Resource capture, plant morphological traits and maize photosynthesis in intercropping

Bei Dong

Propositions

1. Recommendations for arable crop fertilization require adjustment when applied to intercropping.
   (this thesis)
2. Yield increases of maize in strip intercropping rely solely on complementary resource capture.
   (this thesis)
3. Rejecting a hypothesis is crucial to exploring the world.
4. A profound learning on fundamental principles is essential for inspiring creative ideas.
5. Getting away from modern media is getting away from anxiety.
6. In explaining interpersonal interactions, diversity in personality traits trumps cultural diversity.

Propositions belonging to the thesis, entitled

Resource capture, plant morphological traits and maize photosynthesis in intercropping

Bei Dong
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Abstract

Intercropping is the planned cultivation of multiple crop species in a field for the whole or part of their growing periods. In Europe, intercropping research has concentrated on alternate row or within-row mixtures at low input levels. There is comparatively less information available on the potential of strip intercropping under conventional growing conditions in Europe. In strip intercropping, plants are grown in strips of multiple rows of the same species to facilitate species-specific management. I study eco-physiological responses of plants to intercropping in different species combinations under conventional management to explore the potential of strip intercropping for an ecological intensification of conventional agriculture.

Experiments were done with four crop species: maize (*Zea mays*), wheat (*Triticum aestivum*), faba bean (*Vicia faba*), and pea (*Pisum sativum*), combined in bi-specific strip intercrops in all six possible combinations. All species were fertilized in their strip according to conventional agriculture standards. Maize is the only C_4 species, having higher temperature requirement and a longer growing period than the other three C_3 species. Due to the late sowing and long growing period of maize, intercrops with this species are relay intercrops; the other intercrops are (nearly) simultaneous. Faba bean and pea are legumes that require only low or no nitrogen (N) fertilizer input because they can symbiotically fix N from the atmosphere. Thus different combinations of these species provide different possibilities for complementarity, based on differences in growing period and N acquisition mechanisms. I focused first on the N uptake in all six intercrops. Then, I studied species trait responses in wheat-maize and faba-maize relay intercropping. I studied the effects on shoot traits in the three species and the effects on leaf photosynthetic traits in maize.

In simultaneous intercrops, faba bean was a competitive species that decreased N uptake of its companion wheat or pea. In relay intercrops, N uptake of the early-sown species (i.c. wheat, faba bean, and pea) was increased compared to the sole crop. Intercropped wheat had an increased number of tillers and faba bean had an increased number of branches and shorter internodes in response to higher red to far-red ratio (R:FR) and increased transmitted photosynthetically active radiation (PAR) during early growth. N uptake in maize was increased if it was grown with wheat or pea, but not with faba bean. Maize grown with faba bean had longer leaf sheaths, responding to the shading and lowered R:FR experienced during vegetative growth. The shading by faba bean furthermore resulted in thinner maize leaves

with reduced leaf N, lower light-saturated net photosynthetic rate (A_{1800}), and lower light-saturated gross photosynthetic rate ($A_{g,max}$). Those thinner maize leaves also had lower values of the maximum rate of ATP production and the maximum carboxylation rate of Rubisco. During the maize reproductive stage, maize leaves in relay intercrops with wheat or faba bean were exposed to improved light conditions, however, leaf N, A_{1800}, and $A_{g,max}$ were not increased compared to maize leaves in sole crops.

The results elucidate several plant trait responses to strip intercropping under conventional management in the Netherlands. Some of these responses, particularly in the early-sown species in relay intercrops, are conducive to resource capture and production, but others, particularly those in simultaneous intercrops with faba bean, are not. Overall, the results indicate that increases in resource capture due to temporal complementarity between species underlie the productivity increasing effects of relay intercropping. Resource capture and conversion is modulated by plant trait responses to changes in the light environment and available N that result from temporal complementarity.

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Chapter 1

General Introduction

1.1 What is intercropping?

Agriculture is facing sustainability issues in terms of food security, biodiversity loss, environmental degradation and climate change (Hossain et al., 2020). A promising way to address sustainability in agriculture is intercropping. Intercropping is the mixed cultivation of multiple crop species in a field for the whole or part of their growing periods (Brooker et al., 2015). It can serve as a sustainable intensification of agriculture due to advantages in improved land productivity and improved efficiency in light use (Gou et al., 2017a; Wang et al., 2015), water use (Mao et al., 2012; Zhang et al., 2019), and nutrient use (Li et al., 2011a; Xu et al., 2020), as well as better pest and disease control (Boudreau, 2013), and weed suppression (Gu et al., 2021, 2022). Various intercropping strategies have emerged and have been practiced by farmers around the world, depending on production purposes and production constraints (Brooker et al., 2015; Hong et al., 2017; Li et al., 2020b). While the advantages of intercropping have been widely documented in studies in developing countries, comparatively little information on a limited range of systems is available in Europe (Li et al., 2020b; Li et al., 2023).

1.2 Intercropping benefits from species interactions

1.2.1 Complementarity in resource capture

Complementarity in resource capture is a key factor in increasing land and resource use efficiency in intercrops compared to sole crops (Stomph et al., 2020). In an intercrop, complementarity is achieved by combining crop species that occupy different niches in growing periods, above- or below- ground morphology, and functional traits for resource capture (Homulle et al., 2022; Li et al., 2006, 2011b; Yu et al., 2015). The intercropped species can thus exploit light, water, and nutrient resources differently in time and space, resulting in an enhanced resource capture by the species in intercrops compared to sole crops (Liu et al., 2015, 2020; Ma et al., 2020; Wang et al., 2015).

Temporal complementarity exists if intercropped species use resources (light, water, and nutrients) during different periods of the season (Yu et al., 2015). For instance, in temperate regions, length of the growing season is sufficient to grow two species that only partially overlap their growing seasons in a relay intercrop, while insufficient for growing two crops in sequence (double cropping) (Gou et al., 2017a; Li et al., 2020b). An example of relay intercropping in temperate climates is combining maize (*Zea mays*) with a C_3 cereal or legume.

In such a system, maize, being a C_4 species, has a higher temperature requirement and a longer growing period than C_3 species, which is sown and harvested substantially later than C_3 species. Both early-sown C_3 species and late-sown maize are reported to have enhanced light and soil nitrogen (N) capture in relay intercrops compared to sole crops (Li et al., 2001a, 2001b; Liu et al., 2015, 2020; Wang et al., 2015).

Temporal niche differentiation (TND) is an index to characterize the proportion of the periods when intercropped species are growing alone in the field compared to the whole growing period of the intercrop (Yu et al., 2015) (Box 1.1). A larger TND means less overlap in resource use in time and a longer period for both component species growing alone in the field. This enables intercropped species to exploit extra resources in the strips of companion species compared to those in pure stands, where they experience intraspecific competition (Wang et al., 2023; Zhao et al., 2023). In intercropping with C_3/C_4 combinations, advantages in both land productivity and absolute yield gain, compared to sole crops, are positively correlated with TND values (Li et al., 2020b; Xu et al., 2020; Yu et al., 2015).

Complementarity in soil N capture mechanisms, resulting from the ability of N_2 fixation by legumes, exists in cereal/legume intercrops. Most grain legumes need limited mineral N as starter dose for their early establishment, and 60%-80% of their final plant N is derived from N_2 fixation (Giller, 2001). Cereal-legume intercropping has been studied and practiced in regions such as Africa, Asia, and Europe, making use of the ability of N_2 fixation by the legume and decreasing soil N competition for the cereal (Bedoussac and Justes, 2010; Ghaley et al., 2005; Hauggaard-nielsen et al., 2009; Li et al., 2020b; Matusso et al., 2014; Rusinamhodzi et al., 2012).

1.2.2 Factors affecting species interactions in intercrops

Various intercropping designs have been applied, aiming to improve resource use efficiency of the system while decreasing chemical inputs (Bedoussac et al., 2015; Brooker et al., 2015), or to maximize land productivity and absolute yield gain of intercrops compared to sole crops (Li et al., 2020b). Complementarity in resource capature in intercrops is affected by agronomic practices such as the selection of component species, row arrangement, and fertilization strategies.

Species combination. Growth environments of a species vary with the type of companion species in an intercrop (Homulle et al., 2022; Zhao et al., 2023). For instance, maize experiences stronger competition for soil N when intercropped with a cereal such as wheat (*Triticum aestivum*) than it does in the sole crop (Gou et al., 2018). This competition is diminished when maize is intercropped with a legume (Li et al., 2001a, 2001b, 2006).

Row arrangement. Row arrangement in intercrops determines the potential for complementarity as it determines which crop rows interact with conspecific and which interact with companion species (Gou et al., 2017a). In strip intercropping, where component species are grown in alternating strips consisting of several rows, there is a gradient of interspecific interaction with the strongest interactions in the border rows of the strip while much weaker interactions in the adjacent inner rows (Wang et al., 2020). Plants in border rows in a strip intercrop can capture more light and soil resources compared to the adjacent inner row plants and plants in sole crops (Zhang et al., 2022; Zhu et al., 2015). From strip intercropping to alternate-row intercropping or mixed intercropping (with no distinct row arrangement), the degree of interactions between component species increases (Homulle et al., 2022; Jensen et al., 2020)

Fertilization strategy. Nitrogen (N) is an essential element for plant development and growth, which is a main limiting nutrient for crop production (Leghari et al., 2016). Competitive relationship between component species in an intercrop is affected by N fertilizer inputs. For instance, complementarity in N capture between cereals and legumes diminishes when increasing N inputs to intercrops, as high N inputs discourage N_2 fixation by the legume, leading it to take up more soil N (Jensen et al., 2020; Naudin et al., 2010).

1.3 The importance of species-tailored N fertilization in intercropping for sustainable agriculture

1.3.1 Intercropping in conventional high-input agriculture vs. low-input organic farming

Intercropping has been practiced under various N fertilization strategies with different purposes. Intercropping in conventional agriculture (i.e., with artificial fertilizer and biocides for controlling pests, diseases, and weeds) with high input has been widely practiced in China (Hong et al., 2017; Li et al., 2020b). These intercrops usually receive high rates of N fertilizer, in order to prevent N limitation and achieve maximum yield advantages compared to sole crops.

Intercrops in such conventional high-input agriculture often use a strip design, where the strips are wide enough to facilitate management while narrow enough (usually between 1.5 and 3 m) to ensure interspecific interactions (Li et al., 2020a; van Oort et al., 2020; Xu et al., 2020).

In Europe, intercropping has mostly been done by combining cereals and legumes in organic farming, where species receive low or no N fertilizer (Bedoussac and Justes, 2010; Ghaley et al., 2005; Jensen et al., 2020). These intercrops usually use mixed- or alternate-row designs, and full synchronization (TND=0) known as a simultaneous system, allowing a high degree of rhizosphere interactions and whole field mechanized management. They make use of the ability of N_2 fixation by the legumes, decreasing N competition for the cereals and resulting in an acceptable protein content in the cereal grain at limited soil N levels (Brooker et al., 2015; Rodriguez et al., 2020). However, the yield advantages of such a system can be unstable and constrained due to limited N fertilizer (Li et al., 2020b).

Besides the typical high-input and low-input systems, there is a growing interest in appropriately applying mineral fertilizer to intercrops (Falconnier et al., 2023). This approach aims to minimize environmental impact while maintaining stable and high productivity of intercrops. A tailored N fertilization to component species based on their growth needs in an intercrop may be a solution. This strategy aligns with European standards for “Good Agricultural Practices”, i.e., moderate fertilization (Baghasa, 2008; FAO, 2003). One of the emphasized advantages of intercropping to farmers is improved and stable yields (Bedoussac et al., 2015). Strip intercropping with species-tailored N fertilization can serve as an option to increase crop performance while simultaneously avoiding high N losses to the environment. However, there is limited information available on this, and most of it has been studied in wheat-maize strip intercropping with moderate inputs in the Netherlands (Gou et al., 2016, 2017a, 2018; Zhu et al., 2014, 2015, 2016).

1.3.2 Further analysis is required on strip intercropping in conventional agriculture in Europe

In an intercrop, species interactions result in modified access to light and soil resources, leading to different eco-physiological responses of crop species compared to those in the sole crop (Gou et al., 2018). The effects of intercropping exhibit at multiple levels- from resource capture per unit area of a population of plants at the field level (Gao et al., 2020; Gou et al., 2017a; Liu et al., 2015, 2020), to morphological traits at the plant level (Li et al., 2021; Zhu et al., 2014, 2016), and further to leaf photosynthesis at the leaf level (Feng et al., 2020; Gou et al., 2018; Li et al., 2020d) (Fig. 1.1). Information on these multiple-level responses provides a comprehensive understanding of crop performance in an intercrop and potentially optimize intercropping systems for European growing conditions. Recent work in the Netherlands has shown yield advantages of wheat-maize relay strip intercropping at locally conventional fertilizer inputs (Gou et al., 2016). As agronomic practices (i.e., species combinations and N fertilization) affect species interactions in intercropping, further analysis is required on crop performance in other species combinations and compare this to wheat-maize intercropping in a single study to obtain results consistent with European standards for “Good Agricultural Practices”.

1.4 The effects of intercropping at field level- N uptake of intercrops

Previous studies have indicated the effects of intercropping on N uptake under a high N fertilization strategy in China. Two types of fertilization strategies have been studied: i) additive N fertilization in cereal/legume intercrops, where the N input in the intercrop is the sum of that in the two sole crops (Du et al., 2018; Feng et al., 2020; Yang et al., 2017); ii) at the system level, intercrops receive an identical amount of N fertilizer (> 225 kg ha^{-1}) as sole crops. This strategy has been applied in both cereal/cereal and cereal/legume combinations (Gao et al., 2022; Li et al., 2011a; Li et al., 2001a, 2001b; Xing et al., 2023).

The land equivalent ratio for N uptake (LER_N) is used to assess intercropping advantages on N uptake compared to corresponding sole crops (Bedoussac and Justes, 2010; Li et al., 2011a) (Box 1.1). An LER_N greater than one indicates that the combined sole crops need more land than their intercrop to achieve the same N uptake, so the intercrop can use land more efficiently for N uptake than the correponding sole crops. Studies conducted under high N fertilization strategy indicated that values of LER_N greater than one when the intercropped species are in a relay sequence (Li et al., 2011a). In cereal/legume simultaneous intercrops with high N inputs, cereals are usually highly competitive for soil N, decreasing N uptake by the companion legumes compared to sole crops (Gao et al., 2022; Han et al., 2023). This competition can be diminished by sowing legumes before cereals in a relay intercrop (Yu et al., 2016). In such case, both species over-yield in N uptake compared to their sole crops (Li et al., 2001a, 2001b; Xu et al., 2023).

However, a cocern regarding the effiency of applying such large amounts of N fertilizer to legumes, which require limited N fertilization, is the potential for N leaching from the soil, and such high N inputs is not acceptable in Europe. Yet, strip intercropping with tailored fertilizer N at a moderate level that aligns with species' demand is rarely studied. This raises the questions of: i) which species combination yields intercropping advantages in N uptake compared to corresponding sole crops; ii) what the responses of cereals and legumes are in intercrops when applied with moderate fertilizer N, and how these compare to growing two cereals or two legumes in intercrops; iii) whether complementarity in N capture in intercrops is associated with temporal complementarity or complementarity in N capture mechnisms resulting from the ability of N_2 fixation by legumes.

1.5 The effects of intercropping at plant and leaf levels- plant morphological traits and leaf photosynthesis

Plants can adjust their morphological and physiological traits to environmental conditions (Sultan, 2000; Walters, 2005). Crop species in intercropping have altered growth environments compared to that in pure stands (Gou et al., 2017a). Relay strip intercropping involving maize with high N inputs usually has overyielding compared to corresponding sole crops (Li et al., 2020b). It is therefore relevant to study how crop species respond morphologically and physiologically in relay intercrops involving maize to better understand their overall high performance. However, limited information is available regarding such a system when conducted under European growing conditions (Gou et al., 2018; Zhu et al., 2014, 2015, 2016).

In this thesis, I study the responses of shoot traits in both species in relay intercrops involving maize and focus on the responses of maize leaf photosynthetic traits. In the remaining part of this general introduction, I will first give background information on the current knowledge of trait responses in a plant or in a plant stand, and then review previous intercrop studies to highlight what is known and what remains unknown in this domain.

1.5.1 Shoot plasticity and light signaling in relay strip intercropping

Plants have the ability to change their traits in response to changes in the environmental conditions, a phenomenon known as phenotypic plasticity (Sultan, 2000). In plant stands, important indicators of competition for light with neighboring plants are the red to far-red ratio (R:FR) and photosynthetically active radiation (PAR) within the canopy. In a stand, plant tissues absorb red light and reflect and transmit far-red light. The reduction in R:FR perceived by a plant can trigger a suite of shade avoidance responses, such as increased stem or petiole length, reduced tillering or branching (Ballaré and Pierik, 2017; Demotes-Mainard et al., 2016; Evers et al., 2006; Huber et al., 2021). PAR within the canopy serves not only as the light energy for photosynthesis and biomass production, but it also can trigger phototropic and elongation responses in plants (Ballaré and Pierik, 2017; Huber et al., 2021). Thus, shoot plasticity can be related to altered R:FR and PAR signals, and this is the case in an intercrop as well.

In strip intercrops, R:FR perceived by a plant in the radiation received from different directions carry different competitive information. For instance, in an intercrop, R:FR perceived in the radiation received from the direction of neighboring species rows is an indicator of interspecific competition, while that from the direction of adjacent conspecific species rows is an indicator of intraspecific competition. However, in previous studies on R:FR in strip intercropping, there is a lack of information on the position within the canopy where R:FR was measured (Raza et al., 2019). In addition, previous studies did not distinguish R:FR perceived from neighboring species and from conspecifics in strip intercrops (Liu et al., 2017; Yang et al., 2014). Consequently, there is a lack of information on the extent to which changes in R:FR in intercrops compared to sole crops are associated with interspecific competition.

Shoot plasticity and light signaling have been well studied in maize-soybean (*Glycine max*) strip intercropping under conventional agriculture mostly with high-input fertilization. This research includes both relay intercrops in subtropical climates, where maize is sown and harvested earlier than soybean (Yang et al., 2014), and simultaneous intercrops in continental climates (Li et al., 2021; Li et al., 2020c; Liu et al., 2017, 2018). The authors in the latter case reported shade avoidance responses in soybean plants in intercrops, showing longer internodes compared to plants in pure stands. Such plasticity in intercropped soybean has been attributed to a lower PAR within the canopy and a lower R:FR perceived by plants in intercrops than in sole crops (Liu et al., 2017, 2018).

In the Netherlands, maize is sown and harvested later than a C_3 species due to the temperate climate, giving the C_3 plants a better starting position for resource capture in relay intercrops. For the late-sown maize in such intercrops, they initially experience shading and then have improved access to light when they overtop the companion early-sown species and more so after the companion species has been harvested (Gou et al., 2017a). A study in wheat-maize relay strip intercropping conducted in the Netherlands reported increased numbers of tillers in wheat and longer sheaths in maize during its early growth compared to plants in corresponding sole crops (Zhu et al., 2014, 2016). Further analysis is required to investigate shoot plasticity and light signals in relay intercrops where maize is grown with a legume and to compare them with a wheat-maize relay intercrop in a single study. Information on this helps understand crop performance in relay strip intercropping maize with species of different traits under European growing conditions.

1.5.2 Maize leaf photosynthetic traits in relay strip intercropping

The responses of maize leaf traits and leaf photosynthetic capacity

In a stand, the distribution of PAR within the canopy affects the distribution of N content per unit leaf area, with the leaves that are well exposed to the sun having larger leaf N content than shaded leaves (Lambers et al., 2008). Leaves grown at high light conditions have increased leaf photosynthetic capacity, and they are thicker with a larger leaf mass area (LMA) (or a lower value of its inverse, specific leaf area; SLA) compared to leaves grown at low light conditions (Evans and Poorter, 2001; Pengelly et al., 2010; Poorter et al., 2009). These thicker leaves have more chloroplasts per unit area and increased photosynthetic components such as Rubisco content per area (Pengelly et al., 2010; Tazoe et al., 2006).

Leaf photosynthetic capacity can be obtained from field measurements, requiring that leaves are well adapted to ambient CO_2 and artificial light at saturated levels, which allows measuring the light-saturated net photosynthetic rate of leaves. However, previous intercrop studies either measured actual rate of leaf photosynthesis (Liu et al., 2018), or measured the rate of leaf photosynthesis at lower than saturated light levels (Nasar et al., 2020, 2021, 2022), or lacked information on the artificial light level and the adaptation time used to obtain leaf photosynthetic rate (Feng et al., 2020; Ma et al., 2020; Yang et al., 2017). Therefore, the effects of intercropping on the leaf photosynthetic capacity of maize leaves are still unclear.

The acclimation of leaf photosynthetic parameters: insights into the processes of photosynthesis

The processes of leaf photosynthesis consist of light reaction (which converts light energy into ATP) and dark reaction (which assimilates CO_2 to sugars). Photosynthesis-light response curves of leaves are affected by light environments, with leaves grown under high light conditions having a higher light-saturated gross photosynthetic rate ($A_{\mathrm{g,max}}$) than leaves grown under low light conditions (Lambers et al., 2008). Based on the model of von Caemmerer and Furbank (1999), Yin et al. (2011) proposed a biochemical C_4 photosynthesis model that predicts maize leaf photosynthetic rate as limited by the maximum rate of ATP production (J_{atpmax}), the maximum carboxylation rate of phosphoenolpyruvate carboxylase (PEPc) in the C_4 cycle (V_{pmax}), and the maximum carboxylation rate of Rubisco in the C_3 cycle (V_{cmax}). A study conducted on maize plants reported that values of leaf biochemical parameters increased with leaf N content per unit area (Wang et al., 2022).

In an intercrop, maize leaves are expected to acclimate to altered light and soil N conditions, resulting in modified photosynthesis-light response curves compared to leaves in the sole crop. It is anticipated that changes in values of $A_{\mathrm{g,max}}$ in intercropped maize compared to sole maize are explained by the acclimation of maize leaf biochemical parameters to relay intercropping. However, previous intercrop studies did not elucidate such a deep mechanistic understanding of intercropping effects on maize leaf photosynthesis. Information on this provides insights into the responses of traits related to Rubisco carboxylation capacity and ATP production capacity in maize leaves in intercropping, as well as offers potential options for selecting traits in breeding routes for intercropping.

Further analysis for results relevant to European growing conditions

In an intercrop, there are three factors affecting maize leaf photosynthetic traits: (i) the relative sowing time between crop species; (ii) the type of companion species; and (iii) N fertilization strategy. Intercrop studies on maize leaf traits and leaf photosynthesis have been focused on simultaneous intercrops or relay intercrops, where maize was the first sown species, typically under high-input fertilization (Feng et al., 2020; Li et al., 2019; Yang et al., 2017). For instance, in a maize-soybean simultaneous intercrop with additive fertilizer N inputs, intercropped maize had increased leaf N concentration compared to maize in the sole crop (Feng et al., 2020). Such enhanced leaf N of intercropped maize could result from: (i) improved light conditions in intercrops compared to the sole crop, given that maize, a tall species, was sown simultaneously with a shorter legume; (ii) complementarity in N capture between maize and soybean; and (iii) high N inputs in intercrops which increased N uptake by intercropped maize than sole maize.

The story can be different when conducting relay intercrops in the Netherlands. In this case, maize as the late-sown species initially responds to shading by the early-sown C_3 species, showing thinner leaves compared to maize leaves in the sole crop (Gou et al., 2018). After the early-sown species has been harvested, maize leaves in intercrops are exposed to better light conditions compared to leaves in the sole crop (Gou et al., 2017a; Zhu et al., 2014). Such improved light conditions may lead to increased leaf N in intercropped maize compared to sole maize, or this benefit is offset by N deficiency in intercropped maize during its reproductive stage due to moderate fertilizer inputs. Further analysis on the responses of maize leaf photosynthetic traits to dynamic growth conditions in relay intercrops, as well as a comparison of maize responses when grown with a cereal or a legume in an intercrop, could contribute to a comprehensive understanding of maize physiological responses under Dutch growing conditions.

1.6 Overall knowledge gap of this thesis and research questions

The better performance of strip intercropping has been well studied in conventional high-input agriculture. Further analysis is required on the eco-physiological responses of crop species in strip intercropping with agronomic practices that are consistent with European standards for “Good Agricultural Practices”. This thesis addresses following research questions:

i. What are the effects of combining four commonly grown species- maize, wheat, faba bean, and pea (*Pisum sativum*) in bi-specific intercrops on N uptake, when species receive tailored fertilizer N in the Netherlands? (Chapter 2)
ii. What plastic responses do maize, wheat, and faba bean plants show in wheat-maize and faba bean-maize relay intercrops? To what extent is such plasticity related to differences in light signals between intercrops and sole crops? (Chapter 3)
iii. What are the responses of maize leaf traits and leaf photosynthetic capacity to relay intercropping with wheat or faba bean? (Chapter 4)
iv. How do maize leaf photosynthetic parameters acclimate to relay intercropping with wheat or faba bean? To what extent are changes in values of the parameters related to altered leaf thickness or leaf N? (Chapter 5)

1.7 Outline of the thesis

I investigate the eco-physiological responses of crop species to strip intercropping that are conducted under species-tailored N fertilization in the Netherlands. The intercropping effects are tested at multiple levels: i) field level- N uptake per unit area of species strip that comprising three rows of maize or six rows of wheat, faba bean, and pea; ii) plant level- plant morphological traits of maize, wheat, and faba bean in the two relay intercrops; and iii) leaf level- leaf photosynthetic traits of maize in relay intercrops with wheat or faba bean (Fig. 1.1).

