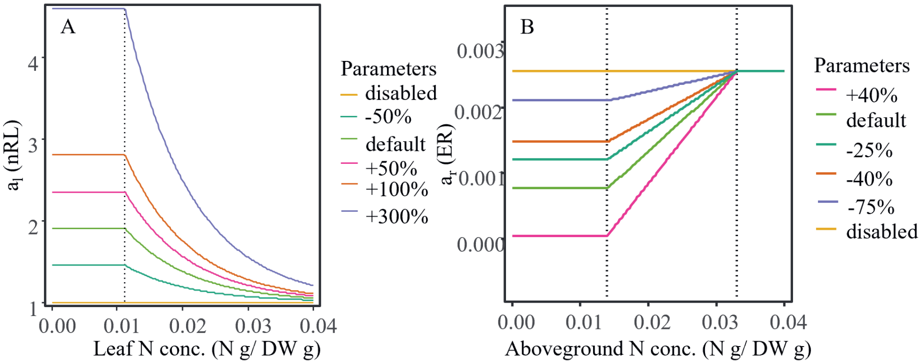

Figure B. 7: Magnitudes of plastic response. Panel A represents different strengths of plastic response in root-to-leaf ratio implemented in the model. Panel B represents different strength of plastic response in root emergence rate implemented in the model. Colors represent different plastic strengths. Vertical lines represent the beyond the values, constant values were assigned to the plastic responses.

Simulations for single plants

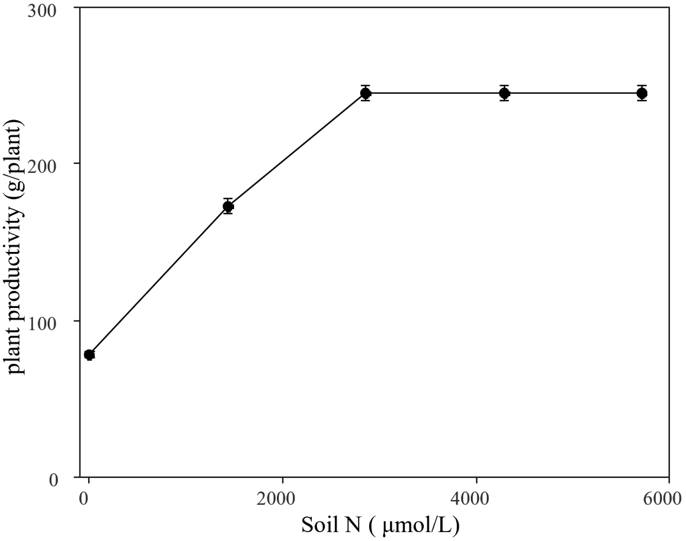

Figure B. 8: Simulated plant productivity for single plant across various N levels. Each point represents the average of simulations for a single plant (plant without neighbors) over four continuous years (2010 to 2013). Error bars are the standard error among the four years.

Appendix C

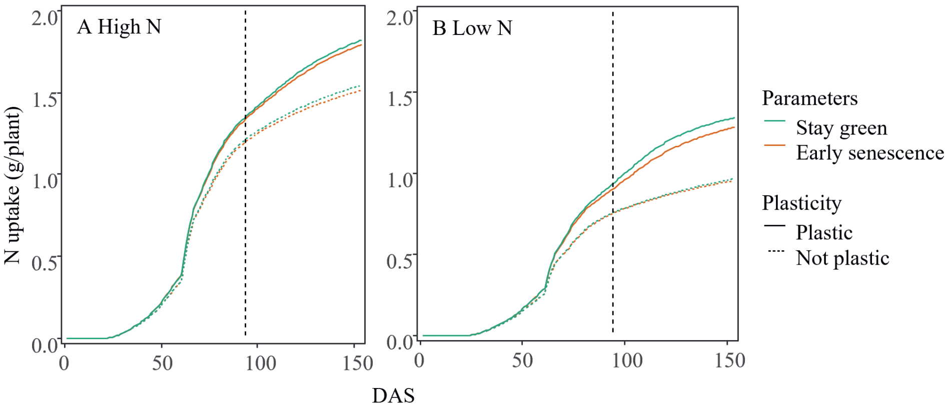

Figure C. 1: Plant N uptake of being plastic to plant N vs not plastic and for plants with early senescence or without senescence (stay green) grown under high N (Panel A, 240 kg/ha) and low N (Panel B, 120 kg/ha). The vertical line represents the silking date (94th DAS).