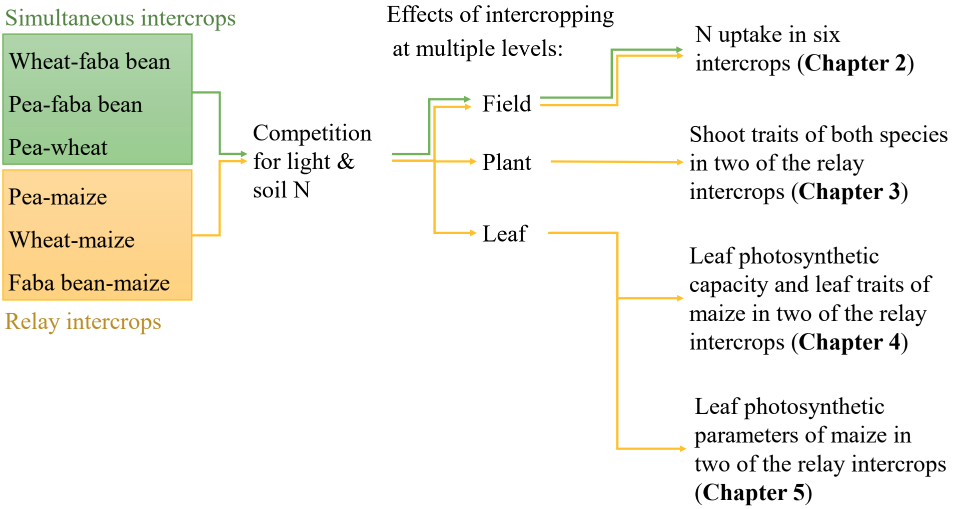

Fig. 1.1 Schematic representation of the relation between the topics reported in this thesis.

Chapter 2 investigates the effects of strip intercropping on N uptake when species are fertilized in accordance with recommendations for arable crop fertilization in the Netherlands for non-organic agriculture (Ministerie van Landbouw, Natuur en Voedselkwaliteit, 2019). I focus on six bi-specific combinations of four species (maize, wheat, faba bean, and pea) and their corresponding sole crops. The effects of intercropping are tested in different functional groups: i) relay intercrops involving maize vs. simultaneous intercrops without maize; ii) cereal/cereal vs. cereal/legume vs. legume/legume combinations.

Chapter 3 investigates shoot plasticity and light signals (R:FR and PAR) in relay strip intercropping in the Netherlands. Here I focus on border row plants in wheat-maize and faba bean-maize intercrop and plants in corresponding sole crops. Specifically, R:FR perceived in the radiation from the direction of neighbor species and from conspecific species in intercrops are distinguished.

Chapter 4 investigates the responses of maize leaf photosynthetic capacity (A_{1800}) (Box. 1.1) and the related leaf traits, i.e., SLA, leaf N concentration (LNC), and specific leaf N (SLN; N content per unit leaf area), in relay strip intercrops with wheat or faba bean. I compare light distribution within the maize canopy between intercrops and sole crops. I compare traits of maize leaves at different positions in the canopy and at different times in the season. I distinguish responses of maize leaf traits in border rows and inner rows in the intercrop strips.

Chapter 5 investigates the effects of relay strip intercropping on maize leaf photosynthetic parameters, i.e., $A_{\mathrm{g,max}}$, J_{atpmax}, V_{pmax}, and V_{cmax}. These parameters are compared in three cropping systems: wheat-maize intercrop, faba bean-maize intercrop, and maize sole crop. I distinguish the acclimation of maize leaf photosynthetic parameters in border rows and inner rows in the intercrop strips. The relationships between leaf photosynthetic parameters and leaf traits (LMA, LNC, and SLN) are studied to link the acclimation of leaf photosynthesis with competition for light and soil N.

In Chapter 6, I first integrate the results of the above chapters, comparing them with previous studies to elucidate how this work advances current understanding in intercropping. Secondly, I discuss the implications for understanding N uptake and plant trait responses in relation to the productivity of the studied intercrops. Finally, I derive suggestions for optimizing intercrop systems tailored to western-European growing conditions.

Box 1.1 Metrics used in Chapter 1

Land equivalent ratio for N uptake ($\mathbf{LER_N}$)

The land equivalent ratio for N uptake (LER_N) is used to assess intercropping advantages on N uptake as compared to corresponding sole crops (Bedoussac and Justes, 2010; Li et al., 2011a):

$$\mathrm{LER_N} = \frac{N_{\mathrm{I},1}}{N_{\mathrm{M},1}} + \frac{N_{\mathrm{I},2}}{N_{\mathrm{M},2}} \tag{1}$$

where $N_{\mathrm{I},i}$ is the N uptake of species i in an intercrop, expressed per unit of total area of the intercrop, $\mathrm{N}_{\mathrm{M},i}$ is the N uptake of species i in its sole crop, expressed per unit area of the sole crop.

An index for temporal niche differentiation (TND)

Temporal niche differentiation (TND) is calculated using the sowing and harvest dates of each species in the intercrop (Yu et al., 2015):

$$\mathrm{TND} = 1 - \frac{P_{\mathrm{overlap}}}{P_{\mathrm{system}}} \tag{2}$$

where P_{overlap} is the co-growth duration of the intercropped species, and P_{system} is the duration of the intercropping system, from the sowing of the first crop until the harvesting of the second crop.

A_{1800} (μmol $\mathbf{m^{-2}\ s^{-1}}$): light-saturated net photosynthetic rate of maize leaves, which is used to quantify leaf photosynthetic capacity in Chapter 4. Values of A_{1800} were obtained in the field using a LI-COR chamber that provided a constant irradiance of 1800 μmol m^{-2} s^{-1} and a constant CO_2 level of 400 μmol mol^{-1}.

Chapter 2

Temporal complementarity drives N uptake in crop species mixtures with or without a legume

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Abstract

Nitrogen (N) fertilization strategy in intercropping varies depending on the production purpose and production situation. There is limited information on N uptake when species receive tailored N fertilization in strip intercropping. Here we determined N uptake of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), faba bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) in six bi-specific intercrops and the corresponding monocrops when the species received locally recommended fertilizer rates for conventional agriculture. We also determined fertilizer N use efficiency of different intercrops compared to the respective monocrops, using fertilizer N equivalent ratio (FNER). In intercrops, the fertilizer was applied to the strip of the species at a rate (kg m^{-2}) equal to that in the monocrop. In relay intercrops involving maize, the early-sown wheat, faba bean, and pea had higher N uptake than the respective monocrops, especially in the border rows of the strips. N uptake in maize intercropped with wheat and pea was increased in the year with six weeks sowing delay compared to companion species but not in the other year, in which the sowing delay was only five weeks. Intercropping with faba bean did not result in increases in N uptake of the two cereals. Relay intercrops involving maize had land equivalent ratios for N uptake (LER_N) mostly higher than one, while simultaneous intercrops not involving maize had LER_N values mostly similar to one. Relay intercrops had FNER values mostly higher than one, while simultaneous intercrops had FNER values mostly similar to one. N uptake and fertilizer N use efficiency of intercrops increased with temporal complementarity. Relay systems had a higher N uptake than expected based on corresponding monocrops, largely because of higher N uptake by the early-sown species. Intercrops with faba bean did not result in increases in N uptake for either cereals or other legumes compared to monocrops. While inclusion of legumes was not required for achieving complementarity for N capture, inclusion of legumes does allow a reduction in N input. Relay intercrops achieved yield advantages with a moderate N fertilizer rate compared to monocrops, indicating that relay intercropping with recommended fertilizer N input is a pathway towards more sustainable N use in agriculture.

Keywords: Conventional agriculture, Tailored N fertilization, N uptake, Cereal-legume intercropping, Relay intercropping.

2.1 Introduction

Intercropping is the mixed cultivation of multiple crop species, growing on the same field and coexisting for a time (Willey, 1990). Intercropping is gaining adoption as an agricultural intensification strategy worldwide, aiming at achieving a balance between productivity and resource inputs (Bedoussac et al., 2015; Brooker et al., 2015). It has advantages in improved use efficiencies in land, light, water, and nutrients (Li et al., 2023; Stomph et al., 2020; Xu et al., 2020; Zhang et al., 2022).

Intercropping can be done using conventional inputs such as artificial nitrogen (N) fertilizer and biocides, or it can be done at low input levels or in organic agriculture. High land equivalent ratios have been found in both situations, but the yield levels are higher when higher inputs are used (Li et al., 2020b). Intercropping systems with conventional inputs are often strip intercrops, to allow tailored inputs to each species comprised in the mixtures. On the other hand, systems without such inputs are often within-row or alternate row mixtures, allowing management of the whole field with conventional machinery with a working width of at least 3 m. In Western Europe, ample experience has been gained with application of such within-row or alternate row mixtures under organic growing conditions (Bedoussac et al., 2015; Fischer et al., 2020; Hauggaard-Nielsen et al., 2009), but less experience has been gained in Western Europe with intercropping with inputs that are used by conventional farmers such as artificial N fertilizer and biocides. There is therefore a lack of knowledge on the potential of strip intercropping systems with conventional inputs to enhance productivity and resource use efficiency under Western-European conditions.

Complementarity in resource capture between component species that explore different niches is a cornerstone of overyielding in intercropping (Brooker et al., 2015; Homulle et al., 2022). For instance, most grain legumes need limited N from the soil because 60%-80% of their final plant N is derived from N fixation (Giller, 2001). Combining cereals with legumes in intercropping can decrease the competition for soil N experienced by the cereal, resulting in increased N uptake by cereals compared to their monocrops (Rodriguez et al., 2020). At the same time, legumes may not be affected much by the strong competition for soil N from the cereal because they can capture N from the atmosphere (Iannetta et al., 2016).

Another important complementarity in intercropping is complementarity in growing period (Yu et al., 2015). When two species are combined in a relay sequence, the earlier sown species experiences relaxed competition during its early growth, while the later sown species may experience relaxed competition during its later growth. This results in a potential for complementarity for all resources, including light, water, and nutrients (Gou et al., 2017a; Liu et al., 2020; Zhu et al., 2015). Such temporal complementarity is relevant particularly in regions with a growing season that is longer than the growth duration of the main crops, but shorter than the growth duration of two crops grown in a double cropping sequence. In such climate conditions, relay intercropping has potential to increase the efficiency of land use and the productivity per unit area substantially (Li et al., 2020b).

N is an essential element for plant development and growth, and is a main limiting nutrient for crop production (Leghari et al., 2016). The effects of intercropping on N uptake have been studied under various N input strategies for different production objectives (Jensen et al., 2020; Rodriguez et al., 2020; Xu et al., 2020). In regions such as China, where the primary aim is to maximize absolute production, N fertilizer is often applied at high rates to prevent N limitation (Li et al., 2020b). Under this strategy, N rates for intercrops are established either at a specific high dose, as the total N rate for the entire system (Li et al., 2011a; Xing et al., 2023), or determined as the weighted average of the respective N doses of the component species grown as monocrops, based on their land proportions in the intercrop (Gao et al., 2022). Another N fertilization strategy involves setting the intercrop N fertilization rate as the sum of those in the corresponding monocrops (Du et al., 2018; Feng et al., 2020; Yang et al., 2017). This approach is often used in additive design intercrops, ensuring a consistent N rate per plant, and maximizing intercropping yield advantages (Yang et al., 2017).

Increased N uptake per unit land area is found in these high-input intercrops on both species and system levels (Li et al., 2011a; Xing et al., 2023), due to various mechanisms leading to reduced interspecific N competition. For instance, reduced N competition can arise from temporal differences in growing periods, spatial differences in root distribution, functional differences in N acquisition, and complementary use of light and water, all of which stimulate enhanced N capture (Li et al., 2006; Liu et al., 2015, 2020). Moreover, fertilizer N was found to be used more efficiently in these intercrops, which is indicated by a fertilizer N equivalent ratio (FNER) greater than one (Li et al., 2020b; Xu et al., 2020). This is primarily due to the increased yield produced per unit land area, with an intercrop N fertilizer rate that falls between those used for monocrops (Xu et al., 2020). However, the application of large amounts of N fertilizer has drawbacks, particularly in cereal-legume intercropping. On the one hand, high N input can discourage N fixation of legumes (Coskun et al., 2017). On the other hand, cereals tend to become overly competitive, leading to a situation where the yield gain of cereals is offset by the yield loss they cause in legumes, resulting in no overall yield advantage for the entire intercrop (Yu et al., 2016). Finally, there is an increased possibility for leaching under this high N fertilization (Wang et al., 2022).

Western-European intercrops, which are typically cereal-legume combinations, have shown increased N uptake with low or zero N fertilization. In conditions of low soil mineral N, legume N fixation is exploited, allowing cereals to access more soil N than in their monocrops (Bedoussac et al., 2015; Bedoussac and Justes, 2010; Ghaley et al., 2005). These intercrops offer advantages such as increased land productivity, enhanced cereal seed protein content, and improved resilience to weeds and pests in comparison to equally fertilized monocrops (Bedoussac et al., 2015). They can also improve fertilizer N use efficiency compared to the corresponding monocrops under low inputs (Li et al., 2020b). Species in these intercrops are usually grown as full mixtures or alternating rows, allowing intimate rhizosphere interactions and easy mechanized management (Bedoussac et al., 2015). However, due to the limited N input, especially for cereal components, the absolute yield gain can be unstable and constrained (Hauggaard-Nielsen et al., 2008; Li et al., 2020b).

Besides the typical high-input and low-input intercrops, intercropping can also be practiced at moderate fertilization levels consistent with European standards for “Good Agricultural Practices” (Baghasa, 2008; FAO, 2003). In this case, species receive tailored N fertilization at moderate levels that align with their growth needs. As a result, at the system level, the rate of fertilizer N in the intercrop is intermediate between that in the two respective monocrops. The aim of this fertilization strategy is to minimize environmental costs while maintaining stable intercropping advantages. Yet, it is unclear whether intercropped species can benefit in their N uptake from intercropping under this species-tailored N strategy, and whether the entire intercropping system can achieve improved fertilizer use efficiency.

Tailoring fertilizer input to species in intercropping is facilitated by using a strip design, with species grown in alternating strips consisting of several plant rows. Such strip intercropping systems are widely used in China (Li et al., 2020b). The strips are usually between 1.5 and 3 m, which is wide enough to facilitate management while narrow enough to ensure interspecific interaction, especially at the border rows of adjacent species strips (van Oort et al., 2020). In a strip intercrop, the border rows of the species strips experience stronger interspecific interactions compared to the adjacent inner rows (Wang et al., 2020), which may result in different N uptake in plants from different rows within a species strip.

In this study, we aim to investigate the effects of strip intercropping on N uptake and fertilizer N use efficiency when species are fertilized in accordance with recommendations for conventional agriculture in the Netherlands. We aim to quantify the responses of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), faba bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) in six bi-specific intercrops, where strips are 1.5 m wide with three (maize) or six (wheat, faba bean and pea) rows per strip. Here the three C_3 species have the same sowing dates and similar harvest dates, while maize as a C_4 species is sown and harvested later than the C_3 species. The three relay intercrops (involving maize) and three simultaneous intercrops (not involving maize) exemplify differences in temporal complementarity. We study whether C_3 cereals benefit from growing with a legume in a simultaneous intercrop and compare this to growing a legume or cereal with maize in relay intercrops. We explore the responses of legumes in simultaneous intercrops with C_3 cereals and compare these responses to growing as early-sown species in relay intercrops, as well as growing the two legumes together. We study the responses of late-sown maize when relay intercropped with a cereal or a legume. Furthermore, we distinguish the responses of each species in border rows and inner rows in the intercrop strips. Finally, we determine fertilizer N use efficiency of all intercrops.

2.2 Materials and Methods

2.2.1 Site description

A two-year field experiment was conducted at Droevendaal Experimental Farm of Wageningen University & Research, Wageningen, the Netherlands (51° 59’ 20” N, 5° 39’ 16” E). Experiments were conducted in one field in 2018 and a nearby field in 2019. Both fields had a sandy soil with a pH of 5.7, containing 3.4% organic matter with a C/N ratio of 11 in the top 30 cm. The climate is temperate oceanic. Prior to the 2018 experiment, the field was cultivated with winter wheat (*Triticum aestivum* L.) in 2017, followed by a green manure crop mixture of bristle oat (*Avena strigosa* Schreb.) and fodder radish (*Raphanus sativus* L.). Prior to the 2019 experiment, the field was cultivated with sugar beet (*Beta vulgaris* L.), after which it remained an uncropped fallow during late autumn and winter.

2.2.2 Experimental design

Ten cropping systems were compared, including all six bi-specific intercrops of four crop species: maize (*Zea mays* L. cv. LG30.223), wheat (*Triticum aestivum* L. cv. Nobless), faba bean (*Vicia faba* L. cv. Fanfare), and pea (*Pisum sativum* L. cv. Astronaute) (Fig. 2.1), and their corresponding monocrops. The three intercrops with maize were relay intercrops, with maize being sown and harvested later than wheat, faba bean, or pea (Fig. 2.1). The other three intercrops were nearly simultaneous, with the same sowing dates and similar harvesting dates for the component species, and the latter based on species maturity (Fig. 2.1). Due to the cool and wet spring of 2019, the sowing dates of the four species were later than in 2018, with a larger delay for the early sown species leading to a reduced temporal complementarity (Table 2.1).

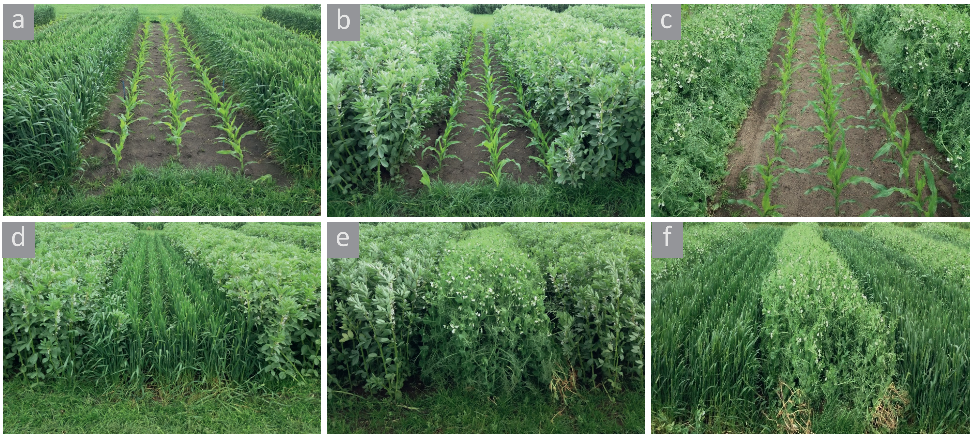
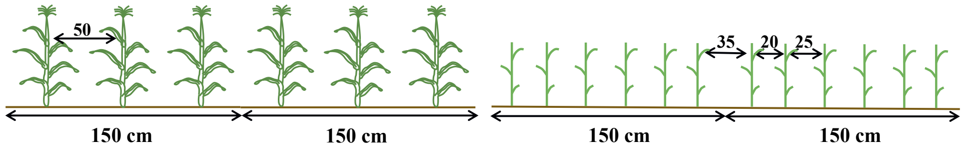
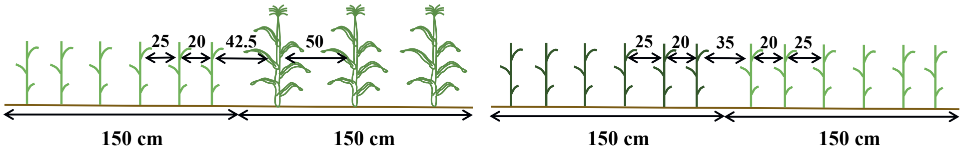


Fig. 2.1 Six intercrops on June 15, 2019 (Wang et al., 2023). Wheat-maize (a), faba bean-maize (b), and pea-maize (c) are relay intercrops, and wheat-faba bean (d), pea-faba bean (e), and pea-wheat (f) are simultaneous intercrops. Due to heavy rain in June, pea lodged to one side in the pea-maize intercrop (c).

Species strips were 1.5 m wide in all plots, comprising three rows of maize or six rows of wheat, faba bean, and pea (Fig. 2.2). A narrow sowing machine (Belt Cone Seeder, Haldrup Co., Germany) and a tractor with a track width of 133 cm (Fendt 207, Fendt Co., Germany) were used for sowing. The row distance of maize was 50 cm, and that of the other three species was 25 cm, except for the distance between the border rows and the neighboring inner rows within the strip, which was 20 cm to allow the wheels of the tractor to span the six rows. The sowing density was 10 seeds m^{-2} for maize, 44 seeds m^{-2} for faba bean, and 83 seeds m^{-2} for pea in both years. Wheat was sown at a density of 383 seeds m^{-2} in 2018 and 369 seeds m^{-2} in 2019. For each species, the sowing density was the same within species strips in both intercrops and monocrops. The intercrops followed a replacement design, and the relative density (density in the intercrop relative to the monocrop) of each species was 0.5.



(a) maize monocrop (b) wheat, faba bean, or pea monocrop



(c) wheat-maize, faba bean-maize, or pea-maize strip intercrop (d) wheat-faba bean, pea-faba bean, or pea-wheat strip intercrop

Fig. 2.2 Row configuration in the monocrops (a and b) and intercrops (c and d). Species were grown in 1.5 m-wide species strips. The row distance of maize was 50 cm. The row distance of wheat, faba bean, and pea was 25 cm, except for the border rows within each strip, which were adjusted inward by 5 cm to accommodate the tractor with a track width of 133 cm. In intercrops, each maize strip had two border rows (one at each side of the strip) while the middle one was the inner row. In each strip of wheat, faba bean, and pea, rows 1 and 6 were border rows, rows 2 and 5 were the inner row I, and rows 3 and 4 were the inner row II.

In 2018, the plot size was 9 m in east-west × 11 m in north-south direction, comprising six species strips. In 2019, the plot size was 12 m in east-west × 11 m in north-south direction for monocrops, comprising eight species strips. For intercrops, the plot size was 15 m in east-west × 11 m in north-south direction, comprising ten species strips. The row orientation was approximately north-south in both years. The experiment had a randomized complete block design with six blocks in 2018 and four blocks in 2019.

From June to August in both years, sprinkler irrigation was applied whenever the water storage in the top 25 cm soil approached 25 mm. Weeds were controlled manually and chemically. Diseases and pests were controlled chemically. Potassium (K) in the form of $K_2SO_4 \cdot MgSO_4$ and phosphorus (P) in the form of $Ca(H_2PO_4)_2 \cdot H_2O$ were applied homogeneously throughout the field before sowing wheat, faba bean, and pea. The rate of K was 87 kg K ha^{-1} in both years. The rate of P was 29 kg P ha^{-1} in 2018 and 34 kg P ha^{-1} in 2019.

In the top 30 cm soil, the residual mineral N was 22 kg N ha^{-1} in 2018 and 12 kg N ha^{-1} in 2019, and the N released from soil organic matter during the growing season was estimated at 90 kg N ha^{-1} in both years. Supplementary N was applied in the form of $NH_4NO_3 \cdot CaMg\,(CO_3)_2$, and in accordance with the recommendations for arable crop fertilization in the Netherlands for non-organic agriculture (Ministerie van Landbouw, Natuur en Voedselkwaliteit, 2019) (Table 2.1). In both years, faba bean and pea received 20 kg N ha^{-1} at emergence as a starter N fertilization. Wheat received 125 kg N ha^{-1} in both years, with the first dose (80 kg N ha^{-1}) at emergence and the second dose (45 kg N ha^{-1}) during stem elongation. Maize received 170 kg N ha^{-1} in both years, with the first dose (80 kg N ha^{-1}) at sowing and the second dose (90 kg N ha^{-1}) during stem elongation. All N fertilizer was applied as top dressing between rows within the species strips, ensuring that species strips in both monocrops and intercrops received the same amount of N fertilizer. The first dose of N fertilizer in maize was applied using a tractor driven machine (ENTI Co., the Netherlands) while the second dose was applied manually to avoid fertilizer from entering into the maize whorl.

Table 2.1 Sowing and harvesting date, application date and rate of top dressing fertilizer N.

Year	Species	Sowing	Harvesting	First top dressing N	Second top dressing N
2018	Pea	Mar 21	Jul 10	Apr 11, 20 kg ha^{-1}	-
	Faba bean	Mar 21	Jul 30	Apr 11, 20 kg ha^{-1}	-
	Wheat	Mar 21	Jul 17	Apr 11, 80 kg ha^{-1}	May 04, 45 kg ha^{-1}
	Maize	May 04	Sep 10	May 04, 80 kg ha^{-1}	Jun 11, 90 kg ha^{-1}
2019	Pea	Apr 01	Jul 29	Apr 15, 20 kg ha^{-1}	-
	Faba bean	Apr 01	Aug 14	Apr 15, 20 kg ha^{-1}	-
	Wheat	Apr 01	Aug 08	Apr 15, 80 kg ha^{-1}	May 06, 45 kg ha^{-1}
	Maize	May 07	Sep 18	May 06, 80 kg ha^{-1}	Jun 14, 90 kg ha^{-1}

2.2.3 Above-ground biomass and N concentration measurements

Measurements were conducted during the growing season and at the final harvest. In both years, the harvests were conducted within one species strip in each plot for each species. Specifically, the plants were harvested separately for each row within one strip. These measurements were made in both intercrops and monocrops.