Table C. 1: Absolute root length density (RLD, m/m^3) for plasticity and leaf senescence under both high N and low N

Soil layers (m)	high N				low N			
	With response		Without response		With response		Without response	
	Stay-green	Early leaf senescence	Stay-green	Early leaf senescence	Stay-green	Early leaf senescence	Stay-green	Early leaf senescence
0.15	3930.7 ± 24.2	3775.6 ± 15.4	3630.9 ± 13.1	3518.5 ± 22.6	3993.5 ± 33.5	3836.7 ± 20.1	3413.4 ± 23.6	3322.5 ± 29.3
0.30	1184.0 ± 39.8	1036.8 ± 42.1	776.5 ± 34.3	710.9 ± 26.4	1765.5 ± 65.3	1522.3 ± 34.4	666.8 ± 13.7	610.7 ± 25.2
0.45	240.5 ± 8.3	219.9 ± 6.3	140.2 ± 4.7	134.7 ± 5.5	292.4 ± 11.2	270.7 ± 8.8	128.7 ± 6.8	122.4 ± 4.5
0.6	28.7±2.5	22.3±3.4	3.0 ± 1.3	1.8 ± 1.1	59.9±5.5	46.8±4.2	0.8 ± 0.4	0.5±0.35
0.75	NA	NA	NA	NA	0.0067 ± 0.02	NA	NA	NA

Appendix D

Input parameters

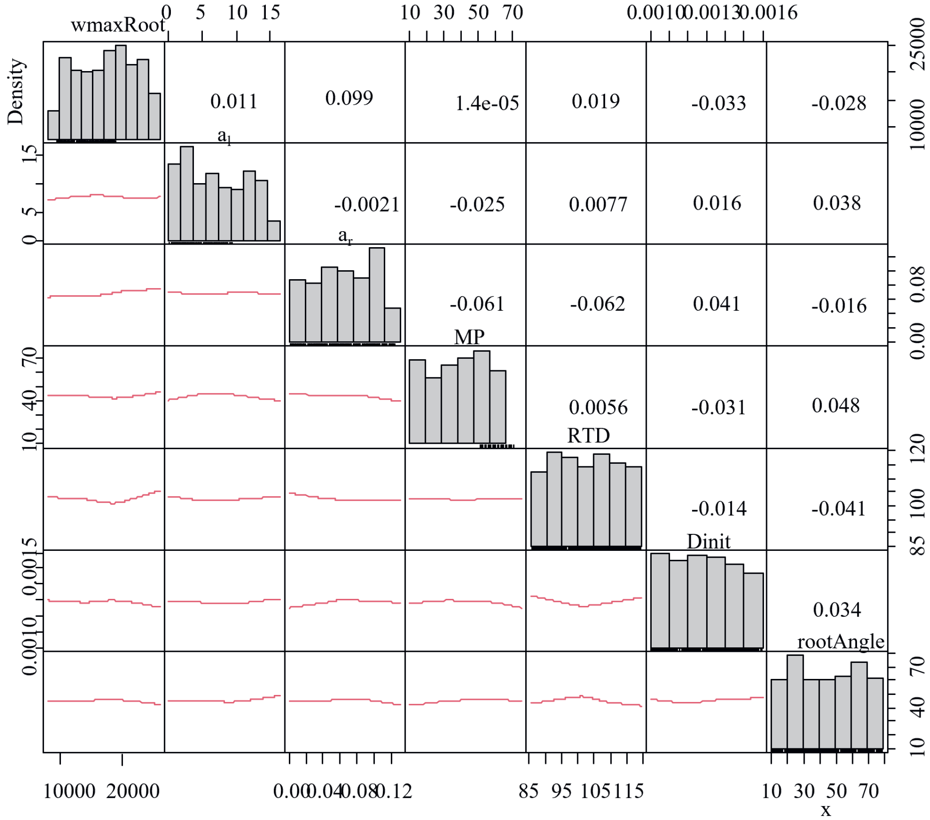

Figure D. 1: Correlations and distributions for the seven root traits. Where *wmaxRoot* (*g/plant*) represents potential root dry weight which determines root sink strength; a_l represents the coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen; MP represents maximum potential root numbers; a_r represents coefficient of root emergence rate in response to shoot nitrogen; RTD represents root tissue density (g/dm^3); $Dinit$ (m) represents average first order root diameter; rootAngle (^{o}) represents the initial axial root angle between stem and root. The values in the upper triangle represent the Pearson correlation coefficients between two of the target plants traits. The lower half of the triangle presents correlations as bivariate scatter plots with fitted lines. The bar graphs in the diagonal represent distributions of values of individual plant traits. 300 model input parameter combinations were independently selected from seven uniform distributions. The correlations among the seven independent input parameters were relatively small ranging from -0.062 to 0.099. The values of the selected individual parameter values were distributed uniformly

Comparison among four root phenotype on root outputs and inputs

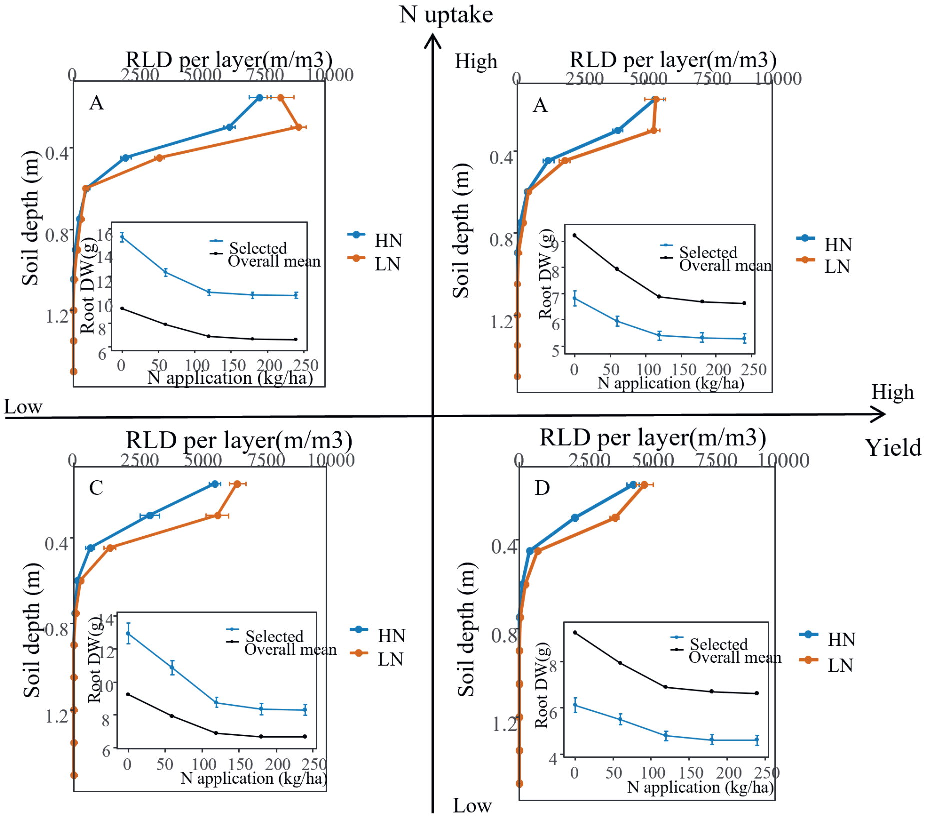

Figure D. 2: The root length density (RLD, m/m^3) and root dry weight (root DW, $g/plant$) for the all four N uptake and yield categories. Blue lines and symbols in the root dry weight panels represent the average root dry weight of selected common simulations for high yield with high N uptake, high yield with low N uptake, low yield with high N uptake and low yield with low N uptake across all N treatments. The black lines and symbols in the root dry weight panels represent the overall mean of all 300 simulations across all N treatments. For the RLD panels, blue lines and symbols represent the RLD of selected common simulations under the highest N treatment (N=240 kg/ha) and orange lines and symbols represent the RLD of selected common simulations under the lowest N treatment (N= 0 kg/ha). Symbols and whiskers represent mean ± SEs (n_A=25, n_B=19, n_C = 20, n_D=21).