In 2019, intermediate harvests were conducted five times in wheat, faba bean, and pea and four times in maize whereas in 2018, one intermediate harvest was made in wheat, faba bean, and pea and two were made in maize. In 2018, two meters row length was harvested for wheat, faba bean, and pea. Maize plants were harvested over a 4 m row segment in the first harvest, and a 3 m row segment in the second harvest. In 2019, for all species, 1.6 m of each row was harvested. All species were also harvested at maturity. Four meters row length was harvested, except for pea in 2018, for which 3 m row length was harvested.

In both intermediate and final harvests, plants were harvested and cut at ground level. Samples of intermediate harvests were analyzed without distinguishing organs, but plants were disassembled into organs at the final harvest. We measured biomass of the shoot samples from intermediate harvests and organ samples from the final harvest after drying at 105 °C for 48 h. N concentration of these samples was determined. The samples were dried at 70 °C for 48 h. After that, samples were ground by a laboratory mill (Peppink Mills 200 AN, Olst, the Netherlands) to coarse particles, and then were ground to a fine powder in a ball mill (Mixer Mill 200, Retsch). N concentration was determined using an element C/N analyser based on the Micro-Dumas combustion method, which was conducted at the School of Biosciences, University of Nottingham, UK.

2.2.4 Data analysis

We compared the above-ground biomass (Mg ha^{-1}), N uptake (kg ha^{-1}), and N concentration (mg g^{-1}) of each species in intercrops and monocrops. The above-ground N uptake was calculated as the product of shoot biomass and shoot N concentration. For intercrops, these calculations were made for each component species, by using total biomass per m^2 species strip and N concentration in the calculations, and also per row, by using the biomass per m row of a species in the intercrop. The latter enables analyzing differences in N uptake between border and inner rows. Comparisons were made at each harvest in each year.

Data were analyzed per species, using linear mixed effects models in which the treatment, or row position (border, inner I, inner II) was specified as a fixed effect and block was a random effect. The function lmer from the “lme4” package (Bates et al., 2015) in R (R Core Team, 2023) was used to fit the models. Multiple comparisons of means were conducted using Fisher’s Least Significant Difference (LSD) in the “emmeans” package (Lenth, 2021).

We assessed whether plant N concentration limited the growth of maize and wheat by comparing the N dilution curves estimated in our experiment to the critical N dilution curves reported in the literature (Plénet and Lemaire, 1999). This analysis was made using the 2019 data because of the greater number of intermediate harvests. The critical N dilution curve of maize is (Plénet and Lemaire, 1999):

If $B_{\mathrm{m}} < 1\ \mathrm{Mg\ ha^{-1}}, \%N_{\mathrm{c,m}} = 3.4$; If $1 \leq B_{\mathrm{m}} \leq 22\ \mathrm{Mg\ ha^{-1}}, \%N_{\mathrm{c,m}} = 3.4 \times {B_{\mathrm{m}}}^{-0.37}$ Eq. 2.1

where B_{m} is the maize above-ground biomass per unit area of the species strip in both monocrops and intercrops, and $\%N_{\mathrm{c,m}}$ is the critical N concentration in maize shoots.

We used the following equation to represent the critical N dilution curve for spring wheat (Ziadi et al., 2010):

If $B_{\mathrm{w}} < 1\ \mathrm{Mg\ ha^{-1}}, \%N_{\mathrm{c,w}} = 3.85$; If $B_{\mathrm{w}} \geq 1\ \mathrm{Mg\ ha^{-1}}, \%N_{\mathrm{c,w}} = 3.85 \times {B_{\mathrm{w}}}^{-0.57}$ Eq. 2.2

where B_{w} is the wheat above-ground biomass per unit area of the species strip in both monocrops and intercrops, and $\%N_{\mathrm{c,w}}$ is the critical N concentration in wheat shoots.

A power equation was used to fit the actual N dilution curves of maize and wheat in our experiment:

$\%N_i = a_i \times B_i^{-b_i}$ Eq. 2.3

where i is m for maize and w for wheat, a_i and b_i are two parameters, and $\%N_i$ and B_i are the above-ground N concentration and biomass per species strip, respectively. When fitting models, data for maize with biomass within the range of $1 \leq B_{\mathrm{m}} \leq 22\ \mathrm{Mg\ ha^{-1}}$, and data for wheat with biomass $B_{\mathrm{w}} \geq 1\ \mathrm{Mg\ ha^{-1}}$were used.

Interactions with companion species in intercrops may affect N uptake and the N dilution curve. To assess this, we estimated dilution curves for each treatment in which a species occurred, and determined by model selection which grouping of treatments best characterized treatment effects on N dilution. We fitted Eq. 2.3 for wheat and maize receptively using: (i) the data of each treatment, resulting in four fitted curves; (ii) the combined data from two of the four treatments, and separately fitted to the remaining two, resulting in three curves; (iii) the combined data from three of the four treatments, and separately fitted to the remaining one, resulting in two curves; (iv) the combined data of all the four treatments, resulting in one curve. Under each of model version (i), (ii), and (iii), we also tested the combination of the two parameters differing between groups of data. There were three cases for each of them: first, both parameters a_i and b_i differed between groups. Second, parameter a_i was different between groups while parameter b_i was the common value. Third, parameter b_i was different while parameter a_i was common.

The *Akaike Information Criterion* (Akaike, 1998) was used to select the best model, i.e., the model with the optimal balance between goodness of fit and number of parameters. We selected the model with the lowest AIC. Models with AIC values within 2 units of each other (ΔAIC < 2) were considered similar, in which case the simpler model was selected (Bolker, 2008). We calculated the AIC value of each model by obtaining maximum likelihood estimates of the parameters using the mle2's formula interface in the "bbmle" package (Bolker, 2022) of R version 4.3.1 (R Core Team, 2023). All variables were assumed normally distributed. Optimization algorithm "Nelder-Mead" was used when estimating parameters. Details showing the AIC values of all models are given in Supplementary Table S2.1.

The land equivalent ratio for N uptake ($\mathrm{LER_N}$) was used to assess intercropping advantages on N uptake as compared to monocrops (Bedoussac and Justes, 2010; Li et al., 2011a):

$$\mathrm{LER_N} = \frac{N_{\mathrm{I},1}}{N_{\mathrm{M},1}} + \frac{N_{\mathrm{I},2}}{N_{\mathrm{M},2}} = \mathrm{pLER_{N,1}} + \mathrm{pLER_{N,2}} \qquad \text{Eq. 2.4}$$

where $N_{\mathrm{I},i}$ is the N uptake of species i in an intercrop at final harvest, expressed at per unit of total area of the intercrop, $N_{\mathrm{M},i}$ is the N uptake of species i in its monocrop at final harvest, expressed at per unit area of the monocrop, and $\mathrm{pLER}_{\mathrm{N},i}$ is the partial land equivalent ratio for N uptake of species i. An $\mathrm{LER_N}$ greater than one indicates that the combined monocrops need more land than their intercrop for the same N uptake, so the intercrop uses land more effienctly for N uptake than the combined monocrops. In a replacement design, a $\mathrm{pLER}_{\mathrm{N},i}$ greater than the relative density of species i (0.5 for all species in our case) indicates that N uptake per plant in the intercrop of species i is greater than in its monocrop. To test if values differed from one for $\mathrm{LER_N}$ values and from 0.5 for $\mathrm{pLER_N}$ values, we assessed if one (or 0.5) fell outside the 95% confidence interval of $\mathrm{LER_N}$ (or $\mathrm{pLER_N}$). The 95% confidence interval was obtained using linear mixed effects models in which species combination was a fixed effect and block was a random effect. The analysis was conducted separately for each year.

Relationships between $\mathrm{LER_N}$ and temporal niche differentiation (TND; Yu et al., 2015) were estimated using linear mixed effects models. To do so, TND was calculated as:

$$\mathrm{TND} = 1 - \frac{P_{\mathrm{overlap}}}{P_{\mathrm{system}}} \qquad \text{Eq. 2.5}$$

where P_{overlap} is the co-growth duration of the intercropped species, and P_{system} is the duration of the intercropping system, from sowing of the first crop until the harvest of the second crop.

We tested whether the relationships differed between intercrops with maize in a relay design and intercrops without maize in a simultaneous design, between intercrops that were a cereal/legume combination and intercrops that were a cereal/cereal or a legume/legume combination, and between all different species combinations. Seven models were compared (Table 2.2), including the simplest model in which the relationship was the same for all intercrops. The R function anova was used to compare AIC values of all models. The AIC values are given in Supplementary Table S2.2.

Table 2.2 Models used to quantify the relationships between $\mathrm{LER_N}$ and TND. In the equations, i, j, and k represent year ID, species combination ID, and block ID, respectively. In all equations, b_{ik} is the block effect nested in year, and ε_{ijk} is the residual random error. Meaning of covariables: Relay: an intercrop was a relay system or not; Cereal.Legume: an intercrop was a cereal/legume combination or not; Comb.: all six species combinations as levels.

Model	Equations
1	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + b_{ik} + \varepsilon_{ijk}$
2	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Relay}_{ijk} + b_{ik} + \varepsilon_{ijk}$
3	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Cereal.Legume}_{ijk} + b_{ik} + \varepsilon_{ijk}$
4	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Relay}_{ijk} + \beta_3 * \mathrm{TND}_{ijk} * \mathrm{Relay}_{ijk} + b_{ik} + \varepsilon_{ijk}$
5	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Cereal.Legume}_{ijk} + \beta_3 * \mathrm{TND}_{ijk} * \mathrm{Cereal.Legume}_{ijk} + b_{ik} + \varepsilon_{ijk}$
6	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Comb.}_{ijk} + b_{ik} + \varepsilon_{ijk}$
7	$\mathrm{LER}_{ijk} = \beta_0 + \beta_1 * \mathrm{TND}_{ijk} + \beta_2 * \mathrm{Comb.}_{ijk} + \beta_3 * \mathrm{TND}_{ijk} * \mathrm{Comb.}_{ijk} + b_{ik} + \varepsilon_{ijk}$

The fertilizer N equivelant ratio (FNER) was used to assess intercropping advantages on saving fertilizer N inputs for grain yields as compared to monocrops (Xu et al., 2020):

$$\mathrm{FNER} = \left(\frac{Y_{\mathrm{I},1}}{Y_{\mathrm{M},1}} \times \frac{F_{\mathrm{M},1}}{F_{\mathrm{IC}}}\right) + \left(\frac{Y_{\mathrm{I},2}}{Y_{\mathrm{M},2}} \times \frac{F_{\mathrm{M},2}}{F_{\mathrm{IC}}}\right) \qquad \text{Eq. 2.6}$$

where $Y_{\mathrm{I},i}$ is the grain yield (per unit of total area of an intercrop) of species i in the intercrop, $Y_{\mathrm{M},i}$ is the grain yield (per unit area of respective monocrop) of species i in the monocrop, $F_{\mathrm{M},i}$ is the rates of fertilizer N applied per unit area of the monocrop of species i, and F_{IC} is the fertilizer N input per unit area of the intercrop. The results of grain yield have been presented in Wang et al. (2023).

In this study, all species received tailored fertilizer N applied within their species strips in both intercrops and monocrops. As the intercrop comprised 50% of the land area for component species, F_{IC} of an intercrop was therefore calculated as the average of fertilizer inputs in the respective monocrops. An FNER greater than one indicates that the combined monocrops need more fertilizer N inputs to obtain the same grain yield as the intercrop. To assess if value of FNER was significantly different from one, linear mixed effects models were used to calculate 95% confidence interval of FNER. This analysis was conducted within each year, with species combination as a fixed effect and block as a random effect.

2.3 Results

2.3.1 Above-ground N uptake per species strip at final harvest

Maize intercropped with wheat or pea had higher N uptake than its monocrop in 2018 but no differences were found in 2019 or between maize intercropped with faba bean and the monocropped maize in 2018 (Fig. 2.3 i). Wheat and faba bean had higher N uptake than in their monocrops if they were intercropped with maize; the same was found for pea in 2018, but not in 2019 (Fig. 2.3 ii-iv). In the simultaneous intercrops, faba bean had increased N uptake when intercropped with pea in 2018 (Fig. 2.3 iii), and pea that was intercropped with faba bean had in both years lower N uptake than monocropped pea (Fig. 2.3 iv). No other effects of simultaneous intercropping on N uptake were found.

The N uptake by monocropped maize and intercropped maize was in the order of 250 to 300 kg ha^{-1}, highly similar to the available soil N (solid line in Fig. 2.3 i), while N uptake by wheat in the simultaneous intercrops (about 50 kg N ha^{-1}) was substantially lower than the available quantity of soil N, except in the relay intercrop with maize (Fig. 2.3 ii). N uptake by faba bean was even greater than that of maize in all intercrop combinations, and far exceeded the available soil N, indicating substantial biological fixation, of about 250 kg ha^{-1}. N uptake by pea exceeded the soil available N by about 150 to 250 kg ha^{-1} with the lowest N uptake in the intercrop with faba bean.

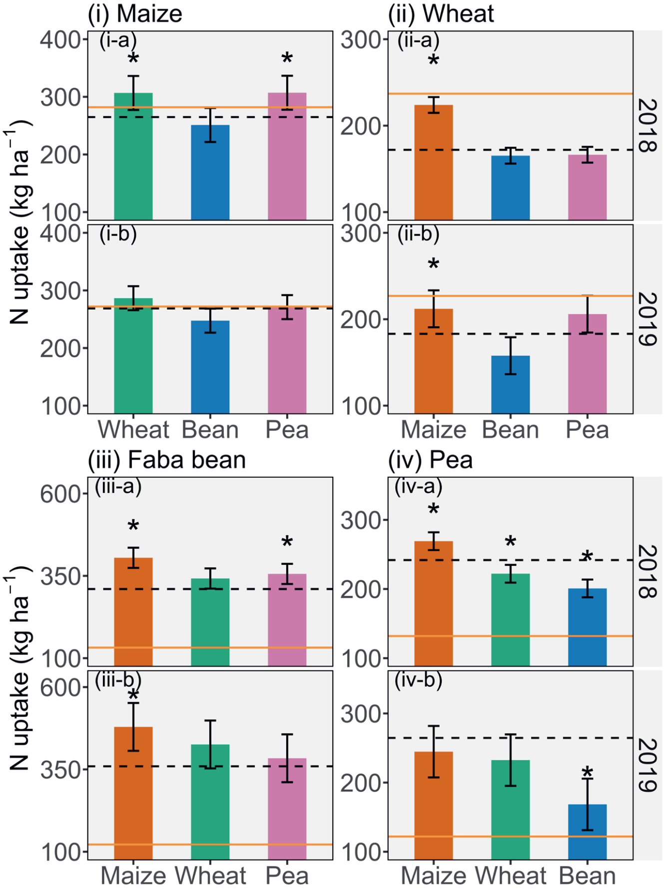


Fig. 2.3 Above-ground N uptake per species strip at final harvest of maize (i), wheat (ii), faba bean (iii), and pea (iv) when grown with different companion species in 2018 (a) and 2019 (b). The X-axis represents the companion species in intercrops. Solid lines represent the total estimated available soil N in species strips, comprising residual mineral N in the top 30 cm soil, N released from soil organic matter during the growing season, and fertilizer N. Black dashed lines represent N uptake of the monocrops of the focal species in each panel. Colored bars represent N uptake of the focal species when it was with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (pink). Multiple comparisons of means were conducted within each year. Asterisks indicate significant differences between the intercrops and the monocrops at $P < 0.05$ according to Fisher's Least Significant Difference (LSD) test. Error bars indicate the 95% confidence intervals. Details showing the multiple comparisons are presented in Supplementary Table S2.3.

2.3.2 Above-ground N uptake of different rows in intercrops at final harvest

The effects of intercropping on different rows within species strips were consistent with those observed at the species strip level (Fig. 2.4). N uptake was higher in the border rows of wheat, faba bean, and pea in relay intercrops than in the neighboring inner rows and in the corresponding monocrops (Fig. 2.4 ii-iv). Border row maize in the intercrop with faba bean had decreased N uptake compared with the adjacent inner row maize and monocrop maize in 2019 (Fig. 2.4 i-b). Border row faba bean had increased N uptake in all intercrops compared with its monocrop (Fig. 2.4 iii).

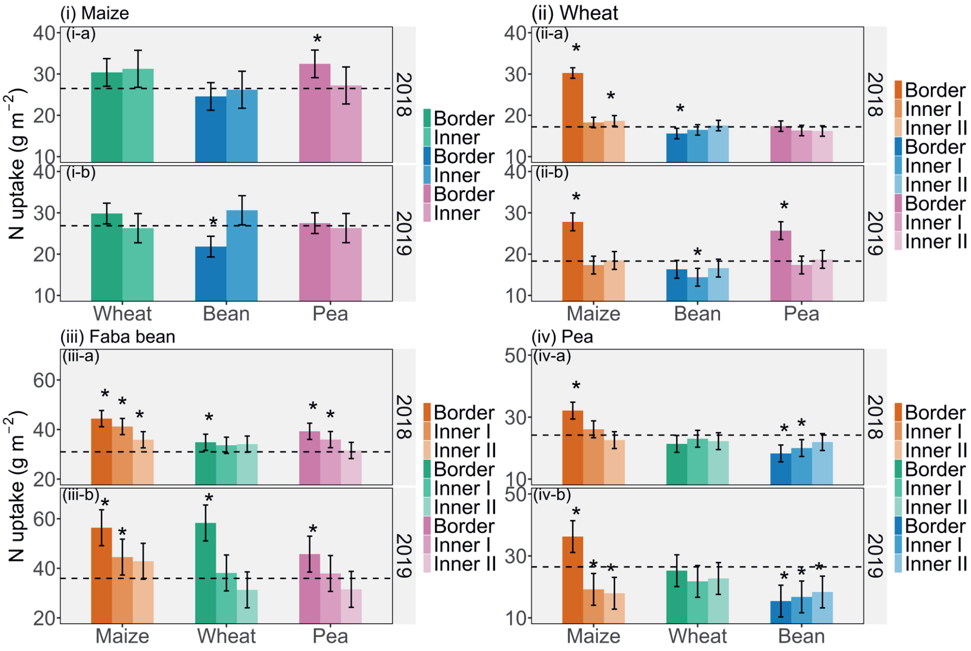


Fig. 2.4 Above-ground N uptake of different rows of intercropped maize (i), wheat (ii), faba bean (iii), and pea (iv) in 2018 (a) and 2019 (b). Companion species are indicated along the X-axis. Dashed lines indicate N uptake per row of the monocrops of the focal species in each panel. Colored bars represent N uptake of the focal species when it was with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (pink). Each color intensity represents a different row. Multiple comparisons of means were conducted within each year with "Species Combination_ Row" as the fixed effect. Asterisks indicate significant differences between the intercrop rows and the monocrops at $P < 0.05$ according to Fisher's LSD test. Error bars indicate the 95% confidence intervals. Details showing the multiple comparisons are presented in Supplementary Table S2.4.

2.3.3 Above-ground N concentration per species strip at final harvest

In relay intercrops, the early-sown species had higher or similar N concentration compared with corresponding monocrops (Fig. 2.5 ii-iv). Intercropped maize had consistently lower N concentration than monocropped maize, though the difference was not significant for the bean-maize intercrop in 2019 (*P* = 0.225) (Fig. 2.5 i). Wheat intercropped with faba bean had significantly higher N concentration than its monocrop in both years and also when intercropped with maize in 2019 (Fig. 2.5 ii). N concentration of intercropped faba bean and pea was similar among treatments and similar to that in the monocrops (Fig. 2.5 iii, iv).

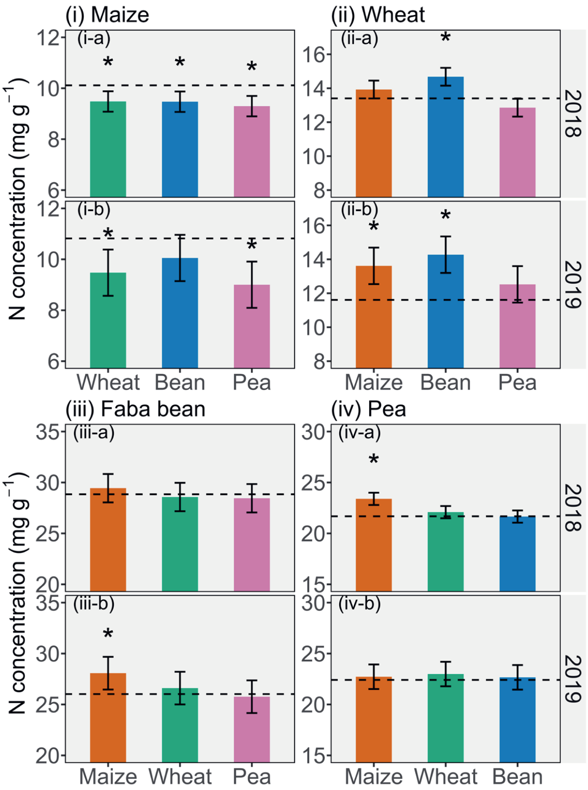


Fig. 2.5 Above-ground N concentration per species strip at final harvest for maize (i), wheat (ii), faba bean (iii), and pea (iv) in 2018 (a) and 2019 (b). Companion species are indicated along the X-axis. Dashed lines indicate N concentration of the monocrops of the focal species in each panel. Colored bars represent N concentration of the focal species when it was with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (pink). Multiple comparisons of means were conducted within each year. Asterisks indicate significant differences between the intercrops and the monocrops at $P < 0.05$ according to Fisher's LSD test. Error bars indicate the 95% confidence intervals. Details showing the multiple comparisons are presented in Supplementary Table S2.5.

2.3.4 Dynamics of above-ground biomass and N uptake per species strip

Measurements were made at more times in 2019 than in 2018. The 2019 data are presented in Fig. 2.6 first. The 2018 data are given in Table 2.3 thereafter to evaluate consistency of effects between seasons.

Relay intercrops

Maize. In both years, when maize and the early-sown species were grown together as intercrop, the intercropped maize had lower biomass and N uptake than its monocrop (Fig. 2.6 i; Table 2.3). The negative effect was largest for maize intercropped with faba bean, both for biomass and N uptake. At maize maturity, maize in all intercrops had recovered to some extent in both biomass and N uptake. The recovery was substantial for maize biomass in the intercrops with wheat and pea in both years, as well as for maize N uptake in the intercrops with wheat and pea in 2018.

Wheat, faba bean, and pea. In both years from flowering onwards, wheat had higher N uptake in relay intercrops with maize than in its monocrop (approx. DOY 196 in 2019 and DOY 155 in 2018) (Fig. 2.6 ii; Table 2.3). Intercropped wheat had a higher biomass than monocropped wheat at maturity in 2018 (Table 2.3). A consistent pattern was observed for faba bean in relay intercrops with maize in both years; from flowering onwards the intercropped bean had higher biomass and N uptake than the monocropped bean (approx. DOY 168 in 2019 and DOY 155 in 2018) (Fig. 2.6 iii; Table 2.3). In both years, pea intercropped with maize had higher biomass and N uptake than the monocropped pea at flowering only (approx. DOY 168 in 2019 and DOY 155 in 2018) (Fig. 2.6 iv; Table 2.3). Thus, the early-sown species in relay intercrops had increases in biomass and N uptake during the intermediate stages of growth.

Simultaneous intercrops

Wheat-pea and wheat-faba bean intercrops. Wheat intercropped with pea had similar biomass and N uptake as its monocrop (Fig. 2.6 ii; Table 2.3). Wheat intercropped with faba bean had lower biomass than the monocropped wheat from flowering onwards (approx. DOY 182 in 2019 and DOY 155 in 2018), while N uptake by the intercropped wheat was not substantially decreased (Fig. 2.6 ii; Table 2.3). Intercropped pea had lower biomass and N uptake than the monocropped pea at maturity in 2018 (Table 2.3). As for the faba bean intercropped with wheat, no differences in biomass and N uptake compared with monocropped faba bean were found (Fig. 2.6 iii; Table 2.3).

Pea-faba bean intercrop. Around flowering the intercropped faba bean had increased biomass and N uptake compared to its monocrop (approx. DOY 182 in 2019 and DOY 155 in 2018) (Fig. 2.6 iii; Table 2.3), and these increased values were also observed at maturity in 2018 (Table 2.3). Simultaneously, intercropping with faba bean led to decreased biomass and N uptake of pea after flowering in 2019 (approx. DOY 182) (Fig. 2.6 iv), as well as at maturity in both years (Fig. 2.6 iv; Table 2.3).

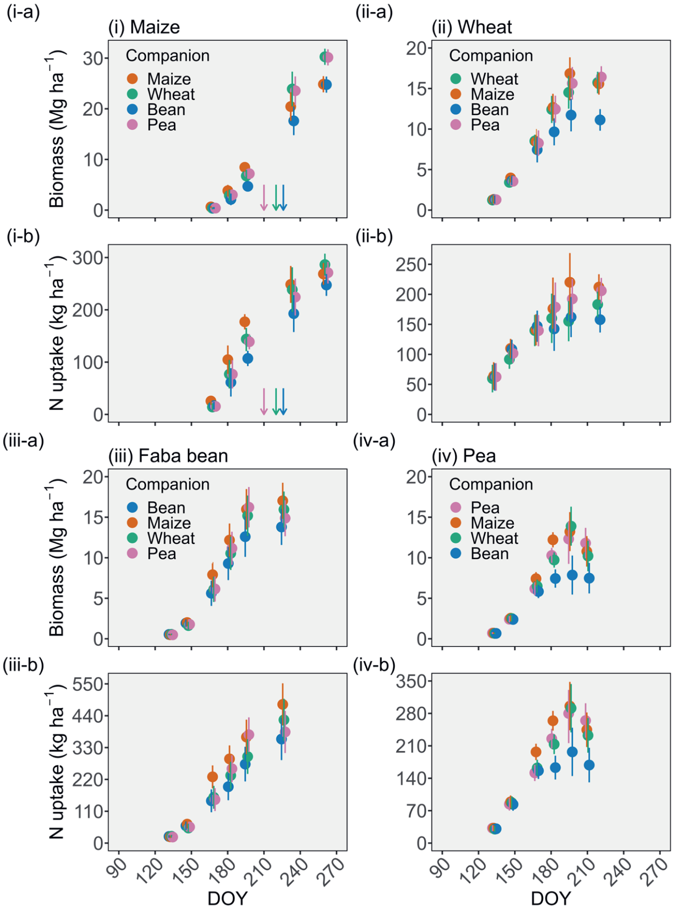


Fig. 2.6 Dynamics of above-ground biomass (a) and N uptake (b) per species strip for maize (i), wheat (ii), faba bean (iii), and pea (iv) in 2019. Colors within each panel represent biomass and N uptake of the focal species when it was with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (pink). Arrows in panel (i-a) and (i-b) indicate the final harvest of pea (pink), wheat (green), and faba bean (blue). Multiple comparisons of means were conducted within an individual harvest. Error bars indicate the 95% confidence intervals. Details showing the multiple comparisons are presented in Supplementary Table S2.6.