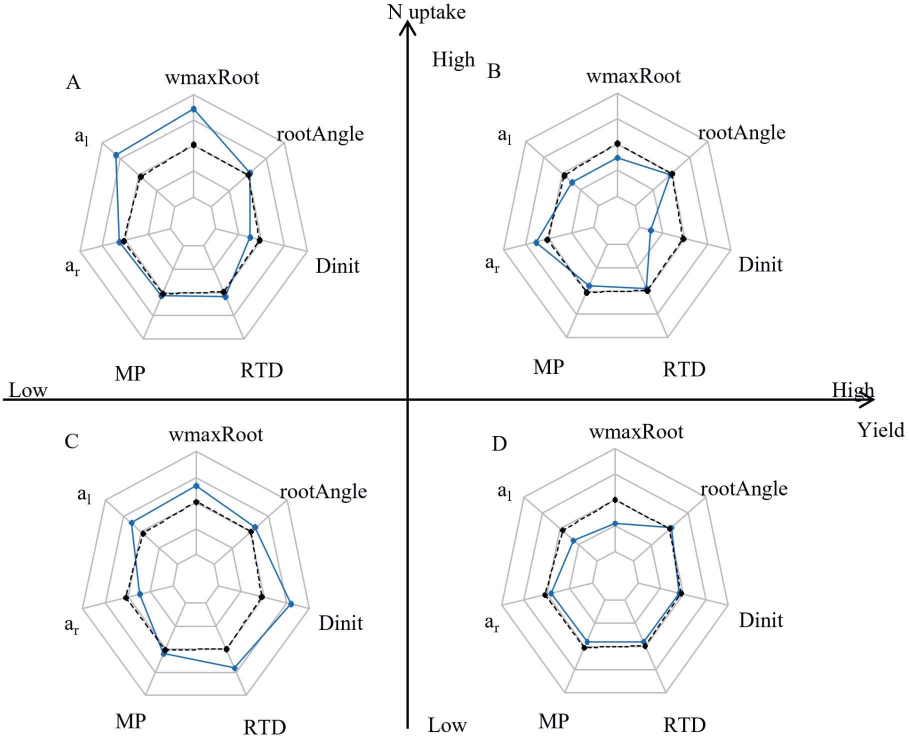

Figure D. 3: The input parameter values for the all four N uptake and yield categories. The *wmaxRoot* ($g/plant$) represents potential root dry weight which determines root sink strength. The a_l represents the coefficient of root-to-leaf biomass partitioning coefficient in response to leaf nitrogen. The MP represents maximum potential root numbers. The a_r represents coefficient of root emergence rate in response to shoot nitrogen. The RTD represents root tissue density (g/dm^3). The *Dinit* (m) represents average first order root diameter. The rootAngle (^{o}) represents initial axial root angle between stem and root. Blue color represents input parameter combinations of selected common simulations for high yield with high N uptake, high yield with low N uptake, low yield with high N uptake and low yield with low N uptake. Black color represents average of the input parameter of all 300 simulations without any selections.

Summary

Excessive nitrogen (N) can be lost to the ecosystem and result in acidification and/or eutrophication of natural ecosystems, leading to in global warming. Reducing N application in agricultural systems is necessary to mitigate environmental pollution. Breeding for crops that are highly N use efficient can contribute to maintaining or even increasing yield with lower amounts of nitrogen fertilizer applied. Nitrogen use efficiency is co-determined by N uptake and physiological use efficiency (PE, grain biomass per unit of N taken up), to which soil processes as well as plant architectural, physiological, and developmental traits contribute.

To achieve the general goal of providing quantified insights to assist breeding for high N use efficient maize, in the General Introduction (**Chapter 1**), I presented three categories of plant traits that may be important in influencing plant N use, including plant traits related to stay-green, roots, and plasticity. Rather than using field observations, a modeling approach is introduced in this chapter and plans to further develop and apply this for exploration and quantification of the contribution of the incorporated plant traits to plant performance.