Table 2.3 Above-ground biomass and N uptake per species strip of maize, wheat, faba bean, and pea in 2018. Multiple comparisons of means were conducted within an individual harvest. Different letters denote significant differences at $P < 0.05$ according to Fisher's LSD test.

Species	Companion	Above-ground biomass (Mg ha^{-1})			N uptake (kg ha^{-1})		
		DOY 155	DOY 197	Final Harvest	DOY 155	DOY 197	Final Harvest
Maize	Maize	0.825 a	7.98 a	26.3 b	34.7 a	140 a	265 b
	Wheat	0.571 a	7.15 a	32.4 a	23.8 a	104 a	307 a
	Bean	0.541 a	5.51 a	26.5 b	23.6 a	88.5 a	251 b
	Pea	0.472 a	6.99 a	33.1 a	19.9 a	105 a	307 a
Wheat	Wheat	7.04 ab	-	12.8 b	151 b	-	172 b
	Maize	8.06 a	-	16.1 a	185 a	-	224 a
	Bean	6.22 b	-	11.3 c	139 b	-	165 b
	Pea	7.28 ab	-	12.9 b	154 ab	-	166 b
Faba bean	Bean	4.29 a	-	10.7 c	121 a	-	310 c
	Maize	5.26 a	-	13.8 a	150 a	-	405 a
	Wheat	3.79 a	-	12.0 bc	102 a	-	342 bc
	Pea	4.90 a	-	12.5 ab	135 a	-	356 b
Pea	Pea	4.50 b	-	11.1 a	118 b	-	242 b
	Maize	5.40 a	-	11.5 a	146 a	-	269 a
	Wheat	4.77 b	-	10.1 b	124 b	-	222 c
	Bean	4.70 b	-	9.29 c	123 b	-	201 d

2.3.5 N dilution curves of maize and wheat

Maize

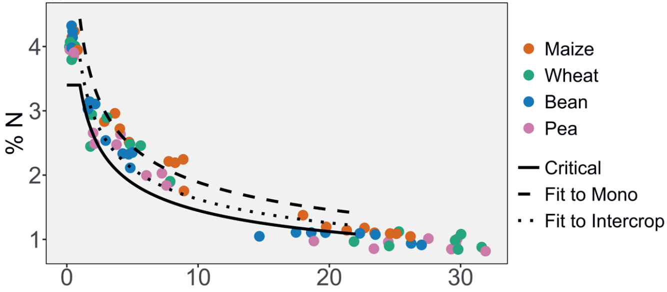
We compared the N dilution curves estimated from our experiment with the maize critical N dilution curve (Fig. 2.7 i). The above-ground N concentration decreased with biomass, following a slope (determined by the parameter b_m) (Eq. 2.3) similar to the published critical dilution curve (Eq. 2.1). We observed a higher value for the intercept parameter a_m (Eq. 2.3), resulting in an increase in N concentration in our experiment compared to the critical N concentration at the same biomass level. The results indicate that the plant N was sufficient during maize early growth in both intercrops and monocrops. However, we observed a lower N concentration of maize in all intercrops than in the monocrop at the same biomass level.

Later during the growth process, at grain filling and with biomass values above approx. 20 Mg ha^{-1}, the measured N concentration of intercropped maize tended to be below the critical curve (Fig. 2.7 i), indicating that plant N likely reduced productive capacity in maize in all intercrops during the later phase.

Wheat

We compared N dilution curves estimated from our experiment with the wheat critical N dilution curve (Fig. 2.7 ii). We observed higher values of above-ground N concentration in both intercrops and monocrops compared to the critical N concentration at the same biomass level. When comparing N dilution curves between wheat in intercrops and monocrops, a higher N concentration in intercropped than monocropped wheat at the same biomass level was observed. This phenomenon was particularly observed after wheat produced approx. 8 Mg ha^{-1} biomass at the heading stage. Thus, the plant N was sufficient for wheat in both intercrops and monocrops during the whole season, and in the later growth stages wheat in all intercrops had a higher N concentration than in monocrop at per the same biomass.

(i) Maize



(ii) Wheat

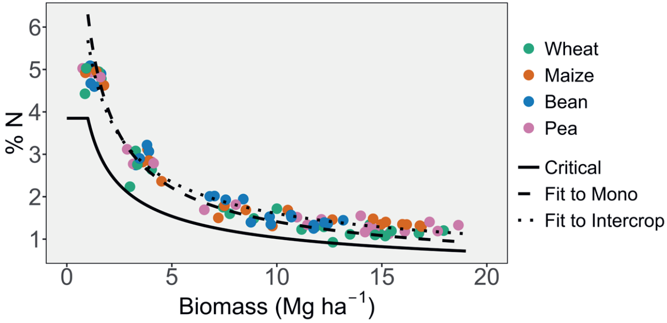


Fig. 2.7 Measured (points) and modelled (curves) N dilution for maize (i) and wheat (ii). The X-axis is the above-ground biomass per species strip. The %N is the above-ground N concentration per species strip. Colored points represent measured %N and biomass of the focal species when it was grown with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (pink). The solid line in each panel is the critical N dilution curve (Eq. 2.1 for maize, Eq. 2.2 for wheat). The dashed (monocrop) and dotted (intercrop) lines are the estimated N dilution curves (Eq. 2.3) for each species. For maize (i), parameter a_m was higher for monocrop maize than for maize in all intercrop treatments, parameter b_m was similar. Thus, two curves were drawn. Also for wheat (ii), the curves differed between monocrop wheat and wheat in all intercrops. Both parameters a_m and b_m are higher for the monocrop than for the intercrops. For parameter values and their standard errors see Supplementary Table S2.1.

2.3.6 LER$_N$ and its relationship with TND

In 2018, all relay intercrops obtained LER$_N$ values larger than one, ranging from 1.14 ± 0.09 (mean ± 95% confidence interval) in bean-maize to 1.24 ± 0.09 in wheat-maize (Fig. 2.8). In 2019, both bean-maize (1.13 ± 0.11) and wheat-maize (1.12 ± 0.11) achieved LER$_N$ values larger than one. In relay intercropping systems, cereal/legume combinations did not obtain a larger LER$_N$ value than the cereal/cereal combinations. In bean-maize intercrop, the partial LER$_N$ value of faba bean contributed more to the system LER$_N$ value than that of maize. As for simultaneous intercrops, LER$_N$ values mostly were not different from one, ranging from 0.94 ± 0.09 in pea-wheat to 1.04 ± 0.09 in wheat-bean in 2018, and from 0.86 ± 0.11 in pea-bean to 1.03 ± 0.11 in wheat-bean in 2019 (Fig. 2.8).

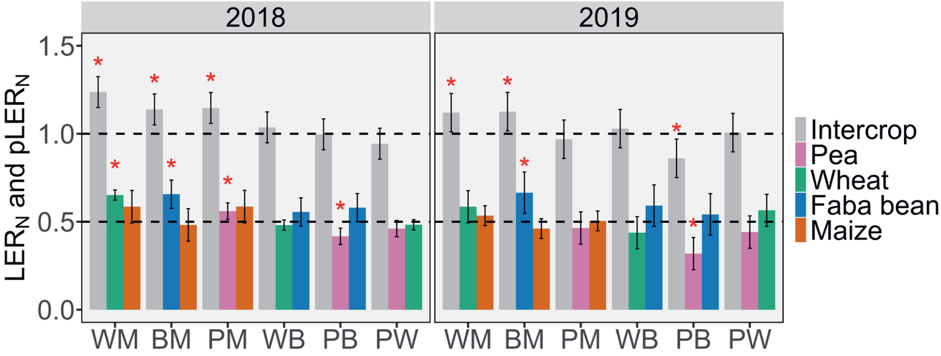


Fig. 2.8 Land equivalent ratio for N uptake (LER$_N$) of intercrops (grey bar) and partial land equivalent ratio for N uptake (pLER$_N$) of component species (colored bar) in 2018 and 2019. X-axis is the abbreviaton for the intercrops, in which P for pea, W for wheat, B for faba bean, and M for maize. Colors represent four component species in intercrops: pea (pink), wheat (green), faba bean (blue), and maize (orange). Error bars indicate the 95% confidence intervals. Asterisks indicate significant differences from one for LER$_N$ values and from 0.5 for pLER$_N$ values, by testing if one (or 0.5) fell outside the 95% confidence interval of LER$_N$ (or pLER$_N$).

A positive correlation between LER$_N$ and TND was observed (Fig. 2.9). The most supported model was Model 6 (Table 2.2), with a common slope across intercrops, with species combination specific intercepts. This indicates that the effect of TND on LER$_N$ was consistent across species combinations, regardless of whether the intercrop systems were relay or simultaneous designs, or whether they involved a cereal/legume combination or not. Thus, both species combination and TND were key factors driving intercropping advantages in N uptake in our experiment.

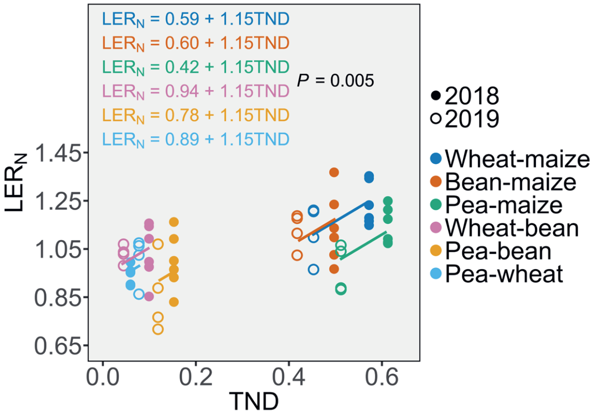


Fig. 2.9 Relationships between LER_N and TND. After comparing AIC values, Model 6 was the most supported model to describe the data. This model indicates that regressions had a common slope and different intercepts across intercrops. The *P*-value is related to the slope (Student's t-test).

2.3.7 Fertilizer N equivalent ratio (FNER)

FNER ranged from 0.76 ± 0.05 (wheat-faba bean) to 1.24 ± 0.07 (pea-maize) in 2018, while it ranged from 0.64 ± 0.06 (wheat-faba bean) to 1.13 ± 0.03 (pea-maize) in 2019 (Fig. 2.10). Despite the inter-year variances within the same treatment, relay intercrops, on average, were more efficient in using fertilizer N compared to simultaneous intercrops. The average FNER over two years was 1.14 ± 0.03 for relay intercrops, whereas it was 0.85 ± 0.03 for simultaneous intercrops.

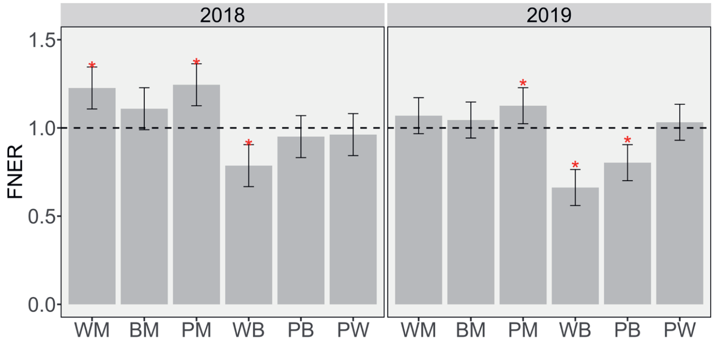


Fig. 2.10 Fertilizer nitrogen equivalent ratio (FNER) of intercrops in 2018 and 2019. X-axis is the abbreviation for the intercrops, in which WM for wheat-maize, BM for faba bean-maize, PM for pea-maize, WB for wheat-faba bean, PB for pea-faba bean, and PW for pea-wheat. Error bars indicate the 95% confidence intervals. Asterisks indicate significant differences from one for FNER, by testing if one fell outside the 95% confidence interval of FNER.

2.4 Discussion

Here we found that C_3 cereals had increased N uptake when grown as an early-sown species in relay intercrops with maize, as compared to monocropped cereals, but they did not have increased uptake when grown with a legume in simultaneous intercrops (Fig. 2.3). Legumes, i.e., faba bean and pea, had higher N uptake in intercrops than in monocrops when grown with maize in relay intercrops but not when grown simultaneously with wheat. N uptake by maize was increased only when relay intercropped with wheat or pea in a year with a larger temporal niche differentiation. Despite inter-year differences, the relay intercrops had land equivalent ratios for N uptake (LER_N) and fertilizer N equivalent ratios (FNER) mostly higher than one, while simultaneous intercrops had LER_N and FNER values mostly similar to one. The results thus show that the relay intercrops were more efficient in capturing N and using fertilizer N than simultaneous intercrops. These results suggest that temporal complementarity between species is a key driver for complementary N uptake and efficient fertilizer N use under the conditions of the study, while complementarity in N uptake mechanisms in relation to the capacity for biological N fixation was not.

Temporal complementarity is known to be important for increasing resource capture and promoting production in intercropping because it allows the component species to grow alone for a period, reducing competition compared to monocrops (Yu et al., 2015). Thus, species grow better during the “grow alone” period when the companion species is absent due to reduced competition for all growth resources, including primarily light, but also water and nutrients. Temporal complementarity drives increased land productivity, absolute yield gain, and utilization of water and nutrients (Li et al., 2020b; Ma et al., 2022; Raza et al., 2020; Wang et al., 2023). In the present study, we found that temporal complementarity is also enhancing N uptake in strip intercropping under a species-tailored N strategy (Fig. 2.9).

A “win-win” situation has been reported in relay intercrops, in which both early- and late-sown species achieved increased biomass and N uptake compared to the monocrops (Li et al., 2001a, 2001b; Xu et al., 2023). In relay intercrops, the early-sown species has better light conditions during early growth than the same species grown as a monocrop (Gou et al., 2017a; Zhu et al., 2015), which could stimulate their biomass accumulation (Fig. 2.6 ii-iv; Table 2.3). Biological N fixation of legumes is known to be positively affected by light capture and above-ground biomass (Carvalho et al., 2019). The higher N uptake of the two legumes, especially faba bean, that we found in the relay intercrops could be due to the better light conditions and increased biomass growth, which is likely to increase root growth and nutrient demand. Intercropped wheat had higher %N than monocrop wheat at per the same biomass (Fig. 2.7 ii), indicating that the relative increase in N uptake due to intercropping was greater than the relative biomass increase. This may be due to better access to soil N. Given that each species had identical N fertilization within its species strips in intercrops and monocrops, it is likely that wheat foraged for N from the neighboring maize strips via lateral root extensions, as previously reported (Li et al., 2006; Liu et al., 2015).

Previous studies on relay intercrops, conducted under high N fertilization strategies in which both intercrops and monocrops received the same rate of fertilizer N (N > 225 kg ha^{-1}) at the system level, indicated that N uptake of late-sown maize was higher than that of monocrop maize at maturity. This research includes both maize relay intercropped with wheat or soybean (Li et al., 2001a, 2001b) and maize grown with faba bean (Li et al., 2011a). In our trials, the higher N uptake of maize was only observed in the relay intercrop with wheat and pea in one year, but not with faba bean (Fig. 2.3). We applied a moderate N rate for maize, with the second topdressing applied at the onset of maize stem elongation. Consequently, the mineral N was largely taken up before maize entered grain filling, reflected by the %N that gradually dropped below the critical level from grain filling in both intercrops and monocrops (Fig. 2.7 i). Our N application strategy did not result in significantly increased N uptake by maize, possibly due to low N availability during maize reproductive growth.

The simultaneous intercrops involving faba bean were relatively inefficient in capturing N because the increased N uptake of faba bean did not compensate for the N uptake loss in the accompanying species (Fig. 2.8). The tall-statured cultivar “Fanfare” of faba bean has a rapidly developing and dense canopy (Andersen et al., 2020), which severely shaded wheat and pea (Wang et al., 2023), resulting in substantial biomass decreases in these companion species (Fig. 2.6 ii and iv; Table 2.3). In contrast to previous studies in which cereals were mostly the taller species in cereal/legume intercrops (e.g., maize-soybean intercrop in Liu et al. (2017, 2018)), wheat in our experiment experienced shading when grown with faba bean (Wang et al., 2023). Nevertheless, the wheat intercropped with faba bean captured sufficient soil N in comparison to its biomass production (Fig. 2.7 ii), indicating that the reduced N uptake was due to decreased biomass caused by light competition, rather than N competition. Maize also experienced substantial reductions in biomass and N uptake in the early stage in the intercrop with faba bean (Fig. 2.6 i). We therefore conclude that combining a cereal with a vigorous legume decreases N uptake by the cereal as a consequence of reduced crop need related to lower biomass accumulation, either in simultaneous or relay systems.

We did not detect N limitations in wheat throughout the entire season in 2019 in any of the treatments (Fig. 2.7 ii). Maize in intercrops or monocrop did not experience N limitation until maize grain filling began (Fig. 2.7 i). The intercropped legumes maintained %N at levels no lower than their monocrops (Fig. S2.1). Given the same N and water application strategy, identical soil conditions, and similar weather conditions, we can infer that N availability should have been similar in 2018 as it was in 2019 for each species. Therefore, we conclude that the species-tailored N strategy ensured non-limiting N conditions for both cereals for most of the season in both years.

Relay intercrops showed generally substantial N capture complementarity, as evidenced by value of LER_N greater than one (with one exception; pea-maize in 2019), however the combination of a cereal and legume was not a key factor driving this complementarity as strong complementarity (high value of LER_N) was found in an intercrop comprising two cereals, wheat and maize. The LER_N of this combination was similar to that of faba bean-maize and higher than that of pea-maize. The lack of response to presence of a legume does seem to be a direct consequence of the N fertilization strategy satisfying needs of combined species. In previous studies, the mineral N applied to legumes in high-input intercrops (Li et al., 2001a, 2001b), or the stored soil N in low-input intercrops (Bedoussac et al., 2015; Rodriguez et al., 2020), largely acted as additional N for cereals because of N fixation of legumes. However, in the current study, when cereals can be satisfied by the N fertilizer applied within their own strips, and rhizosphere interactions, especially at the early stages, were limited by the strip design, N fixation by legumes contributed little, if anything, to the N uptake of the cereals. The main contribution to complementary N uptake in our systems was likely the temporal complementarity, which caused increased biomass growth, which in turn may have driven increased root proliferation and N uptake in relay-intercropped species, compared to the monocrops.

The two cereals captured almost all available mineral N in relay intercrops, and the small quantity of "starter" N fertilizer also required the two legumes to thoroughly exploit N fixation, as reflected by their final N uptake that far exceeded the quantity that was applied (Fig. 2.3). Compared with a high-input N strategy where N surplus, N leaching, and inhibition of legume N fixation have been observed (Wang et al., 2022; Yu et al., 2021), we suggest that a species-tailored N strategy is good for intercropping to reduce environmental costs and make full use of N fixation. Moreover, the species-tailored N strategy does also allow for a higher cereal production compared with the low or unstable yield and N uptake found in low-input intercrops (Hauggaard-Nielsen et al., 2008; Li et al., 2020b; Wang et al., 2023).

Despite the finding that N fixation by legumes was not a key driver of complementary N uptake, combining legumes with maize in relay intercropping still allowed a reduction in N inputs compared with the wheat-maize intercrop (Fig. 2.8). In 2019, pea lodged due to heavy rains, and this lodging was more severe in the pea-maize intercrop than in the pea monocrop because the neighboring young maize plants failed to support the lodging pea as other species did (Fig. 2.1). Thus, combining maize with a firm-stem species in relay strip intercropping is a recommended practice to ensure N uptake advantages. Species with a weak stem are more appropriate for intercropping with firm-stem companion species in full mixtures (Barillot et al., 2012).

Fertilizer N saving effects were observed previously in both the high-input and low-input intercrops (Li et al., 2020b; Xu et al., 2020). In the current study when tailored N fertilization was used, on average over the two years, relay intercrops had an FNER of 1.14 ± 0.03, indicating a 14% saving in fertilizer N compared to monocrops for the same grain yield. In contrast, simultaneous intercrops did not show such a fertilizer saving effect. In essence, combining of temporal complementarity and an intercrop N rate being intermediate to monocrop N rates enabled each species to capture more light, N, and probably also other resources, leading to increased yield per unit land area compared to monocrops and thus enhancing fertilizer N use efficiency.

Our findings align with meta-analyses that show that intercrop performance increases more from temporal complementarity if the N nutrition is higher (Li et al., 2020b; Yu et al., 2015). Our research seems to indicate that it is advisable to combine species with only partially overlapping growth seasons and apply N tailored to the individual species demands. The advantages in light capture offer opportunities to increase biomass with non-limiting N availability and accordingly promote the capacity of the plants to acquire N from the soil. Nevertheless, there could be potential for increasing N uptake and production advantages in late-sown maize because N limitation became evident during maize late growth stage in the current study (Fig. 2.7 i). Applying an additional N dose to maize when harvesting the early companions has been shown to significantly facilitate N uptake recovery in maize (Hu et al., 2016). In our species tailored fertilization approach, to boost a "win-win" situation in relay intercrops, an additional N input after maize tasselling might be a recommendation; however, it remains uncertain whether this approach would be sustainable in terms of N uptake efficiency during this late growth phase. Postponing the second N fertilization could also be an option, but it could also lead to N limitation in maize around flowering. There seems to be room for additional experimentation before making recommendations to practice. Choosing the appropriate early companions is also important. Overyielding of the later-sown maize in relay intercrops increased with a longer recovery time after harvest of the early sown species. Given the cool climate of the Netherlands, a recommendation would be to use winter-sown rather than spring-sown cereals or legumes as the early-sown species, so they may be harvested early, leaving a longer recovery time for maize to boost its production and take up the soil N.

2.5 Conclusion

Our study investigated the effects of strip intercropping on nitrogen (N) uptake of maize, wheat, faba bean, and pea and intercropping fertilizer N use efficiency under a species-tailored N strategy in the Netherlands. Relay intercrops used land more effienctly for N uptake than the combined monocrops, while such intercropping advantages were not observed in simultaneous intercrops. In relay intercrops, early-sown species captured more N than their monocrops and late-sown maize maintained comparable N uptake. Temporal complementarity between species was the main driver of complementary N uptake in intercrops, irrespective of the presence of a legume. Combining cereals with legumes, however, did allow a reduction in N input to the system. Under the species-tailored N fertilization, relay intercrops used fertilizer N more efficiently than the combined monocrops and simultaneous intercrops. To enhance maize N uptake and likely production, applying additional N after maize tasselling could be required, although analysis of long-term sustainability is to be made first. Proper selection of early companion species is crucial. The species-tailored N strategy effectively ensures the complete capture of available mineral N for cereals, enables and requires legumes to exploit N fixation, and reduces environmental costs in intercropping.

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Chapter 3

Shoot plasticity and light signals in relay strip intercropping

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Abstract

Plants in intercrops experience interspecific competition for light, resulting in plastic responses leading to different trait values compared to sole crops. Important indicators of competition for light are the red to far-red ratio (R:FR) and photosynthetically active radiation (PAR) within the canopy. We aimed to find out to which extent plasticity in shoot traits in intercrops is related to differences in R:FR and PAR between intercrops and sole crops. We compared wheat-maize and faba bean-maize relay intercropping, where maize was the late-sown and late-harvested species, and the corresponding sole crops. Wheat had more tillers, and bean had more branches and shorter internodes in intercrops than in sole crops. Plasticity in wheat and bean coincided with higher PAR and higher R:FR in the radiation from neighboring maize rows than those in sole crops. Maize had longer sheaths in its lower canopy in both intercrops than in sole crops. Sheaths were longer in the maize grown with faba bean than with wheat, responding to stronger shading and lower R:FR in the radiation from bean rows. The results show that earlier sown species responded to improved light environments in relay intercrops compared to sole crops. Intercropping maize with species of different statures results in varying levels of responses. This can provide a better understanding of maize yield performance when intercropped with different species.

Keywords: Wheat-maize intercropping, Faba bean-maize intercropping, PAR, Red to far-red ratio, Shoot plasticity.

3.1 Introduction

Intercropping is the cultivation of multiple crop species on the same field for at least part of their growing season. Relay intercropping involving maize (*Zea mays*) and a C_3 species that partially overlap in their growing season, is more productive than would be expected on the basis of the productivity of corresponding sole crops and their mixing proportion (Li et al., 2020a, 2020b, 2023; Wang et al., 2023). The component species in relay intercrops experience different growth environments compared to sole crops as a result of interspecific competition (Brooker et al., 2015; Homulle et al., 2022; Stomph et al., 2020). Plants have the ability to show phenotypic plasticity in response to environmental conditions (Sultan, 2000). It is therefore relevant to investigate shoot plasticity of maize and its companion species in relay intercropping to better understand their overall high performance.

In Europe, combining a legume and a C_3 cereal in a mixed (no distinct row arrangement) or alternate-row intercrop is commonly practiced within low-input organic systems, where the component species are managed and harvested simultaneously (Bedoussac et al., 2015; Jensen et al., 2020). Such intercrops allow a high degree of rhizosphere interactions between cereals and legumes, exploiting biological nitrogen (N) fixation of legumes and releasing soil N competition for cereals (Bedoussac and Justes, 2010; Ghaley et al., 2005; Rodriguez et al., 2020). Information on plant phenotypic plasticity in such a system has been obtained. For instance, in a wheat (*Triticum aestivum*)-faba bean (*Vicia faba*) mixed intercropping conducted in Denmark, wheat had more tillers per plant in the intercrop than in the sole crop (Ajal et al., 2022). However, there is little information available on shoot plasticity in relay intercropping grown under European conditions, and most of it was obtained in wheat-maize relay intercropping in the Netherlands (Gou et al., 2017a; Zhu et al., 2014, 2015, 2016).

Shoot plasticity in relay intercropping involving maize has been well studied in conventional high-input agriculture, aiming to understand the high crop productivity in such intercrops compared to sole crops (Raza et al., 2019; Wang et al., 2015). In the Netherlands, relay intercropping with maize in conventional agriculture, where species receive a moderate rate of fertilizer N, has shown yield advantages over sole crops (Gou et al., 2016; Wang et al., 2023; Zhu et al., 2016). Species interactions in intercrops are affected by agronomic practices such as the selection of component species. For instance, wheat and faba bean are the two contrasting companion species for relay intercropping with maize, in which wheat competes for soil N more strongly than faba bean does (Gou et al., 2018; Li et al., 2006). Moreover, differences in canopy structures between wheat and faba bean may lead to different morphological responses in intercropped maize. Further analysis is required to compare plant responses in intercrops of maize with legume or non-legume companion species in conventional agriculture under European conditions.

Intercropping within conventional agriculture is usually practiced in strip designs, in which species are cultivated in strips of a few crop rows to facilitate management as well as interspecific interaction (Li et al., 2020b). In strip intercropping, there is a gradient of interspecific interaction, with the strongest interactions and plant responses in the border rows of strip, but much weaker interactions and much weaker or no detectable responses in the inner rows (Li et al., 2020c, 2021; Wang et al., 2020; Zhang et al., 2007; Zhu et al., 2016). Border rows of the species strips in intercrops have been reported to make a larger contribution to the species' yield compared to inner rows (Gou et al., 2016). Therefore, shoot plasticity in border row plants is important for understanding crop performance in relay strip intercropping.

Combining maize and a legume such as soybean (*Glycine max*) in an intercrop results in a more efficient land use than growing the corresponding sole crops (Xu et al., 2020). Maize and soybean are sown and harvested at the same time in a simultaneous intercrop in continental climate region (Li et al., 2020c, 2021; Liu et al., 2017, 2018; Pelech et al., 2022), or maize is sown and harvested earlier than soybean in a relay intercrop in subtropical climates (Yang et al., 2014). In these intercrops, maize is the dominant species having increased acquisition of light, while the soybean exhibits shade avoidance traits, showing longer internodes than soybean plants in sole crops. In the Netherlands, the temperate climate only allows maize to be sown and harvested later than a C_3 species in a relay intercrop, giving legumes a better starting position for resource capture, resulting in improved legume performance (Wang et al., 2023). However, it is not clear to what extent plastic responses in shoot traits of early-sown legumes and late-sown maize occur and can (partly) explain the effects on intercrop performance.

In relay intercrops with maize sown later, maize plants initially suffer from competition for light with the earlier sown species. When the maize outgrows the earlier sown species in height and especially after the earlier sown species has been harvested, maize has improved access to light and soil resources compared to maize in sole crops where they compete with conspecifics (Gou et al., 2017a; Liu et al., 2020; Ma et al., 2020). Zhu et al. (2014) observed that in a wheat-maize relay intercrop, the late-sown maize exhibited shade avoidance traits at low phytomer ranks such as increased final sheath length, while sheath lengths were smaller at higher phytomer ranks, compared to the same ranks in pure maize stands. This finding shows that maize in relay intercrops exhibits contrasting plastic responses between phytomers on the same plant in response to the dynamically changing growth environment. Further analysis is required to examine shoot plasticity in maize when relay intercropping with species having different traits (cereals vs. legumes), to formulate a comprehensive understanding on maize responses in relay intercropping.

The effect of intercropping on the canopy light environment includes effects on both red to far-red ratio (R:FR) and photosynthetically active radiation (PAR) intensity (Yang et al., 2014; Zhu et al., 2014). In plant stands, plant tissues absorb red light and reflect and transmit far-red light. The reduction in R:FR perceived by a plant is an indicator of competition with neighboring plants- a signal of light competition that triggers a suite of shade avoidance responses, such as increased stem or petiole length, reduced tillering or branching (Ballaré and Pierik, 2017; Demotes-Mainard et al., 2016; Evers et al., 2006; Huber et al., 2021). Changes in PAR also affect plant shoot traits, because PAR serves not only as the light energy for photosynthesis and biomass production, but it also affects phototropic and elongation responses in plants (Ballaré and Pierik, 2017; Huber et al., 2021). Thus, if intercrops show different patterns in R:FR and PAR than sole crops, it is expected that shoot plasticity in intercropping is associated with changes in light signals. There is, however, limited information available on it (Yang et al., 2014; Zhu et al., 2014, 2016).

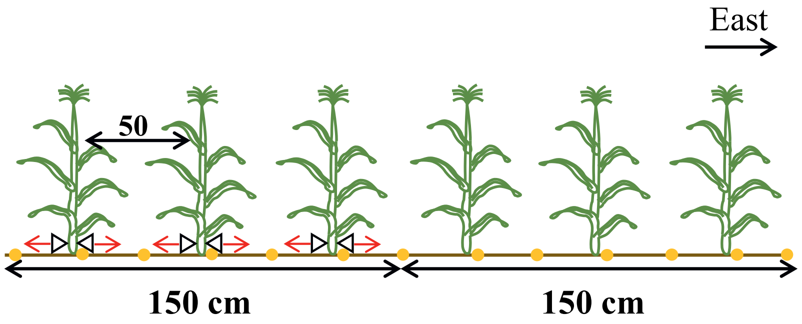
R:FR perceived by a plant in the radiation from different directions in strip intercrops contain different competitive information. In a strip intercrop, R:FR perceived by a plant in the radiation from the direction of neighboring species rows may be an indicator of interspecific competition, while R:FR perceived from the adjacent conspecific species rows is more likely to be an indicator of intraspecific competition. Previous studies on the effect of intercropping on R:FR ignored to distinguish R:FR perceived from neighbor species and from conspecifics in intercrops (Liu et al., 2017; Yang et al., 2014). Thus, they lack information on the extent to which changes in R:FR in intercrops is associated with interspecific competition.

In this study, we aim to investigate shoot plasticity in relay strip intercropping under conventional growing conditions in the Netherlands, and determine to which extent such plasticity is related to differences in R:FR and PAR between intercrops and sole crops. We focused on five cropping systems: sole wheat, sole faba bean, sole maize, wheat-maize relay strip intercropping, and faba bean-maize relay strip intercropping. We examined shoot plasticity in maize when relay intercropped with species having different traits (cereals vs. legumes). We analyzed effects of relay intercropping on wheat tillering, faba bean branching, and the profile of organ morphological traits along the plant stem. In intercrops, we focused on plasticity in border row plants and PAR and R:FR perceived by these plants. Specifically, we distinguished R:FR perceived in the radiation from the direction of neighbor species and from conspecific species in intercrops.

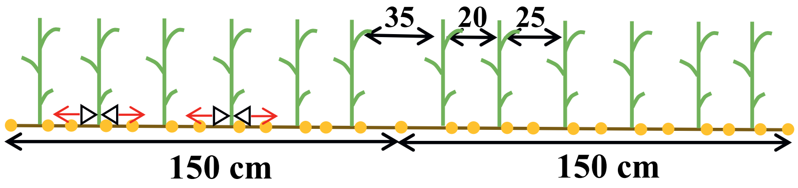
3.2 Materials and Methods

3.2.1 Experimental set-up

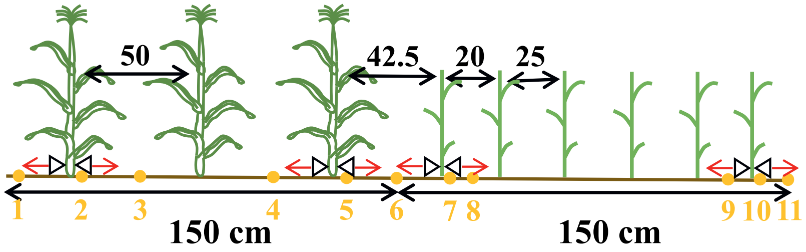
All measurements were made in a field experiment at Droevendaal Experimental Farm in Wageningen, the Netherlands (51° 59’ 20” N, 5° 39’ 16” E) in 2018. The climate at the site is oceanic temperate. The farm has a sandy soil with 3.4% organic matter and a pH of 5.7. Spring wheat (*Triticum aestivum* L. cv. Nobless) was sown on 21 March and harvested on 17 July. Faba bean (*Vicia faba* L. cv. Fanfare) was sown on 21 March and harvested on 30 July. Maize (*Zea mays* L. cv. LG30.223) was sown on 4 May and harvested on 10 September. The sowing density of wheat, faba bean, and maize was 383, 44, and 10 seeds m^{-2} respectively. In wheat-maize and faba bean-maize intercrops, 1.5 m maize strips were alternated with 1.5 m strips of wheat or faba bean (Fig. 3.1). In both intercrops, the rows on both sides of the species strip are border rows while the other rows are inner rows. Maize was grown at a row distance of 50 cm. The row distance of both wheat and faba bean was 25 cm, except for the distance between border rows and the adjacent inner rows, which was 20 cm to allow space for the wheels of the tractor that had a track width of 133 cm. A replacement design was applied in both intercrops. The relative density of all species in intercrops was 0.5. A randomized complete block design with six replicates was used. Plots were 9 m in east-west by 11 m in north-south direction. The row orientation was approximately north-south.



(A) Sole maize



(B) Sole wheat, or sole bean



(C) Wheat-maize, or bean-maize strip intercrop

Fig. 3.1 Row configurations, R:FR measurements, and PAR measurements in sole maize (A), sole wheat or bean (B), and in the wheat-maize and bean-maize strip intercrop (C). Species were grown in 1.5 m-wide strips. In each species strip in intercrops, rows 1 and 3 are border rows for maize, and rows 1 and 6 are border rows for wheat and faba bean. Each triangle indicates the probe that was positioned back against the plant to measure R:FR. Measurements were conducted at the bottom of shoots, with the probe facing west and east, i.e., cross-row towards neighboring rows. Each yellow circle indicates a measuring point with the PAR probe oriented parallel to the rows. In intercrops, from west to east, the PAR measurements of positions 1 to 3 and 4 to 6 were used to calculate the weighted mean fraction of transmitted PAR (f_{trans}) for the western and eastern border row maize respectively. The PAR measurements of positions 6 to 8 and 9 to 11 were used to calculate the weighted mean fraction f_{trans} for the western and eastern border rows of wheat and bean respectively.

Before sowing wheat and faba bean, potassium and phosphorus fertilizer were applied homogeneously throughout the field. Potassium was applied at a rate of 105 kg K_2O ha^{-1}, and phosphorus at a rate of 67.5 kg P_2O_5 ha^{-1}. Mineral N in the 0-30 cm soil layer was 22 kg N ha^{-1} before sowing. Fertilizer N was applied at a rate of 125 kg N ha^{-1} in wheat, 20 kg N ha^{-1} in faba bean, and 170 kg N ha^{-1} in maize. In intercrop plots, fertilizer N was applied within species strips, thus species strips in sole crops and intercrops received identical amounts of fertilizer per unit area of the species (i.e., half the amount compared to sole crops when expressed over the whole intercropping area). Weeds, diseases, and pests were controlled as needed. Sprinkler irrigation was given from June to August to avoid drought stress.

3.2.2 Thermal time and plant height

Thermal time (°Cd) after sowing of each species was calculated at a daily basis from sowing, using the daily mean air temperature recorded at weather station De Veenkampen at 2.5 km distance from the experimental site. The base temperature is 0 °C for wheat and faba bean, and 8 °C for maize.

Plant heights of wheat, faba bean, and maize in intercrops were determined to monitor dominance with respect to light competition. Plant height was determined using a measuring stick along the main stem, from the ground to the base of the last fully developed leaf. The measurements were made four times in wheat and faba bean, and six times in maize. In each plot, one plant in each row in one species strip and at least 1 m away from the plot edge was selected. The measurements were made in six blocks for maize, and six blocks for wheat and faba bean during the first three measurements and three blocks in the fourth measurement.

3.2.3 Plant architecture

We compared traits in border row plants in intercrops and plants in sole crops. A model was used to describe the profile of organ size along the plant stem. Model selection was used to determine whether profiles were different among treatments (see below).

Wheat

A wheat phytomer consists of an internode, a tiller bud at the bottom, a node above the internode, a leaf sheath inserted on the node, and a leaf blade (Fig. 3.2). The number of tillers per wheat plant was determined at heading and maturity. In the field, with the high sowing density of wheat (383 seeds m^{-2}) and the presence of tillering, identifying individual wheat plants within a row, as well as distinguishing wheat tillers from main stems, were challenging. So, total wheat stems (tillers + main stems) within the harvest area were cut at soil level and brought to the lab for counting, without discriminating main stems and tillers. At heading, all wheat stems were harvested within a 2-meter length of each row in a strip in each plot of intercrops and sole crops. At maturity, 4 m of each row in a strip was harvested in each plot of sole crops, and 2 m in intercrops. Measurements were made in three blocks at heading and six blocks at maturity. The number of tillers per wheat plant was calculated as:

$$\frac{\text{All stem numbers per meter - Germinated plant numbers per meter}}{\text{Germinated plant numbers per meter}} \qquad \text{Eq. 3.1}$$

where the germinated plant number (Table S3.1) was determined one week after wheat germination.

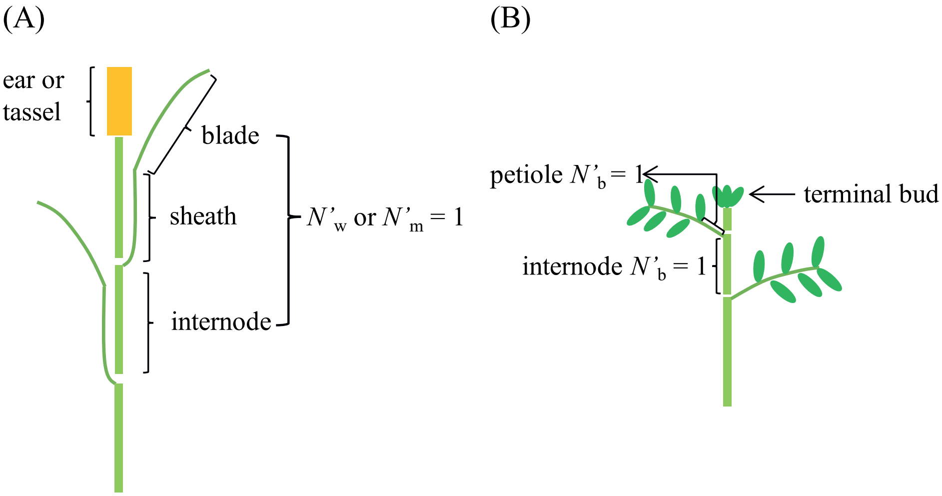


Fig. 3.2 Schematic representation of phytomers of the wheat or maize plant (A) and faba bean plant (B). (A) A wheat or maize phytomer consists of an internode, a leaf sheath, and a leaf blade. N'_w or N'_m = 1 represents the rank of the last developed collar leaf in wheat or maize, and ranks are counted from the top to the bottom of the plant. (B) A faba bean phytomer consists of an internode and a compound leaf composed of a petiole and leaflets. Faba bean exhibits indeterminate habits. The terminal bud of a faba bean plant is always vegetative and keeps growing. N'_b = 1 represents the rank of the last developed compound leaf.

At wheat flowering, lengths of internode and sheath at each phytomer rank of the main stem were measured with a ruler. To distinguish tillers from the main stem, wheat plants, including roots, were harvested manually. We harvested two plants per plot in sole crops and one border row plant in intercrops, in three blocks. The plants were then brought to the lab for destructive measurements.

Faba bean

A faba bean phytomer consists of an internode, axillary buds, and a compound leaf composed of a petiole and leaflets (Fig. 3.2). The number of branches per plant was determined at pod filling and maturity. In the field, the individual faba bean plant within a row was identified due to the low sowing density (44 seeds m^{-2}). At pod filling, in each plot of intercrops and sole crops, total numbers of faba bean plants and total numbers of stems (branches + main stems) were counted within a 2-meter length of each row in a strip. At maturity, measurements were conducted within a 4-meter length. Measurements were made in three blocks at pod filling and six blocks at maturity. The number of branches per faba bean plant was calculated by subtracting the total number of faba bean plants from the total number of stems, and dividing by the total number of faba bean plants.

To determine internode and petiole lengths, we harvested two plants per sole crop plot and one border row plant per intercrop plot, in three blocks at pod filling. Plants were cut at soil level and brought to the lab. Length of internode at each phytomer rank was measured with a ruler. Length of the petiole was measured at random phytomer ranks that were representative of the upper, middle, and lower layer of the stem.

Maize

As in wheat, a phytomer of maize comprises an internode, a node, a leaf sheath, and a leaf blade (Fig. 3.2). Length of internode and sheath at each phytomer was measured using a ruler at maize tasseling. We harvested one plant per sole maize plot and one plant from each of the two border rows in a maize strip per intercrop plot, in three blocks. Maize plants were cut at soil level and brought to the lab for destructive measurements.

3.2.4 Red: far-red ratio (R:FR)

In each plot, wheat, faba bean, and maize plants were tagged when the second leaf was visible. For wheat and faba bean, in each sole crop plot, two plants were tagged in a strip. In each intercrop plot, one plant was tagged in each border row of a strip. For maize, in each sole crop plot, one plant was tagged in each row of a strip. In each intercrop plot, one plant in each border row of a maize strip was tagged (Fig. 3.1). Measurements of R:FR ratio were made weekly or bi-weekly on the tagged plants, and in three blocks in wheat and faba bean and six blocks in maize. A Skye SKR 100/116 Fibre Optic Probe Measuring System (Skye Instruments Ltd, Powys, UK) was used, featuring a glass fibre probe at its tip for measuring R:FR. The probe has an angle of view of 40°. The probe was placed approx. 2 cm above the soil surface, and positioned with the back against the plant to measure R:FR in the radiation from west and east, i.e., cross-row towards neighboring rows (Fig. 3.1). Measurements were made around noon, with either a clear or uniformly overcast sky.

In intercrops, we distinguished between R:FR originating from the direction of the same species and from the companion species. We compared R:FR signals coming from (i) the companion species in intercrops, with the probe facing west for the western border row plants and east for the eastern border row plants; (ii) the same species in intercrops, with the probe facing east for the western border row plants and west for the eastern border row plants (Fig. 3.1).

3.2.5 Photosynthetically active radiation (PAR)

Incoming and transmitted PAR were measured using a SunScan canopy analysis system (SunScan SS1, Delta-T Devices Ltd, Cambridge, UK). A 1-m long SunScan probe with 64 quantum sensors was placed parallel to the rows on the ground. At the same time, a Beam Fraction Sensor (BFS, one quantum sensor) recorded the incoming light. In each sole crop plot, measurements were taken in the middle between the rows and directly adjacent to the plants within the row, and in two strips. In each maize sole crop plot, thirteen measurements were made. In each sole crop plot of wheat and faba bean, twenty-five measurements were taken (Fig. 3.1). In each intercrop plot, as we focused on light conditions of border row plants, measurements were taken at three locations for each border row plant: in the middle between the border row and companion species row, directly adjacent to the plants within the row, and in the middle between border row and inner row. A total of eleven measurements were taken in an intercrop (Fig. 3.1).

All measurements were made weekly or bi-weekly around noon, with either a clear or uniformly overcast sky. Prior to making measurements in each plot, we placed the probe and BFS horizontally in uniform sunlight and recorded one reading, which was used to obtain a correction factor. The fraction of transmitted light (f_{trans}) was then calculated by dividing the corrected probe reading by the BFS reading (incoming light). In intercrops, a weighted mean f_{trans} of border row plants was used for analysis (Zhu et al., 2014). The weighting factors for the three locations within the space occupied by the border row plant (50 cm for maize and 27.5 cm for wheat and faba bean) were calculated based on their representative lengths, as given in Methods S3.2.

3.2.6 Estimation of the profile of organ size along the plant stem

For wheat, the profile of internode length ($L_{\text{I,w}}$) along the main stem was described using a negative exponential equation:

$$L_{\text{I,w}} = a \times e^{(-b \times N'_{\text{w}})} \qquad \text{Eq. 3.2}$$

where a and b are coefficients, and N'_{w} is the phytomer rank counted from the top of the main stem (Fig. 3.2). The profile of sheath length ($L_{\text{S,w}}$) along the wheat main stem was described by a decreasing logistic equation:

$$L_{\text{S,w}} = M_{\text{S,w}} - \frac{M_{\text{S,w}}}{1+e^{-c \times (N'_{\text{w}} - d)}} \qquad \text{Eq. 3.3}$$

where $M_{\text{S,w}}$ is the maximum wheat sheath length, c is a slope parameter, N'_{w} is the phytomer rank, and d is the phytomer rank at half of the maximum sheath length.

For faba bean, a generalized additive model (GAM; Wieling, 2018) was used to describe the relationship between internode length and phytomer rank (N'_{b}) counted from the top of the stem (Fig 3.2). A logistic equation was used for describing petiole length (L_{P}) as a function of phytomer rank (N'_{b}):

$$L_{\text{P}} = \frac{M_{\text{P}}}{1+e^{-f \times (N'_{\text{b}} - g)}} \qquad \text{Eq. 3.4}$$

where M_{P} is the maximum petiole length, f is a slope parameter, and g is the phytomer rank at half of the maximum petiole length.

For maize, a Lorentz peak distribution equation was used to describe the profile of internode length ($L_{\text{I,m}}$; Eq. 3.5) or sheath length ($L_{\text{S,m}}$; Eq. 3.6) along the stem:

$$L_{\text{I,m}} = \frac{M_{\text{I,m}}}{1+\left(\frac{N'_{\text{m}} - h}{i}\right)^2} \qquad \text{Eq. 3.5}$$

$$L_{\text{S,m}} = \frac{M_{\text{S,m}}}{1+\left(\frac{N'_{\text{m}} - j}{k}\right)^2} \qquad \text{Eq. 3.6}$$

where $M_{\mathrm{I,m}}$ and $M_{\mathrm{S,m}}$ represent the maximum lengths of internode and sheath, N'_{m} is the phytomer rank counted from the top of maize stem (Fig. 3.2), h and j are phytomer ranks at the peak length, and i and k are scale parameters.

3.2.7 Model fitting and model selection

Model selection was used to assess whether the profile of organ size along the plant stem need to be distinguished between groups of treatments. To do so, first, multiple versions of each model were made, based on which parameters differed among which groups of treatments.

For wheat and faba bean, we compared the model with the distinction between sole crops and intercrops (type i), i.e., separate parameterization for the data of sole crops and intercrops, and the model without the distinction (type ii), i.e., the model was fitted to all data combined. For type i, for the model with two parameters (Eq. 3.2), three model versions were made: first, both parameter 1 and 2 were different between sole crops and intercrops; second, parameter 1 was different between groups while parameter 2 was the common value; third, parameter 2 was different while parameter 1 was common. For the model with three parameters (Eq. 3.3 and Eq. 3.4) see Methods S3.1.

For maize, there were five types of model version: separate parameterization for the data of (i) sole maize, wheat-maize intercrop, and faba bean-maize intercrop; (ii) wheat-maize, and combined data of sole maize and faba bean-maize; (iii) faba bean-maize, and combined data of sole maize and wheat-maize; (iv) sole maize, and combined data of wheat-maize and faba bean-maize; and (v) the model was fitted to the combined data of all treatments. For each of type i, ii, iii, and iv, for the model with three parameters (Eq. 3.5 and Eq. 3.6), we also test the combination of the three parameters differing between groups of data (see Methods S3.1).

The *Akaike Information Criterion* (AIC) was used to compare model versions, to find the one that fits the data best with as few as possible parameters to be estimated. To do so, first, maximum likelihood estimate of the parameters of each model version was obtained. For Eqs 3.2 to 3.6, the function mle2 in the “bbmle” package (Bolker and R Core Team, 2022) was used with “Nelder-Mead” optimization algorithm, and then the R base function AIC (R Core Team, 2023) was used to calculate AIC value of each model version. For the GAM, the maximum likelihood estimation was made using the function bam in the "mgcv" package (Wood, 2010) with "ML" optimization algorithm, and then the function compareML in the "itsadug" package (van Rij et al., 2022) was used to compare AIC values (Wieling, 2018). The model with the lowest AIC value was considered the best fit to the data. Models with AIC values less than 2 units apart (ΔAIC < 2) were considered equivalent, in which condition the simpler model (with fewer parameters) was chosen (Bolker, 2008). The results of model selection are given in Tables S3.2 to S3.4. The fitted parameter values are given in Table S3.5.