Since the relative contribution of plant traits to N use is not well known but relevant to identify breeding targets in important crop species like maize, I evaluated, in **Chapter 2**, the effect of varying both shoot and root traits on crop N uptake across a range of nitrogen levels using a further developed functional structural plant model for maize. Root architectural traits were found to play a more important role in root N uptake than physiological traits. Phyllochron determined the structure of the shoot through changes in carbon source : sink ratio over time which, in interaction with light and temperature, resulting in a significant effect on PE and N uptake. Photosynthesis traits were more relevant to biomass accumulation rather than yield, especially under high nitrogen conditions. We argue that the traits identified in this chapter can be potential targets in maize breeding for improved crop N uptake and use.

In addition to quantifying plant traits, plant phenotypic plasticity plays an important role in plant resource acquisition under limited N conditions and may have a role under non-limited N conditions. However, the contribution of plastic responses to N uptake and use across N application levels has not been quantified, leaving it unclear whether plastic responses should be considered potential targets for phenotypic selection. In **Chapter 3**, a combined modeling and experimentation approach is taken to assess the potential role of plasticity in maize for N uptake and for the conversion efficiency of N taken up into yield (i.e. PE). Based on a small experiment, I considered plasticity in two traits: root-to-leaf biomass allocation ratio and axial root emergence rate. In a virtual experiment, I individually enabled or disabled both plastic responses for maize stands grown across six N levels to quantify the effect of plasticity on N use. Both plastic responses contributed to maintaining N uptake and plant productivity at N-limited conditions compared to stands with plastic responses disabled. I conclude that plastic responses quantified in this study may be a potential target trait in breeding programs for greater N uptake across N levels while it may only be important for PE under N-limited conditions in maize. Given the complexity of breeding for plastic responses, *a priori* model analysis seems a useful tool to assess which plastic traits to target for enhanced plant performance in specific environments.

Stay-green is an important plant characteristic in maize to increase grain yield by prolonging leaf life. However, a longer leaf life span can reduce N remobilization from leaves to the grain, as leaves senesce later, leading to lower grain N content especially under low N conditions. As studied in Chapter 3, plastic responses to plant N status can increase N uptake. This increase in N uptake could potentially compensate for the negative effect on grain N content in stay-green varieties. To explore to which extent such plastic responses interact with stay-green characteristics and their effect on grain N, I applied, in **Chapter 4**, the FSP modeling approach from Chapters 2 and 3. I first used field observations to evaluate model performance for a range of cultivars differing in leaf senescence rate. Then I ran the simulations with and without plastic responses and with and without leaf senescence (i.e. stay-green and non-stay-green) at a range of N levels. I analyzed the relative contribution of plasticity to N uptake, grain yield, and grain N concentration. The absence of leaf senescence contributed to grain yield under high N, while under low N, the combination of plasticity and stay-green improved grain yield. Plastic responses reduced the negative effect of stay-green on grain N concentration by improving N uptake, especially under low N. Using maize cultivars that plastically respond to plant N status and improve N uptake may therefore mitigate the negative effects of stay-green on grain quality.

After studying the effect of individual plant traits and plasticity on plant N uptake and N use, understanding interactions among multiple traits was considered an interesting next step. Identifying maize genotypes with a high capacity for nitrogen uptake without yield penalty is relevant to maize breeding for sustainable production systems with limited inputs. Identifying the root system phenotype to achieve this is complex in field or greenhouse experiments, due to high variation in root phenotypes within a single genotype. Therefore, in **Chapter 5**, I applied the FSP model developed in chapters 2 and 3 to identify beneficial root system phenotype(s) for maize to achieve high yield and high N uptake simultaneously. I first identified root phenotypes that give either high yield or high N uptake at a wide range of soil nitrogen levels. Data from a field experiment with six maize cultivars released in China between the 1970s and the 2000s were used to evaluate the model results. Subsequently, I performed a virtual experiment to identify root phenotypes that could combine both high N uptake and high yield at a high plant density common in maize cultivation, and across a wide range of nitrogen levels. The results demonstrated trade-offs in root phenotypes for high yield and for high N uptake, between root sink strength for carbon, and root-to-leaf biomass partitioning. Using cluster analysis I identified two root phenotypes that give both high yield and high N-uptake. I showed low root biomass and high root length density at 15 cm to 45 cm depth allowed maize to combine high yield and high N uptake. The study demonstrates that cluster analysis of FSP modeling results can be used to identify improved root system phenotypes across a wide range of environments, which can be useful information when defining targets for breeding.