3.2.8 Analysis of light measurements

Linear mixed effect models were used to compare means of treatments of R:FR and f_{trans}, in which treatment was a fixed effect and block a random effect. Comparisons were made at each measurement time. The function lmer from the "lme4" package (Bates et al., 2015) in R (R Core Team, 2023) was used. Significance of the fixed effects was determined using analysis of variance (ANOVA) at $P = 0.05$. The function Anova from the "car" package (Fox and Weisberg, 2019) was used. Pairwise comparisons were conducted using Fisher's Least Significant Difference (LSD) in the "emmeans" package (Lenth, 2021).

3.3 Results

3.3.1 Plant height

In the wheat-maize intercrop, maize surpassed wheat in height when maize had ten collar leaves at 504 °Cd, after approximately 99 days from sowing wheat (Fig. 3.3), while wheat was at grain filling stage (1406 °Cd). In the faba bean-maize intercrop, maize overtopped faba bean when the maize tassel appeared at 627 °Cd, after approximately 110 days from sowing faba bean, while faba bean was at pod filling stage (1617 °Cd).

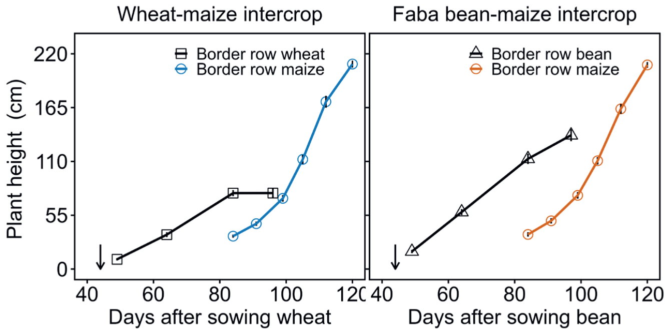


Fig. 3.3 Plant height of border row wheat, faba bean, and maize in intercrops. The x-axis is the days after sowing the first species. The black arrow in each panel represents the time of sowing maize. Each point indicates the mean of plant height, and each error bar represents standard error of the mean.

3.3.2 Wheat plant traits

At wheat heading (999 °Cd) and maturity (1769 °Cd), wheat in the wheat-maize intercrop border rows had more tillers per plant than those in sole wheat (Fig. 3.4A). At wheat flowering (1351 °Cd), internode or sheath lengths along the main stem did not differ between sole crops and intercrops (Fig. 3.4B and C).

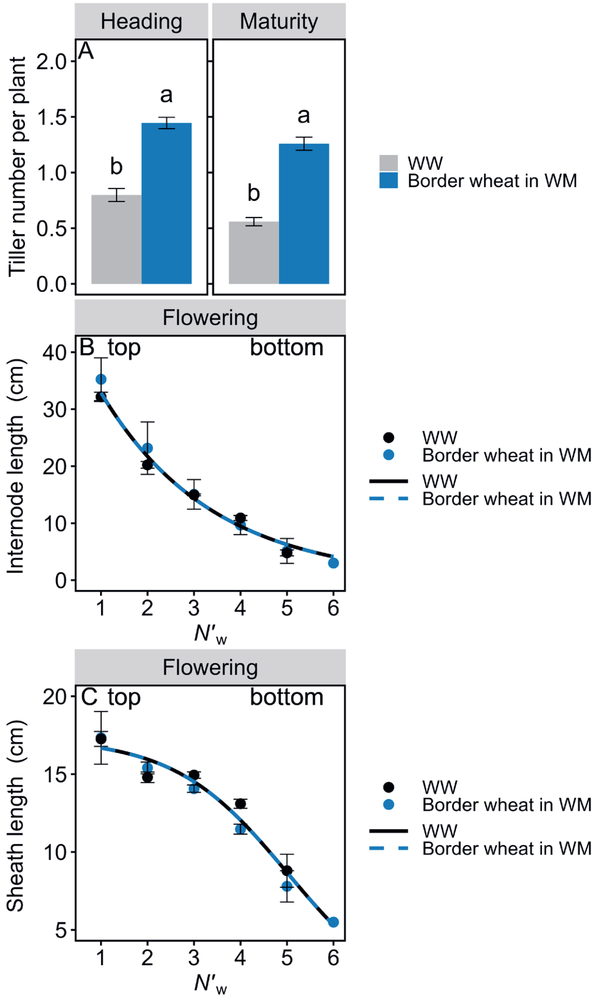


Fig. 3.4 Tiller number per plant (A), internode length as a function of phytomer rank (N'_w) (B), and sheath length as a function of N'_w (C) in sole wheat (WW) and border row wheat in wheat-maize intercrop (WM). The phytomer rank (N'_w) was counted from the top of the wheat main stem to the bottom; $N'_w = 1$: rank of last developed collar leaf. In panel A, for both stages different letters denote significant differences between treatments at $P < 0.05$. In panel B and C, points indicate the means of measured lengths. Curves were drawn using Eq. 3.2 (B) and Eq. 3.3 (C) with estimated parameters (Table S3.5). According to the results of model selection (AIC), values of parameters in both equations were identical between treatments. Thus, the fitted curves of sole wheat and intercropped wheat were identical. Error bars in all panels indicate the standard error of the mean.

3.3.3 Faba bean plant traits

Faba bean intercropped with maize had more branches per plant in the border rows than faba bean in sole crops both at pod filling (999 °Cd) and maturity (2061 °Cd) (Fig. 3.5A). At pod filling (1367 °Cd), internode lengths of faba bean were at most ranks shorter in the intercrop with maize than in the sole crop (Fig. 3.5B). Both reduced branching and longer internodes are typical shade avoidance traits observed in sole faba bean. Petiole lengths along the plant stem were not different between intercrops and sole crops (Fig. 3.5C).

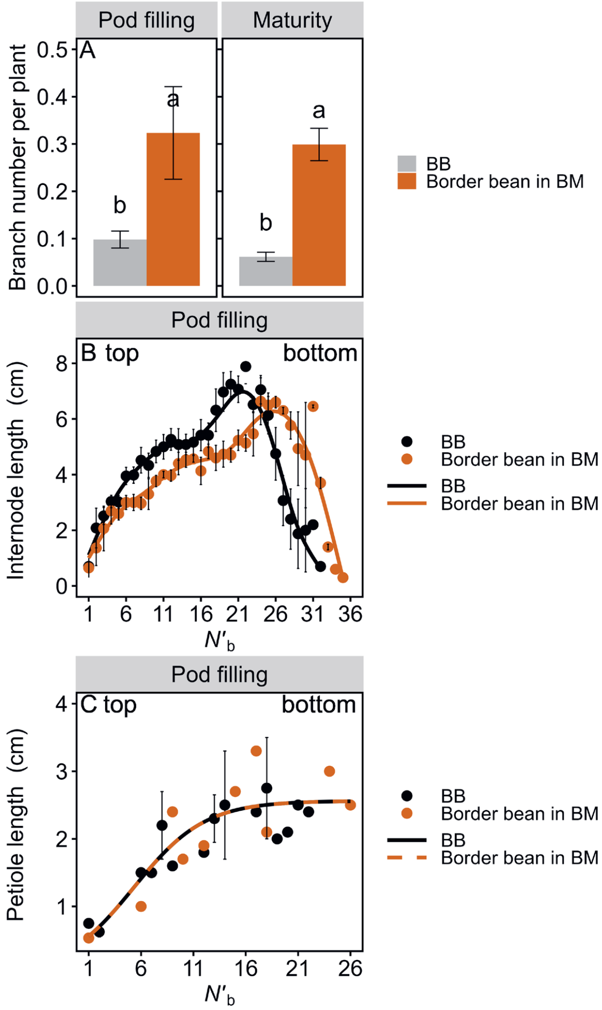


Fig. 3.5 Branch number per plant (A), internode length as a function of phytomer rank (N'_b) (B), and petiole length as a function of N'_b (C) in sole faba bean (BB) and border row bean in faba bean-maize intercrop (BM). The phytomer rank (N'_b) was counted from the top of plant stem; $N'_b = 1$: rank of last developed compound leaf. In panel A, different letters denote significant differences between treatments at $P < 0.05$ in each bean stage. In panel B and C, points indicate the means of measured lengths. In panel B, generalized additive modelling (GAM) was used, and the profile was distinguished between sole crops and intercrops based on AIC. In panel C, the profile was described by Eq. 3.4. According to AIC, values of parameters were identical between treatments, thus curves were identical for sole crops and intercrops. Error bars in all panels indicate the standard error of the mean.

3.3.4 Maize plant traits

At tasseling (644 °Cd), leaf sheaths in the lower canopy of maize were longer in border row maize in both intercrops than in sole maize, and the longest sheaths were observed in maize intercropped with faba bean (Fig. 3.6A). This shade avoidance response ceased earlier and in the lower canopy layers in maize intercropped with wheat than in maize intercropped with faba bean. In the upper canopy of maize, sheath lengths were shorter in the maize next to wheat than in sole maize and maize intercropped with bean. Intercropping with faba bean resulted in shorter internodes in the lower canopy of maize, while intercropping with wheat led to shorter internodes in the upper canopy, compared to the corresponding phytomer ranks in the other treatments (Fig. 3.6B).

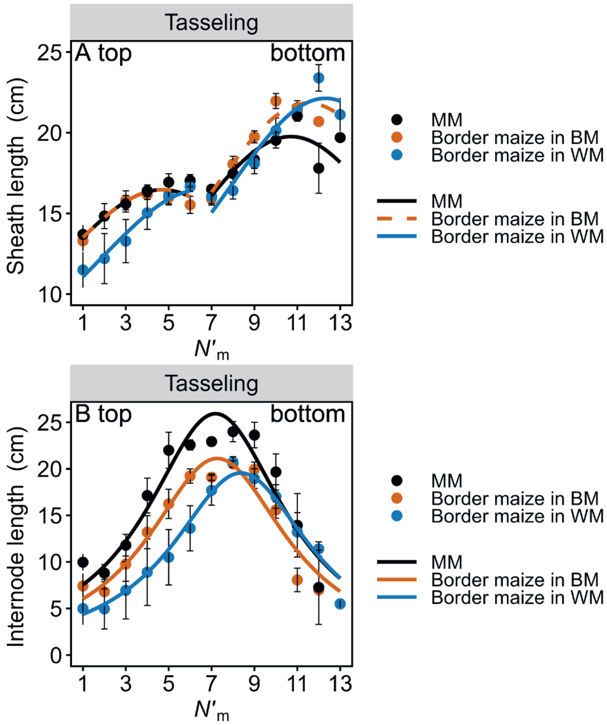


Fig. 3.6 Sheath length as a function of phytomer rank (N'_m) (A), and internode length as a function of N'_m (B) in sole maize (MM), and border row maize in faba bean-maize (BM) and wheat-maize intercropping (WM). The phytomer rank (N'_m) was counted from the top; $N'_m = 1$: rank of last developed collar leaf. Each point indicates the mean of measured lengths. Error bars indicate the standard error of the mean. In panel A, the profile for $N'_m <= 6$, i.e., in the upper canopy of maize, and for $N'_m >= 7$, i.e., in the lower canopy of maize, were fitted separately by Eq. 3.6. The profile for $N'_m <= 6$ differed between wheat-maize and maize in both sole maize and faba bean-maize based on AIC. Thus, curves of sole maize and faba bean-maize coincide for the upper canopy. At $N'_m >= 7$, the profile was differed in each of the three treatments. In panel B, the profile was described by Eq. 3.5, and differed among the three treatments, based on AIC.

3.3.5 Red: far-red ratio (R:FR) and fraction of transmitted PAR (f_{trans})

R:FR and f_{trans} decreased over time as the canopy of wheat and faba bean developed. In the early stages of wheat and faba bean growth, the radiation received by border row wheat and faba bean from the direction of the still largely unvegetated maize strip had a higher R:FR than the radiation coming from the adjacent conspecific in intercrops and the radiation received in sole crops (Fig. 3.7A and B). Thus, the early-sown species perceived a weak signal of light competition from maize rows in relay intercrops before the maize overtopped it. The border row wheat in the relay intercrop also had higher f_{trans} compared to sole wheat during the early phase (Fig. 3.7D).

In both relay intercrops, maize plants in border rows initially suffered from shading by the earlier sown species, showing a lower f_{trans} than maize in sole crops (Fig. 3.7F). The shading was stronger when maize was intercropped with faba bean than with wheat. Also, the radiation received by border row maize from the direction of the bean rows had a lower R:FR than the radiation coming from the adjacent maize rows in intercrops, and the radiation received in the wheat-maize intercrop and sole maize (Fig. 3.7C). Thus, in the early stages of maize growth, faba bean was a stronger light competitor for maize than wheat in relay intercrops. After maize surpassed wheat in height, border row maize perceived a weaker R:FR signal (Fig. 3.7C) and experienced increased f_{trans} (Fig. 3.7F) than border row maize in the faba bean-maize intercrop and maize in sole crops.

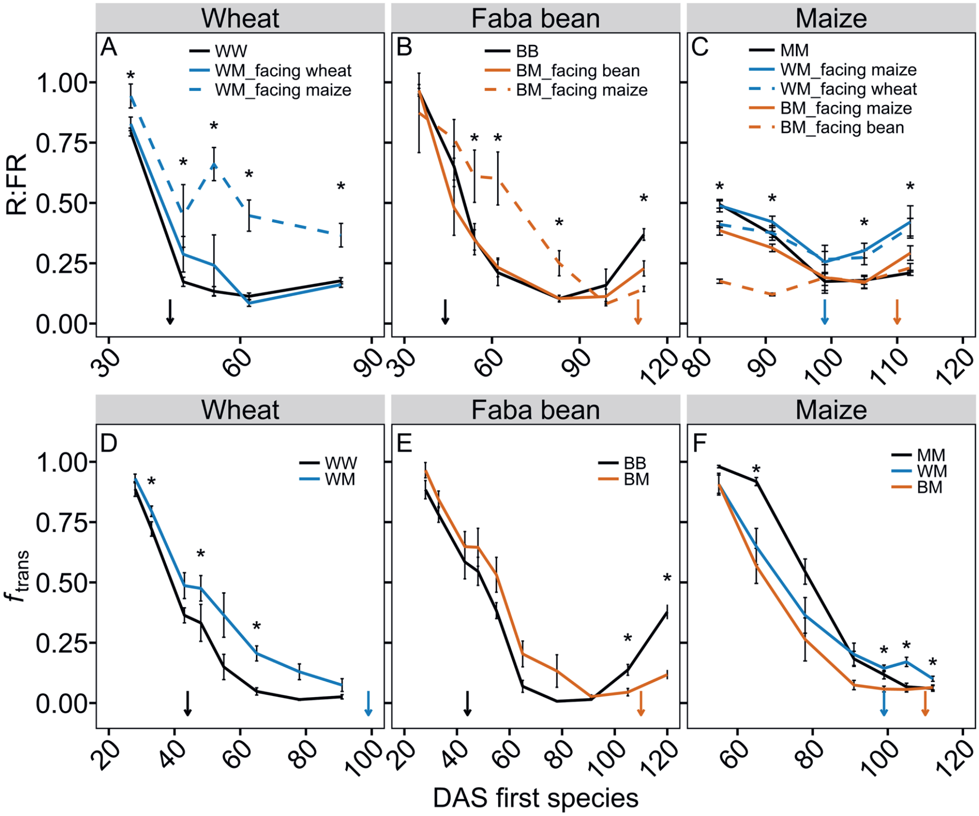


Fig. 3.7 Red to far-red ratio (R:FR) measured at the shoot base, and fraction of transmitted light (f_{trans}) reaching the soil surface in sole crops and in border rows in intercrops. The x-axis is days after sowing (DAS) the first species in relay intercrops: A and D, DAS wheat; B and E, DAS faba bean; C and F, DAS wheat or faba bean. Lines segments connect the means, and error bars indicate the standard error of the mean. We compared R:FR measured under three conditions: the probe facing neighbor species (i) and inner row plants (ii) in intercrops, and the probe facing the adjacent rows in sole crops (iii). The f_{trans} of border row plants in intercrops was the weighted mean f_{trans}. The black arrow indicates the time of maize sowing, the blue arrow indicates when maize overtopped wheat, and the orange arrow indicates when maize overtopped faba bean. Significance of treatment differences was determined at each measurement time, using ANOVA with treatment as a fixed effect and block as a random effect (* = $P < 0.05$). Details showing the pairwise comparisons are presented in Tables S3.6 and S3.7.

3.4 Discussion

In this study, we analyzed effects of relay intercropping on shoot plasticity, R:FR, and transmission of PAR (f_{trans}) of border row plants as compared to plants in sole crops. We focused on wheat-maize intercrop, faba bean-maize intercrop, and their corresponding sole crops. The earlier sown species in relay intercrop border rows responded plastically to being next to late-sown maize, resulting in different shoot traits compared to sole crops. The later sown maize exhibited shade avoidance traits during its early growth, and this response ceased after maize overtopped the companion species, but the extent to which maize exhibited such responses differed when intercropped with wheat or faba bean.

3.4.1 Shoot plasticity in earlier sown species in relay intercrops are related to the combined effects of R:FR and PAR in the canopy

In relay intercrops, the earlier sown species have access to extra resources (i.e., light, water, nutrients) in the strips of neighbor species, during the phase when maize is initially absent and smaller than the earlier sown species (Gou et al., 2017a; Liu et al., 2015; Zhu et al., 2016). The border row plants in intercrops accordingly perceived a weaker signal of light competition (i.e., a higher value of R:FR) than plants in sole crops (Fig. 3.7A and B), confirming observations by Zhu et al. (2016). The absence of a strong light signal could trigger the bud outgrowth in those border row plants (Demotes-Mainard et al., 2016), and result in more tillers (wheat) or branches (bean) compared to sole crops (Figs 3.4 and 3.5). Also, the increased f_{trans} in border row plants (Fig. 3.7) indicates improved light availability, which could improve photosynthesis and thus the amount of substrates that can satisfy the needs for outgrowth of more tillers or branches.

The relay aspect in these intercrop systems with maize is key to these observations. In studies with simultaneous maize-soybean intercrop, soybean exhibited contrasting responses, such as longer internodes along the plant stem than sole crops (Li et al., 2021; Liu et al., 2017; Pelech et al., 2022). In our trials, faba bean, being an earlier sown species in the relay intercrop with maize, perceived a weaker signal of light competition than the bean plants in sole crops (Fig. 3.7B). This prevented typical shade-avoidance responses and likely triggered the intercropped faba bean to allocate a larger proportion of substrates to their roots (Huber et al., 2021). As a result, they had shorter internodes compared to pure stands (Fig. 3.5) and may have had larger roots and capture more soil resources compared with sole crops.

3.4.2 Plasticity in sheath lengths along maize stem coincided with changes in PAR and R:FR in the canopy

A longer sheath during the early growth of maize in relay intercrops with wheat than in pure stands is a shade avoidance response. Zhu et al. (2014) attributed this response to a lower R:FR and f_{trans} at the soil level in intercropped maize than sole maize. In our trials, a longer sheath was observed in the maize next to faba bean than in the border row maize in the wheat-maize intercrop during maize early growth (Fig. 3.6A). This phenomenon coincided with a strong signal of light competition (i.e., lower R:FR) perceived in the radiation coming from the direction of the bean rows (Fig. 3.7C). Additionally, the low f_{trans} perceived by those maize plants (Fig. 3.7F) indicates reduced availability of blue light, which could trigger phototropic responses such as sheath extension (Ballaré and Pierik, 2017; Huber et al., 2021; Kotilainen et al., 2020). Thus, compared to wheat, faba bean was a stronger competitor for light and resulted in longer sheaths in maize in relay intercrops.

The stimulation of sheath growth in the lower canopy of maize next to bean may compete for resources with internode growth, the elongation of which begins near the end of sheath extension (Fournier and Andrieu, 2000; Zhu et al., 2014). This could lead to shorter internodes in the lower canopy of these border row maize compared with pure stands (Fig. 3.6B).

As faba bean was taller than wheat, it took maize longer to overtop faba bean than wheat (Fig. 3.3). Thus, the shade avoidance response (i.e., longer sheaths) ceased at later time in the intercrop with faba bean, and higher up on the plant, than in the intercrop with wheat (Fig. 3.6). Prior to maize overtopping wheat, a higher value of R:FR at the soil level was found in maize intercropped with wheat than in maize in other systems (Fig. 3.7C), preventing shade-avoidance responses and reducing sheath and internode lengths in maize upper canopy (Fig. 3.6A). Therefore, we conclude that maize exhibited different patterns of sheath lengths along the stem when relay intercropped with species of different statures, coinciding with effects on light signals. Our findings contribute to a deeper understanding of maize shoot plasticity in intercropping, and insights into explaining maize productivity in relay intercrops with different species.

3.4.3 Relationship between shoot plasticity and yield in relay intercrops

In this experiment, both wheat and faba bean yielded more per unit area of the species strip in relay intercrops than in sole crops. However, overyielding in the intercropped maize compared to sole maize only occurred in the wheat-maize intercrop (reported in Wang et al., (2023)). Increased tillering of wheat in border rows in wheat-maize relay strip intercropping contributes to increased light capture compared to sole wheat (Zhu et al., 2016), which may have been the case in our study as well. We found a similar response to intercropping in faba bean, with approximately three in ten faba bean plants forming a branch in the intercrop, compared to one in ten faba bean plants in its sole crop (Fig. 3.5). This response will tend to increase both sink and source strength and can increase potential faba bean yield and contribute to overyielding at the field level.

Plasticity in maize shoot traits in response to early competition for light has a cascade effect on whole plant development in relay intercrops (Zhu et al., 2014). For instance, in a wheat-maize relay intercrop, early competition with wheat resulted in a lower leaf appearance rate of maize, which propagated into a decreased final leaf area index and, consequently, reduced light capture compared to maize in pure stands (Zhu et al., 2014, 2015). The faba bean cultivar “Fanfare” has a large total root length and rapid ground cover (Andersen et al., 2020; Homulle, 2020). Compared to growing with wheat, the stronger signal of light competition perceived by maize next to bean likely lead to a lower leaf area index (see Fig. S4.18 in Chapter 4). Also, such a strong light signal may trigger more resource allocation to traits for light-capturing capabilities at the expense of traits related with leaf photosynthetic capacity, such as leaf thickness (Demotes-Mainard et al., 2016; Gou et al., 2018). Therefore, we conclude that the negative effect of intercropping with faba bean on maize yield can be associated with shoot plasticity due to early competition for light.

Late-sown species in relay strip intercropping suffer from competition during the co-growth period, but they may recover after the early-sown species has been harvested (Zhang and Li, 2003). A sufficient temporal niche differentiation is important for the intercrop overyielding, which reduces overlap for interspecific competition and provides a longer period for intercropped species to grow alone in the field, exploiting extra light and soil resources from strips where the component species is absent (Li et al., 2020b; Yu et al., 2015;

Zhao et al., 2023). This indicates the vital role of sowing and harvest dates of component species in intercrops.

In agriculture, shade avoidance responses result in a decrease in crop yield, as relatively more biomass is allocated to tissues that may increase light capture, at the expense of roots and grains (Huber et al., 2021). The cessation of exhibiting longer sheaths in intercropped maize than sole maize occurred earlier in maize intercropped with wheat than with bean, and in the wheat-maize intercrop, this switch occurred already during the vegetative phase (Fig. 3.6), allowing these maize plants to allocate more resources to the tissues responsible for soil resource capture. This is important for maize recovery growth under moderate N input fertilization, as soil N likely be a limiting factor during this period (see Fig. 2.7 in Chapter 2). Thus, in addition to the sowing and harvest dates of component species, the timing of the cessation of exhibiting shade avoidance traits is also important for yield increase of late-sown species in relay intercrops.

The study highlights the need to consider the occurrence of light signals for interspecific competition due to the non-uniformity of intercrop canopies in space and time, as well as the relationships between these signals, plant trait values, and intercrop productivity. Assessing the extent to which shoot plasticity contributes to light capture in intercrops is not easily achieved through experiments. In this case, a functional-structural plant (FSP) model can be used, which simulates plants by combining 3D plant architecture with physiological processes and environmental factors (Evers et al., 2019; Li et al., 2021; Zhu et al., 2015). In the field, changes in PAR caused by neighboring plants can be accompanied or even preceded by R:FR signals (Ballaré and Pierik, 2017). An FSP model may serve as a tool to disentangle the effects of PAR and R:FR on shoot plasticity in intercrops (Evers et al., 2007) and the consequences for intercrop performance and functioning.

3.5 Conclusions

Shoot plasticity in earlier sown species in relay intercrops with maize coincided with higher values of R:FR and PAR at the base in intercrops than in sole crops. Such plasticity could contribute to overyielding of the early-sown species in relay intercrops. The signal of light competition perceived by maize in relay intercrops with faba bean was associated with the formation of long sheaths, which could negatively affect maize yield as compared to maize in the wheat-maize intercrop and sole crops. The timing of the cessation of shade avoidance responses during the vegetative phase may be vital for late-sown maize to achieve overyielding in relay intercrops. Functional-structural plant modelling is a useful tool to assess how resource capture and crop yield depend on plastic trait responses to R:FR and PAR signals in mixed canopies.

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Chapter 4

Competition for light and nitrogen with an earlier-sown species negatively affects leaf traits and leaf photosynthetic capacity of maize in relay intercropping

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Abstract

Mixing crop species in intercrops often results in yield advantages but the underlying processes are not completely understood. Increased resource capture in intercrops, particularly of light and nutrients, has been frequently demonstrated, but there is less information on the effect of intercropping on the photosynthetic capacity of leaves and on the leaf traits related to photosynthesis. Here we determine whether photosynthetic capacity and associated leaf traits are enhanced in intercropped maize (*Zea mays*), a species frequently used in intercrops. We determined leaf photosynthetic capacity (A_{1800}) and leaf traits of maize leaves in different canopy layers and at different growth stages in relay strip intercrops with spring wheat (*Triticum aestivum*) or faba bean (*Vicia faba*) and in the maize sole crop. We also measured the distribution of photosynthetically active radiation (PAR) in the canopies. Intercropping with wheat or faba bean resulted in larger specific leaf area (SLA; thinner leaves), lower specific leaf nitrogen (SLN), and lower A_{1800} of maize leaves during vegetative growth, and differences were larger for maize intercropped with faba bean than wheat, consistent with stronger shading by faba bean than wheat. After the harvest of companion species, maize leaves received more light in the two intercrops than in the sole maize crop, but this did not result in increases in leaf N concentration, SLN, and A_{1800}. Results indicate that shading and lower leaf N caused by relay intercropping maize with an earlier sown species negatively affected leaf photosynthetic capacity of maize leaves. The yield increase of maize in relay intercrops was not due to a higher leaf photosynthetic capacity. Options for mitigating or overcoming these negative intercropping effects are discussed.

Keywords: Interspecific competition, Light distribution, Leaf photosynthetic capacity, Leaf traits, Maize-faba bean intercrop, Maize-wheat intercrop.

4.1 Introduction

Plant production is driven by the photosynthetic conversion of atmospheric CO_2 to structural plant mass, and this process is supported by light energy. Productivity increases can result from greater light capture and (or) from higher light conversion efficiency (Monteith, 1977; Keating and Carberry, 1993). Mixed stands are usually more productive than would be expected on the basis of the productivity of pure stands of the species and their mixing proportion, resulting in a positive net biodiversity effect (Loreau and Hector, 2001). This is true both in natural systems (Isbell et al., 2017; Tilman et al., 2001) and agricultural production systems (Li et al., 2020a,b, 2023; Xu et al., 2020). It is also well established that intercropping can result in enhanced light capture compared to pure stands (Gou et al., 2017a; Zhang et al., 2008). However, there is less information on how leaf photosynthetic capacity is altered in intercropping.

Leaf photosynthetic capacity differs between individuals of the same species in a stand and between different leaves on the same plant according to their position and age (Anten and Hirose, 2003). Compared with shaded leaves, leaves that are well exposed to the sun have a higher nitrogen (N) content per unit leaf area and higher leaf photosynthetic capacity (Lambers et al., 2008; Walters, 2005). Such leaves are thicker, they have a lower specific leaf area (SLA) and they have a greater number of chloroplasts per unit leaf area than shaded leaves (Evans and Poorter, 2001; Oguchi et al., 2003; Pengelly et al., 2010; Poorter et al., 2009).

Maize (*Zea mays*) is frequently used in species mixtures, and maize often contributes substantially to intercropping yield advantages, more than other species do (Li et al., 2020b, 2023). It is therefore relevant to understand the physiological response of maize to intercropping. To date, studies on the response of maize leaf photosynthesis to intercropping have focused on changes in actual rate of leaf photosynthesis (Liu et al., 2018), or the rate of leaf photosynthesis under artificial light at lower than saturated light levels (Nasar et al., 2020, 2021, 2022; Yin et al., 2021). These studies did not elucidate photosynthetic capacity of maize leaves in the field because the photosynthetic capacity is expressed only after a leaf is well adapted to full light, allowing the leaf sufficient time to open the stomata to completely match the CO_2 demand of a well-lit leaf. Previous studies also lack information on the artificial light level and the adaptation time used to obtain leaf photosynthetic rate (Feng et al., 2020; Li et al., 2020d; Ma et al., 2020; Yang et al., 2017). Thus, it is not clear from previous studies whether observed increased rates of leaf photosynthesis in intercropped maize were due to the greater incident radiation, as maize is usually the taller plant in intercrops, or were due to an increased capacity of the leaves to photosynthesize because the leaves were accommodated to higher levels of light, resulting in a greater innate capacity to photosynthesize than leaves that had grown under more shaded conditions.

Intercrops with maize are often grown as relay intercrops with a C_3 species, whereby the C_3 species is sown and harvested earlier than the maize (Li et al., 2020b). However, in warm climates, such as in Sichuan province in China (Feng et al., 2020), maize can be the first-sown species. Which species is sown first greatly affects the competitive relationships and species performance in intercrops (Yu et al., 2016). Relay intercropping is particularly prevalent in China where species are usually grown in narrow strips of a few crop rows to facilitate management as well as interspecific interactions (Li et al., 2020b, 2023). In relay intercrops in which maize is sown later than its companion species, maize plants initially experience shading from the early-sown species (Yu et al., 2016; Zhu et al., 2014). When maize plants overtop the companion species, and more so after the companion species has been harvested, maize has an improved access to light, with light penetrating more deeply into the canopy than in a pure maize stand (Liu et al., 2018). The yield increase of maize in such relay intercrops compared to pure stands has been attributed to the enhanced acquisition of light and soil resources from the strip where the early-sown species is harvested (Liu et al., 2020; Wang et al., 2023; Zhao et al., 2023). However, the improvement in light conditions could also result in accommodation of leaf traits and increased maize leaf photosynthetic capacity after harvest of a companion species, and an increase in photosynthetic capacity could also contribute to yield gain. There is little information on the responses of maize leaves to intercropping in terms of leaf traits and photosynthesis (Gou et al., 2018) and it is therefore unclear to what extent such responses may contribute to the yield performance of maize in relay intercrops.

Previous studies on the response of maize leaf traits to intercropping have mostly been done either in simultaneous intercrops, in which maize and a legume were sown and harvested simultaneously (Li et al., 2019; Liu et al., 2018; Nasar et al., 2020, 2022; Pelech et al., 2022), or in relay intercrops in which maize was the early-sown species (Feng et al., 2020; Nasar et al., 2021; Yang et al., 2017). If maize is sown before the companion species, maize is the dominant species in the intercrop from the beginning, allowing improved resource capture, e.g., nutrients and light, which could explain why it would have enhanced leaf traits compared to sole maize (Feng et al., 2020). In the Netherlands, the oceanic climate allows maize to be sown only after a C_3 species in a relay intercrop. A recent study in the Netherlands reported that in maize-wheat (*Triticum aestivum*) relay intercropping, shading by the early-sown wheat resulted in increased SLA of maize leaves (Gou et al., 2018). They also found that intercropped maize had lower leaf N concentration (LNC) and lower specific leaf N (SLN; N per unit leaf area) than sole maize. Further work is required to elucidate how leaf N and photosynthetic capacity of maize leaves respond to competitive species interactions and the dynamically changing conditions in relay intercropping. This is relevant for understanding how maize achieves overyielding in relay intercropping in the Netherlands and to potentially improve this system.

Leaf traits vary due to species interactions in intercropping that result in modified access to resources (Evers et al., 2019). Feng et al. (2020) and Nasar et al. (2021, 2022) found that maize had increased LNC and SLN when intercropped with soybean (*Glycine max*) or alfalfa (*Medicago sativa*) compared to sole maize. The increases in leaf N may be due to nitrogen fixation by legumes which can fix N from the atmosphere, thus releasing maize from competition for soil N (Brooker et al., 2008; Fujita et al., 1992). It may also be related to a high N input in intercropping compared to sole maize as many studies were conducted in China using an additive N input design, i.e., the N input in the intercrop is the sum of that in the sole crops (Du et al., 2018; Feng et al., 2020; Li et al., 2020b). Under Dutch growing conditions, additive N input in intercropping is not acceptable as there are environmental constraints to N input and N surplus. Hence, to obtain results that are relevant for European growing conditions, the responses of maize photosynthetic capacity and leaf traits need to be determined with agronomic practices that are consistent with European standards for "Good Agricultural Practices", i.e., moderate N input (Baghasa, 2008; FAO, 2003).

In strip intercrops, complementarity and competition between species are most strongly expressed in the border rows of each strip, and so are the responses of plant traits (Li et al., 2020c, 2021; Zhu et al., 2015, 2016). Thus, intercropped plants have different traits according to their position in the strip, which may be true for their leaf photosynthetic traits as well. This border row effect provides an opportunity to assess whether effects in intercropping are due to interactions with the neighboring plants.

In this study, we aim to quantify the extent to which maize leaf photosynthetic capacity and maize leaf traits are affected by resource competition with the companion species, under growing conditions that are relevant for north-west Europe, i.e., with moderate N input and with maize sown later than the companion species such that maize has a competitive disadvantage compared to the companion species. We compare light distribution in the maize canopy in three crop systems: sole maize, maize-wheat and maize-faba bean (*Vicia faba*) relay strip intercropping. We compare traits of leaves at different positions in the maize canopy in the three crop systems and at different times in the season. We distinguish the responses of maize leaf traits in border rows and inner rows in the intercrop strips because border and inner plants experience competitive interactions with different types of neighbors. We selected wheat and faba bean as two contrasting companion species for intercropping with maize because previous work in the Netherlands has shown that wheat-maize relay strip intercropping is a good intercropping system for Dutch growing conditions (Gou et al., 2016). We anticipated that intercropping maize with a legume could have the added benefit of complementary N use due to biological N fixation by the legume (Bedoussac et al., 2015), allowing a reduction in fertilizer input, while legumes are needed for generating more plant based protein for the diversification of food systems and sustainably sourced human diets (van Zanten et al., 2023).

The study tested three hypotheses: (i) During early growth of maize, due to shading by wheat and faba bean, leaves of intercropped maize show shade leaf traits, i.e., larger specific leaf area (SLA), lower specific leaf N (SLN, leaf N content per unit leaf area), and lower leaf photosynthetic capacity than leaves of sole maize. (ii) When maize overtops the companion species, the upper leaves of intercropped maize experience better light conditions and thus show sun leaf traits, with lower SLA, higher SLN, and higher leaf photosynthetic capacity than the upper leaves of sole maize. After the harvest of the companion species, light penetrates more deeply in the maize canopy in intercrops. As a result, leaves in the intercrop from both upper and lower maize canopy show sun leaf traits when compared to leaves of the same rank in sole maize. (iii) In maize-wheat intercrops, competition for N between maize and wheat reduces leaf N concentration (LNC) and SLN of maize compared to plants in sole maize, whereas LNC and SLN of maize in maize-faba bean intercropping increase compared to plants in sole maize due to complementary N capture between maize and faba bean.

4.2 Materials and Methods

4.2.1 Experimental design

Measurements were conducted at Droevendaal Experimental Farm, Wageningen, the Netherlands (51° 59’ 20” N, 5° 39’ 16” E) in 2018 and 2019 under agronomically realistic growing conditions in the field (Wang et al., 2023). We considered three cropping systems: sole maize (*Zea mays* L. cv. LG30.223), a relay strip intercrop of maize and spring faba bean (*Vicia faba* L. cv. Fanfare), and a relay strip intercrop of maize and spring wheat (*Triticum aestivum* L. cv. Nobless). In both intercrops, species were grown in 1.5 m-wide strips, with three rows of maize or six rows of wheat or faba bean per strip (Fig. 4.1). Each species strip had two border rows (one at each side of the strip) while the other rows are inner rows.

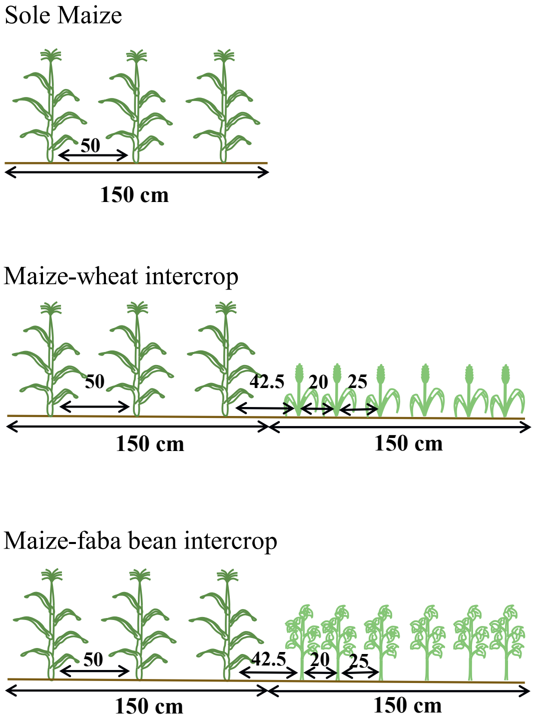

Fig. 4.1 Schematic illustration of row pattern in sole maize and maize intercropped with spring wheat or spring faba bean. Maize was sown at 50 cm row distance in strips comprising three rows. The resulting 1.5 m-wide maize strips were alternated with 1.5 m-wide strips of wheat or faba bean. Wheat and faba bean were planted at a row distance of 25 cm, except for the border rows of each 1.5 m strip, which were moved “inward” into the strip by 5 cm at both sides to allow space for the wheels of the tractor used for sowing. Each species in the intercrop had a relative density of 0.5, thus the intercrops followed a replacement design.

Maize was grown at a 50 cm row distance. In each wheat or faba bean strip, the row distance between inner rows was 25 cm, but the distance between the border rows and the neighboring inner rows was reduced to 20 cm to allow passage of the tractor wheels (track width 133 cm) without causing damaging the plants in the outer rows of the strip. We used a replacement design to avoid confounding intercropping effects with effects of a change in plant density. To obtain a replacement design (de Wit, 1960), the distance between the border rows of maize and wheat or faba bean was 25 + 17.5 = 42.5 cm, where 25 cm was half the row distance of maize and 17.5 cm was obtained by summing 12.5 cm (half the row distance of wheat or faba bean) and 5 cm (the distance over which the border rows in a wheat or faba bean strip was moved "inward" into the strip) (Fig. 4.1). The relative density (density in the intercrop relative to the sole crop; van der Werf et al., 2021) for all species was thus equal to 0.5.

In 2018, wheat was sown on 21 March and harvested on 17 July, faba bean was sown on 21 March and harvested on 30 July, and maize was sown on 4 May and harvested on 10 September (Fig. 4.2). Due to the cool and wet spring of 2019, wheat was sown on 1 April and harvested on 8 August, faba bean was sown on 1 April and harvested on 14 August, and maize was sown on 7 May and harvested on 18 September. Maize was sown at a density of 10 seeds m^{-2} and faba bean was sown at a density of 44 seeds m^{-2} in both years. The sowing density of wheat was 383 seeds m^{-2} in 2018 and 369 seeds m^{-2} in 2019. In 2018, the plot size was 9 m in east-west × 11 m in north-south directions. Each intercrop plot comprised six species strips (three of each species). In 2019, the plot size was 12 m in east-west × 11 m in north-south directions for the sole maize crop, and 15 m in east-west × 11 m in north-south directions for the intercrop. Each intercrop plot comprised ten species strips (five of each species). The row orientation was approximately north-south in both years. The experiment had a randomized complete block design with six replicates in 2018 and four replicates in 2019. Photosynthesis measurements were made in all replicates.

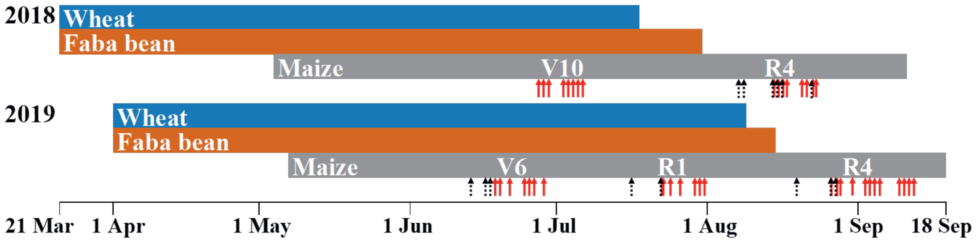

Fig. 4.2 Growing periods of wheat, faba bean, and maize in 2018 and 2019. Measurements on light distribution were made during maize grain-filling (R4) in 2018, and during six-leaf (V6), silking (R1), and R4 in 2019 (black arrows). Photosynthesis measurements were made at ten-leaf (V10) and R4 in 2018 and at V6, R1, and R4 in 2019 (red arrows).

Soil at the experimental site was sandy with 3.4% organic matter and a pH of 5.7. While the climate in the Netherlands is oceanic temperate with mostly cool summers, the summers of the measurements were hot and dry; hence, sprinkler irrigation was given from June to August, 13 times in 2018 and 9 times in 2019, to avoid drought stress (See Figs S4.1-S4.3 for data on daily air temperature, daily photosynthetically active radiation, and monthly precipitation). Potassium was applied in the form of $K_2SO_4{\cdot}MgSO_4$ at a rate of 105 kg K_2O ha^{-1} in both years. Phosphorus was applied in the form of $Ca(H_2PO_4)_2{\cdot}H_2O$ at a rate of 67.5 kg P_2O_5 ha^{-1} in 2018 and 78.75 kg P_2O_5 ha^{-1} in 2019. As soil P levels were high these rates were based on expected uptake. Potassium and phosphorus were applied homogeneously throughout the field before sowing. Mineral N in the 0-30 cm soil layer before sowing was 22 kg N ha^{-1} in 2018 and 12 kg N ha^{-1} in 2019. Supplementary N was supplied in the form of $NH_4NO_3{\cdot}CaMg\ (CO_3)_2$. Total N applied was 20 kg N ha^{-1} in faba bean, 125 kg N ha^{-1} in wheat, and 170 kg N ha^{-1} in maize in both years. N fertilizer in wheat and maize was split into two doses (Table S4.1). In intercrop plots, fertilizer was applied within species strips such that plants in the intercrop and sole crop received the same amount of fertilizer. As the intercrops comprised 50% area of both species, the N input into intercrops per unit intercrop area was equal to the average of the input in the sole crops of the component species. Weeds were controlled chemically and manually as needed. Diseases and pests were managed chemically (Table S4.1).

4.2.2 Leaf traits

Photosynthetic capacity of maize leaves was measured to quantify the effect of interspecific competition during the co-growth period and when early-sown species in relay intercrops were harvested. Photosynthetic capacity of the youngest full-grown leaf during co-growth with the companion species was measured at V10 in 2018 and at V6 in 2019 (Fig. 4.3). In both years, photosynthesis was furthermore measured at maize grain filling (R4), i.e., after harvest of the companion species. In 2018, we made the measurements at R4 on the ear leaf (leaf 10) while in 2019, we measured leaf 14 and a much older leaf (leaf 7) as this older leaf might show a greater contrast between the sole crop and the intercrop due to its lower position in the canopy as light is penetrating more deeply in the maize canopy in the intercrop than in sole maize. Furthermore, we added an intermediate measurement at maize silking stage (R1) in 2019, after maize had overtopped its companion species in both intercrops while these companion species were still present (Fig. 4.3). At R1, both a lower canopy leaf (leaf 7) and a upper canopy leaf (leaf 14) were measured.

We did not make photosynthesis measurements on rainy days or on days with a maximum temperature above 30 °C. In each sole crop plot, one plant in 2018 and three plants in 2019 were randomly selected. In each intercrop plot, plants from the western border row of one maize strip and the adjacent inner maize row were selected. One plant per row was selected in 2018 and three plants per row in 2019. Measurements were made in each of the six blocks in 2018 and each of the four blocks in 2019. The location of the selected plants was at least 1 m away from the plot edge. Measurements were made on fully developed leaves.

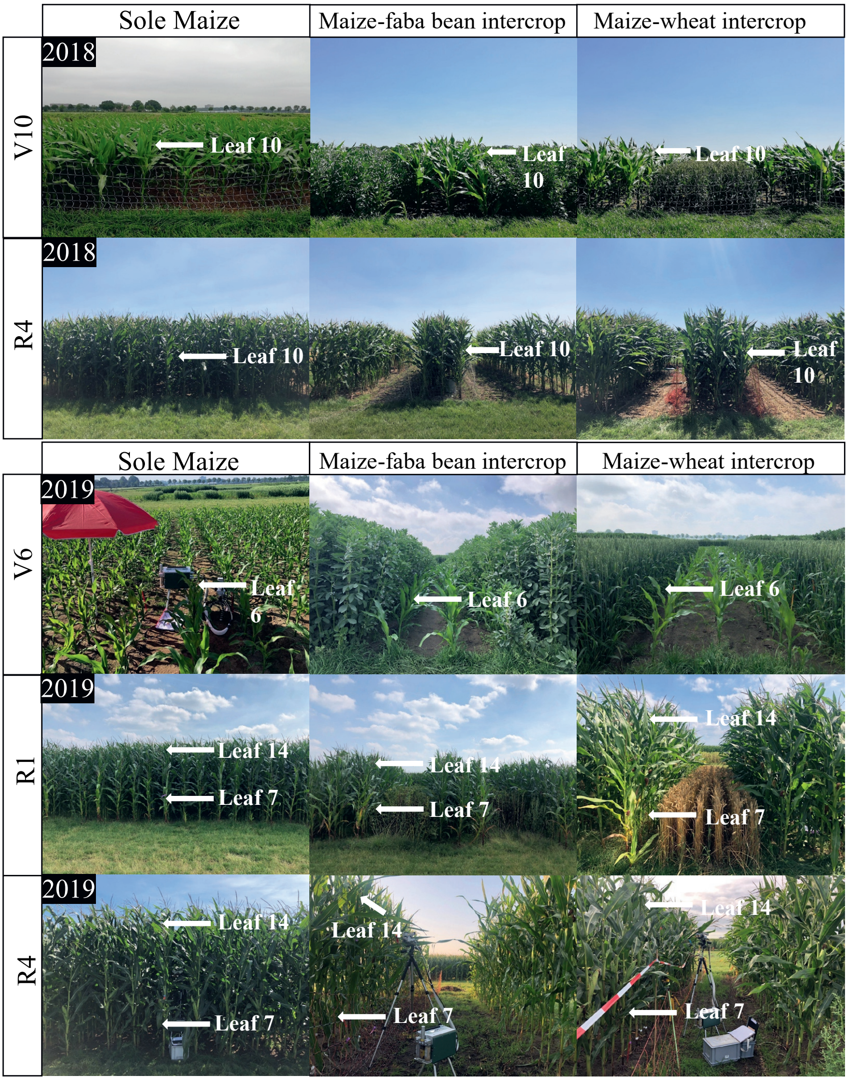

Fig. 4.3 Maize development stages and leaf positions for gas exchange measurements in sole maize, the maize-faba bean intercrop, and the maize-wheat intercrop in 2018 and 2019. White arrows represent the approximate positions of the measured leaves in the maize canopy.

In 2018, we made gas exchange measurements using two portable photosynthesis systems, the LI-COR 6400XT and LI-6800 (Li-Cor Inc., Lincoln, USA). In 2019, we used the LI-COR 6400XT at V6 and R4, and the LI-COR 6400XT and LI-COR 6800 at R1. When the two LI-CORs were used simultaneously, all measurements in one block were made with the same instrument. The LI-COR leaf chamber provided a constant irradiance of 1800 μmol m^{-2} s^{-1} and a constant CO_2 level of 400 μmol mol^{-1}. The photosynthetic rate (A_{1800}; μmol m^{-2} s^{-1}) of maize leaves under this irradiance is close to the light-saturated rate of photosynthesis, or photosynthetic capacity (Yin et al., 2011). The adaptation time for each measurement was 30 min, allowing A_{1800} to reach steady state. Both A_{1800} and stomatal conductance for water (g_{sw}; mol m^{-2} s^{-1}) were then recorded. Leaf temperature during measurements was maintained at 25 °C. Leaf-to-air vapour pressure difference was within the range of 1.0-1.5 kPa.

Leaf blades were removed for further analysis after the gas exchange measurements. Three leaf discs (2.16 cm^2 per disc) were punched around the position at which the gas exchange measurement was made. SPAD measurements were made at ten points on each disc, using a SPAD Meter (SPAD-502, Minolta Camera, Tokyo, Japan) to measure greenness as a proxy for chlorophyll content. The mean of the ten points was recorded as the SPAD value for the disc. The fraction of light absorbed by the leaf, absorptance (*Abs*), was calculated as one minus transmittance minus reflectance. The transmittance and reflectance of each disc was measured in the spectral range of the light source of the gas exchange measurement (red: 625-645 nm; blue 455-475 nm), using a Spectrometer (STS-VIS miniature Spectrometer, Ocean Optics, USA). The midrib was removed and the area of the remaining blade was measured with a leaf area meter (LI-3100 area meter, Lincoln, USA). The remaining blade and the three discs were then oven-dried at 70 °C until constant weight to determine dry weight. Thereafter the three discs were ground and the leaf N concentration (LNC; mg N g^{-1} leaf) was analyzed using an element C/N analyzer (Flash 2000, Thermo Scientific) based on the Micro-Dumas combustion method. Specific leaf area (SLA; cm^2 leaf g^{-1} leaf) was calculated using the area and dry weight of the blade without the midrib. Specific leaf N (SLN; g N m^{-2} leaf) was calculated as LNC divided by SLA.

4.2.3 Light distribution

We measured light distribution using a SunScan canopy analysis system (SunScan SS1, Delta-T Devices Ltd, Cambridge, UK) to determine relationships between leaf traits and exposure to incoming light. In 2018, these measurements were made at maize R4, and in 2019 they were made at V6, R1, and R4 (Fig. 4.2). The measurements were made in one 1.5 m strip in each sole crop plot, and in one intercrop strip comprising two 1.5 m species strips in each intercrop plot. The 1 m long SunScan probe with 64 quantum sensors was placed parallel to the rows in the canopy (Fig. S4.5), while a Beam Fraction Sensor (BFS, one quantum sensor) simultaneously recorded the incoming light. The probe was placed at different heights from the bottom to the top of the canopy in steps of 25 cm, and from west to east across the rows in steps of 25 cm, covering the whole strip width and canopy height (Fig. 4.4). Measurements were conducted with either clear sky or steady overcast sky, within two hours from solar noon (around 11:45 to 15:45) (for details see in Figs S4.6 to S4.9).