In **Chapter 6**, I return the aim of this thesis (Chapter 1) and reflected on the new insights presented in this thesis. I summarize the major findings in Chapters 2-5 and further discuss the validity by applying the FSP modeling approach to understand plasticity and trade-offs in the general context of breeding for high nitrogen use efficient maize cultivars. Combining genomic information with FSP modeling and applying FSP modeling to explore the contribution of physiological mechanisms in the mixture can be a future application of this FSP modeling approach.

Curriculum Vitae

Jie Lu was born on 2 Sep.1991, in Sichuan province, China. In 2014, she obtained her BSc study in Agronomy at both China Agricultural University and Colorado State University. After her bachelor study, she decided to start her master program in Agronomy at Colorado State University. In this program, she studied *"Changes in phenotypic traits and DNA methylation status in cryopreserved seeds of rye (Secale cereale)"*. During this program, she started to realize the potential needs to combine modern computational technology and agriculture. In addition to obtaining the MSc in Agronomy, she started a master program in Applied Statistics in Colorado State University. In 2019, she was admitted to China Agricultural Green Development program (AGD, co-developed by CAU and WUR) to pursue her PhD study in developing 3D plant simulation model to assist high nitrogen-use-efficient maize breeding programs in China. During the study, she spent the first year in China in Key Laboratory of Plant-Soil Interactions, CAU and in 2020, she moved to the Center of Crop System Analysis, WUR, the Netherlands to continue her PhD study.

Peer-reviewed journal publications

- J. Lu, T. Stomph, G. Mi, Y. Yuan, J. Evers (Submitted). Phenotypic plasticity can mitigate the reduction in grain nitrogen associated to stay-green maize

- J. Lu, T. Stomph, G. Mi, Y. Yuan, J. Evers. (Submitted). Finding improved maize root phenotypes for high N uptake and yield at high population density using plant modeling

- J. Lu, J. Lankhost, T. Stomph, H. Schneider, Y. Chen, G. Mi, Y. Yuan, J. Evers. (Submitted). Root plasticity in response to nitrogen availability improves uptake by maize

- J. Lu, T. Stomph, G. Mi, Y. Yuan, J. Evers. (Submitted). Identifying and quantifying the contribution of maize plant traits to nitrogen uptake and use through plant modelling

Other scientific publications

Oral Presentations

- J. Lu, T. Stomph, Y. Yuan, J. Evers. 2023. Optimizing plant traits for efficient nitrogen use in maize using functional-structural plant modelling. FSPM Conference 2023

Poster Presentations

- J. Lu, T. Stomph, Y. Yuan, J. Evers. Can functional-structural plants models contribute to improving maize nitrogen uptake? The 5th European Maize Conference, 2023

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 (4.5 ECTS)

- Integrate FSPM and GWAS to improve nitrogen use efficiency in maize

Post-graduate courses (6.5 ECTS)

- Agricultural green development; CAU (2019)
- Theory of system analysis; CAU (2019)
- Integrate modelling and optimization; WUR (2021)
- Advanced crop physiology; WUR/UF (2022)

Competence, skills and career-oriented activities (2.1 ECTS)

- Scientific writing; WUR (2021)
- Introduction to LATEX; WUR (2022)

Scientific integrity/ethics in science activities (0.3 ECTS)

- Ethic in plant and environmental sciences; WUR (2021)

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

- PE&RC First years weekend (2020)

- PE&RCX Midterm weekend (2021)
- PE&RC Day (2022)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Agricultural green development symposia (2019-2023)
- FSPM group (2022-2023)

International symposia, workshops and conferences (4.4 ECTS)

- 5th European maize conference; Bologna (2023)
- FSPM Conference; Berlin 2023

Lecturing / supervision of practicals / tutorials (1.5 ECTS)

- Soil-plant relation (2022-2023)

BSc/MSc thesis supervision (6 ETCS)

- Root biomass allocation in nitrogen deficient maize
- Effects of root to leaf plasticity on maize growth at different planting densities using plant modelling

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