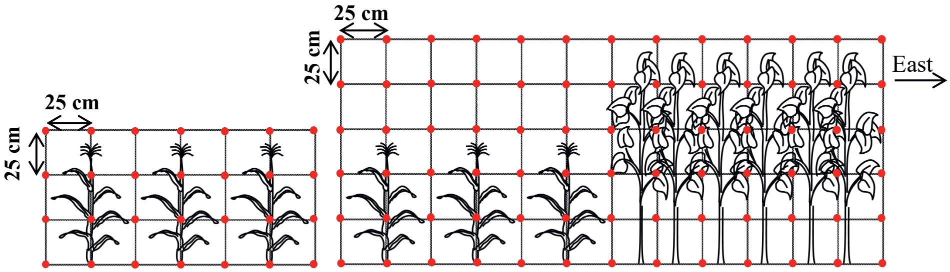

Fig. 4.4 Schematic illustration of the positions of the SunScan probe in the canopy in a maize strip in sole maize and in an intercrop strip in maize-faba bean intercrop at maize V6 stage in 2019. Every red dot was a measuring point with the probe oriented parallel to the rows. The measurements were made at every 25 cm distance in the vertical direction, from the ground level to the top of the canopy, and at 25 cm intervals horizontally, from west to east across the rows. The measurement design in the maize-wheat intercrop was similar to that in the maize-faba bean intercrop (Fig. S4.4).

As the probe and the reference Beam Fraction Sensor (BFS) gave slightly different readings, a correction factor is needed to compare them and determine transmission. Therefore, before light distribution measurements in a plot, the probe and BFS were placed horizontally in uniform sunlight and three readings were taken to obtain this correction factor. The corrected probe readings were then used to calculate the fraction of transmitted photosynthetically active radiation (fPAR) at different positions in the canopy. fPAR represents the light intensity detected at any position relative to the light intensity above the canopy. Visual representations of fPAR distribution in crop canopies were generated in R version 4.2.1 (R Core Team, 2023), using the "ggplot2" package (Wickham, 2016).

4.2.4 Grain yield

Maize was harvested manually at maturity. In each plot, plants from each row in one strip and at least 1 m away from the edge of the plot were harvested over a 4 m row segment. The grain yield was determined after separating the grain from the cobs and drying the grain at 105 °C for 48 hours. The effect of intercropping on grain yield per maize plant was characterized by overyielding (OY_m, %) (Li et al., 2011c; Wu et al., 2022; Zhao et al., 2023):

$$\mathrm{OY_m}\ (\%) = \frac{Y_{\mathrm{im}} - 0.5Y_{\mathrm{sm}}}{0.5Y_{\mathrm{sm}}} \times 100 \qquad \text{Eq. 4.1}$$

where Y_{im} is the grain yield (per unit area of the whole intercrop) of maize in the intercrop; Y_{sm} is the grain yield (per unit area of the sole crop) of sole maize; 0.5 is the land area ratio of maize in the intercrop in this study, which was calculated as the strip width of maize (1.5 m) divided by the width of intercrop strip (3 m; comprises two species strips); $0.5 \times Y_{\mathrm{sm}}$ is the expected yield for maize in the intercrop. As the sowing density (per m^2 maize strip) was identical in pure stands and the intercrop, this metric indicates by which percentage the yield per plant in intercropping exceeds that in the sole crop.

4.2.5 Statistics

We considered border and inner row maize separately when studying the responses of maize leaf traits in intercropping. Linear mixed effect models were used to compare means of five treatments (sole maize, maize-faba bean border, maize-faba bean inner, maize-wheat border, maize-wheat inner) of leaf traits. Comparisons were made for each leaf position at each growth stage and in each year. In the analyses, treatment was a fixed effect and block a random effect. Models were fitted using the function lmer from the “lme4” package (Bates et al., 2015) in R (R Core Team, 2023). Significance of the fixed effects was determined with analysis of variance (ANOVA) ($P = 0.05$), using the Anova function from the “car” package (Fox and Weisberg, 2019). Pairwise comparisons were conducted using Fisher’s Least Significant Difference (LSD) in the “emmeans” package (Lenth, 2021).

To explore relationships between A_{1800} and SLA or LNC across treatments, correlations between A_{1800} and SLA, and A_{1800} and LNC were determined for each stage and leaf position per year, using the combined data from the five treatments. The R base function lm (R Core Team, 2023) was used.

4.3 Results

4.3.1 Maize leaf traits

Measurements of leaf traits were made in more leaf layers and at more maize growth stages in 2019 than in 2018. The 2019 data are presented first. The 2018 data are given thereafter to evaluate consistency.

At V6, A_{1800} of the highest leaf, leaf 6, was lower in the border row maize than in inner row maize or sole maize (Fig. 4.5A). This lower A_{1800} in the border row was associated with shade leaf traits such as larger SLA and lower SLN compared to sole maize (Fig. 4.5E and G; for substantial correlation between SLN and SLA see Fig. S4.10). These shade responses of SLA and SLN were stronger in border row maize in maize-faba bean than in maize-wheat, which was associated with comparatively stronger shading of maize in maize-faba bean than in maize-wheat (see below). On the other hand, border row maize in maize-wheat had lower LNC than sole maize and both border and inner row maize in maize-faba bean, indicating that competition for N was more severe in the intercrop with wheat than in the intercrop with faba bean (Fig. 4.5F).

At R1, leaf 7 was a lower canopy leaf, with seven additional leaves above it (Fig. 4.3). Leaf 7 of inner row maize in maize-wheat had higher A1800 than the same leaf in sole maize (Fig. 4.5A). In intercrops, maize leaf 14 was fully above the canopy of faba bean or wheat. Leaf 14 had higher A1800 than leaf 7 in all treatments. No differences in A1800 of leaf 14 were found among treatments. Leaf 14 showed sun traits in inner row maize in maize-wheat, having lower SLA than in sole maize (Fig. 4.5E). However, leaf 14 in border row maize in maize-wheat had lower LNC than the same leaf in sole maize (Fig. 4.5F). Both LNC and SLN of leaf 14 were reduced in border row maize in maize-faba compared to sole maize (Fig. 4.5F and G). Thus, sun traits occurred in the upper leaves of intercropped maize, but the leaf N was reduced in border row maize in both intercrops, and A1800 of leaf 14 was not increased in any intercrop treatment.

At R4, faba bean and wheat had been harvested. In intercrops, the extra space and resources (i.e., light, water, nutrients) were exclusively available for maize plants. No differences in A1800 of maize leaves were found among treatments (Fig. 4.5A), but leaf traits did differ between treatments. Leaf 14 had lower LNC and SLN in both border and inner row maize in both intercrops than in sole maize (Fig. 4.5F and G; for substantial correlation between SLN and LNC see Fig. S4.14). Thus, both leaf N and A1800 of maize leaves were not increased by intercropping with faba bean or wheat.

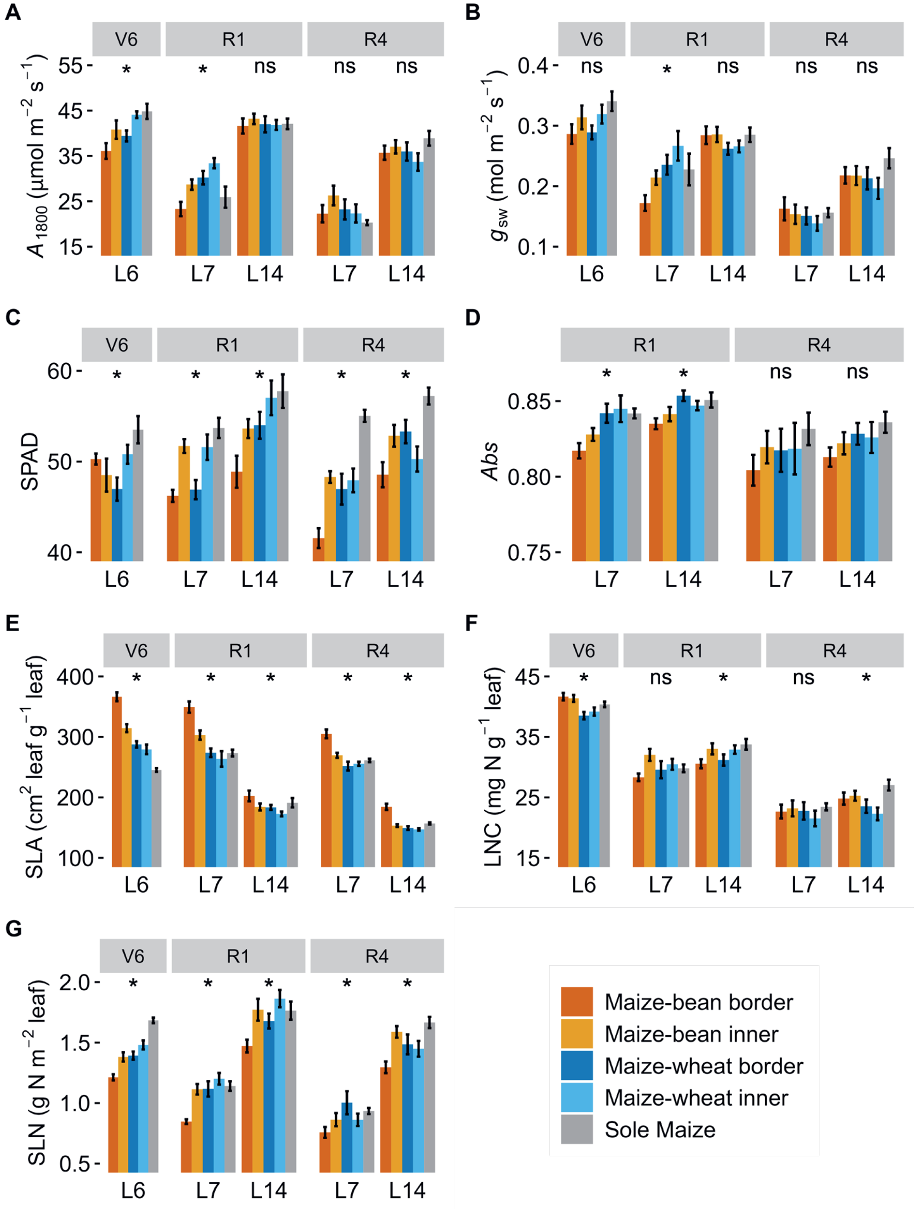

Fig. 4.5 Leaf traits of maize in different treatments at different growth stages and leaf positions in 2019. L6: leaf 6; L7: leaf 7; L14: leaf 14; A_{1800}: leaf photosynthetic capacity; g_{sw}: stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA: specific leaf area; LNC: leaf nitrogen concentration; SLN: specific leaf nitrogen. Error bars indicate the standard errors of means. In each stage and leaf position, significance of treatment effects was determined using ANOVA (* = $P < 0.05$; ns = $P > 0.05$). Details showing the pairwise comparison are presented in Table S4.2.

Findings in 2018 were consistent with those in 2019. During the vegetative phase (V10), the border row maize next to faba bean showed strong shade responses, having lower A_{1800} (Fig. 4.6A), larger SLA (Fig. 4.6E), and lower SLN (Fig. 4.6G) than leaf 10 in sole maize and other intercrop treatments. At R4, maize A_{1800} was not increased in both intercrops after the early-sown species had been harvested.

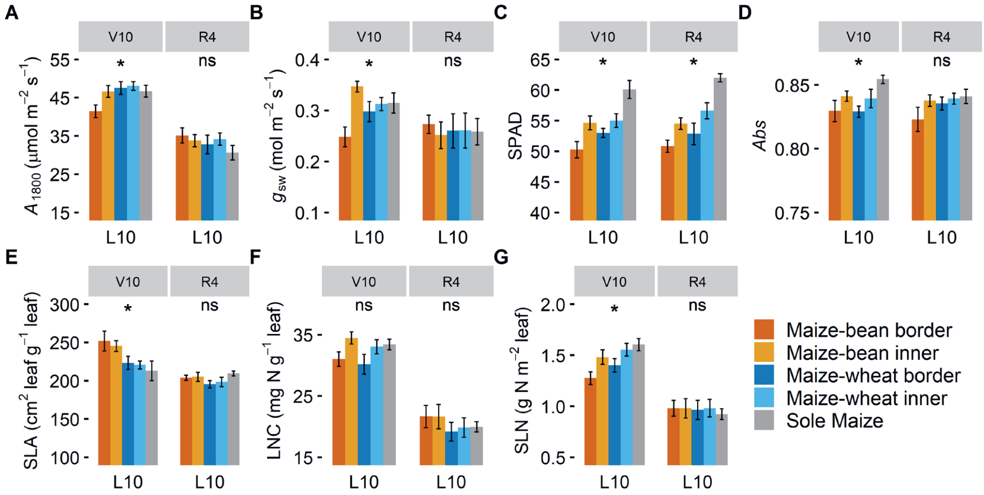

Fig. 4.6 Leaf traits of maize leaf 10 (L10) in different treatments at maize V10 and R4 stages in 2018. In each stage and leaf position, significance of treatment was determined, using ANOVA with treatment as the fixed effect (* = $P < 0.05$; ns = $P > 0.05$). Details showing the pairwise comparison are presented in Table S4.3.

4.3.2 Relationships between leaf photosynthetic capacity and the other leaf traits

We analyzed associations between A_{1800} and SLA or LNC to assess possible causal pathways for effects of intercropping on leaf photosynthetic capacity.

A negative correlation between A_{1800} and SLA was found in leaves at maize V6 and R1 stages in 2019, and at maize V10 stage in 2018 (Fig. 4.7A,C,E, and K), indicating shade response (increased SLA; thinner leaves) as a mechanism for lower leaf photosynthetic capacity during early maize development in intercrops. A positive correlation between A_{1800} and LNC was found in leaf 14 at maize R1 stage, and in leaves at maize R4 stage in 2019 and 2018 (Fig. 4.7F,H,J, and N), indicating competition for N between maize and companion species as a possible mechanism for lower leaf photosynthetic capacity in intercropped maize during later maize development.

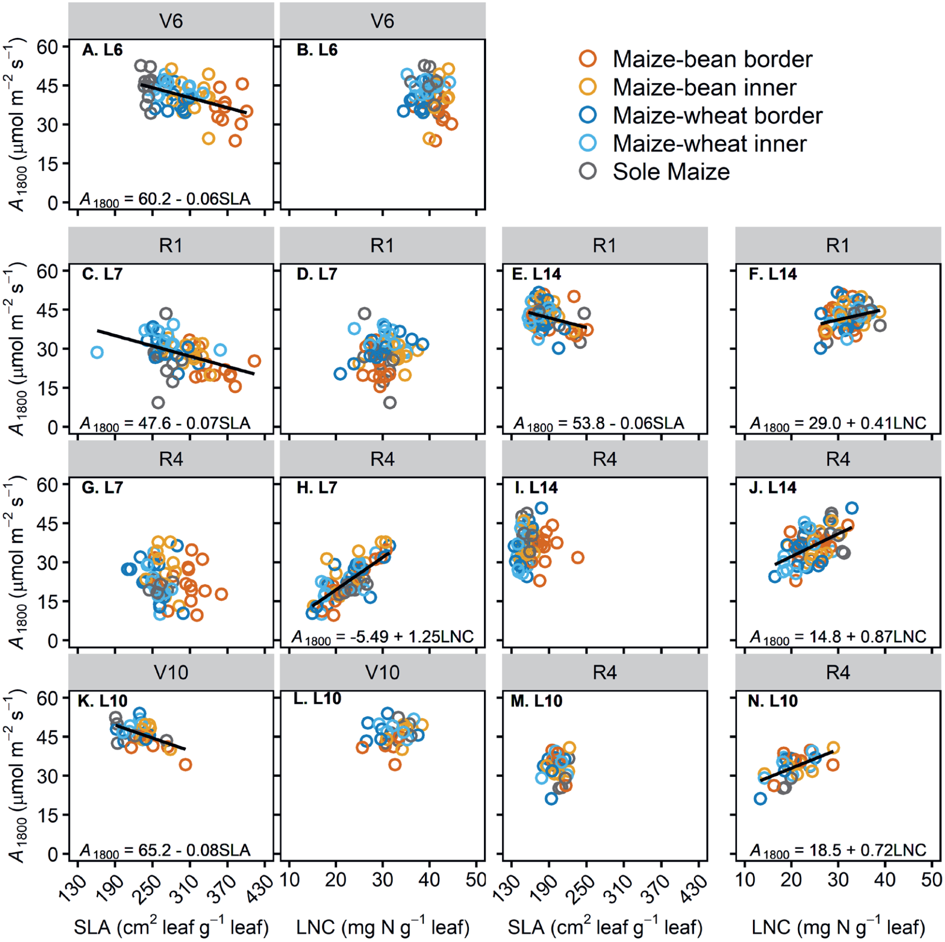

Fig. 4.7 Relationships between leaf photosynthetic capacity (A_{1800}) and specific leaf area (SLA), and A_{1800} and leaf nitrogen concentration (LNC) in 2019 (A to J) and 2018 (K to N). In each stage and leaf position per year, a linear regression was fit through the combined data from the five treatments. Only the lines for regressions with $P < 0.05$ are presented. Details on coefficients (± SE) and *P*-values of the regressions are presented in Table S4.4.

4.3.3 Distribution of PAR in maize canopy

In 2019, at maize V6 stage, maize leaves in border rows with faba bean experienced heavier shading than inner row leaves or leaves in other crop systems (Fig. 4.8A to C). At maize R1 stage, upper leaves in the maize canopy in intercrops were above the wheat or faba bean canopy and experienced better light conditions than leaves with the same rank in sole maize (Fig. 4.8D to F). At R4 in both years, intercropped maize showed a deeper penetration of radiation into the canopy compared to sole maize (Fig. 4.8G to K).

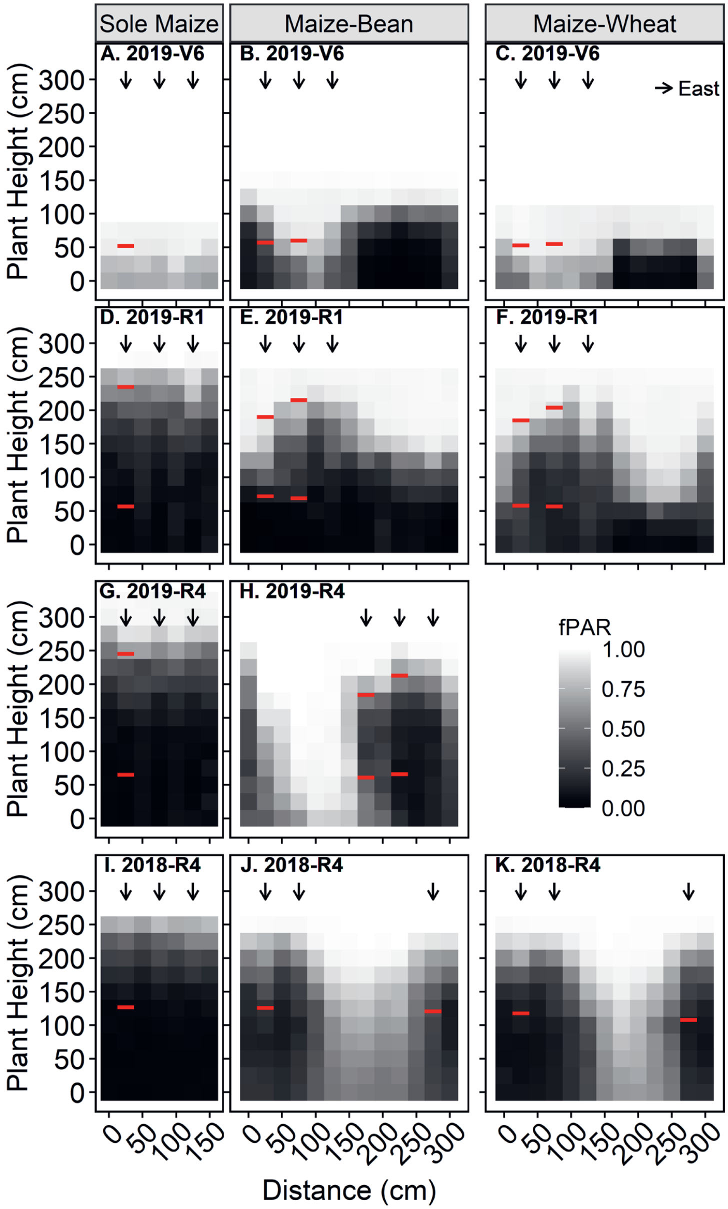

Fig. 4.8 Light distribution in sole maize, maize-faba bean intercropping, and maize-wheat intercropping at maize V6, R1, and R4 stages in 2019 (A to H), and at maize R4 stage in 2018 (I to K). The arrows indicate the position of maize rows. The red lines indicate the position of photosynthesis measurements. The fraction of photosynthetic active radiation (fPAR) represents the light intensity detected at each position relative to the light intensity above the canopy. The fPAR at each position indicates the mean value across blocks. Details showing the light distribution and weather conditions in each block are presented in Figs S4.6 to S4.9.

4.3.4 Maize yield

Maize in maize-wheat intercropping produced 27.3% (2018) ($P = 0.056$) and 16.8% (2019) ($P = 0.005$) more grain yield per plant than maize in pure stands, while the maize yield was not significantly improved in maize-faba bean intercropping (Table 4.1).

Table 4.1 Overyielding (OY_m) of maize grain yield and the standard errors (SE) of means in maize-faba bean and maize-wheat intercropping in 2018 and 2019. *P*-values report the outcome of the Student's t-test to check if the value of OY_m was significantly different from zero ($P = 0.05$).

Year	Companion species	OY_m (%)	SE	*P*
2018	Faba bean	8.3	9.8	0.434
	Wheat	27.3	11.0	0.056
2019	Faba bean	1.7	4.4	0.723
	Wheat	16.8	2.3	0.005

4.4 Discussion

In this study we tested three hypotheses on the effects of intercropping on the photosynthetic capacity of maize leaves in relation to specific leaf area (SLA), leaf N concentration (LNC), and leaf N content per unit leaf area (SLN). Data are in agreement with the first hypothesis that early formed maize leaves respond to shading by an earlier sown companion species, i.e., wheat or faba bean. At maize V6 stage in 2019, shading from wheat and faba bean resulted in larger SLA and lower SLN of maize leaf 6 compared to sole maize. Border row maize in both intercrops had decreased A_{1800} in leaves with shade traits (Fig. 4.5A,E, and G). These responses were also found at maize V10 stage in 2018: the shaded leaf 10 of border row maize in the maize-faba bean intercrop had larger SLA, lower SLN, and lower A_{1800} than sole maize (Fig. 4.6A,E, and G).

The second hypothesis posited that intercropped maize exhibits sun traits in upper leaves formed after maize overtops the companion species, and exhibits sun traits in both upper and lower leaves after companion species harvest. This hypothesis was confirmed for leaf 14 in the inner rows of maize strips when grown with wheat at maize R1 and R4 stages. These leaves had lower SLA than corresponding leaves in sole maize (Fig. 4.5E). However, sun leaf adaptations were not found at other leaf positions and at other developmental stages in intercropping.

We found evidence supporting the third hypothesis that maize leaves have lower leaf N when grown with wheat, due to competition for N between the two cereals, but we did not find evidence that maize leaves grown with faba bean have higher leaf N than leaves of sole maize. In contrast to expectation, LNC and SLN of leaf 14 in border rows of intercrops with faba bean were lower than in sole maize at R1. The same effect was found for SLN at R4 in 2019 (Fig. 4.5F and G). The hypothesis that complementary N use between maize and faba bean increases leaf N was thus not confirmed under the conditions of this study.

The results show that competition for light and N with an early-sown species altered leaf photosynthetic capacity and photosynthesis-related leaf traits of maize in relay intercrops. The effects were most apparent in border-rows in the intercrop. This is expected because plants in border rows are directly exposed to resource competition with the companion species (Wang et al., 2020). Despite some differences in the experimental protocols between the two years, consistent patterns were observed: (i) a shading effect on leaf traits during early maize growth in both intercrops, (ii) evidence for competition for N in maize-wheat intercropping but (iii) lack of evidence for relaxation of competition for N in maize-faba bean intercropping, and (iv) no substantial recovery of maize leaf N and leaf photosynthetic capacity after harvest of the companion species, despite improved light conditions.

Maize experienced lower light levels during its early growth in relay intercrops than in a pure maize stand (Fig. 4.8), confirming earlier studies in the Netherlands (Gou et al., 2017a; Zhu et al., 2014). The maize leaves accordingly showed shade traits during their early growth, such as a large SLA (Figs 4.5 and 4.6), consistent with earlier work (Gou et al., 2018). This shade response was associated with a decreased leaf photosynthetic capacity in border row maize in both intercrops (Fig. 4.7). Thus, the shading resulting from interspecific light competition negatively affected maize leaf photosynthetic capacity. The effects on SLA were stronger in faba bean-maize than in wheat-maize, indicating that light competition was stronger with bean than with wheat. This is consistent with the comparatively tall stature of faba bean plants compared to wheat and maize (Fig. S4.17) and the shade cast by faba bean (Fig. 4.8).

Likewise, in studies on maize-soybean intercropping, shading by the taller maize plants resulted in thinner leaves and lower leaf photosynthetic capacity of soybean leaves compared to the sole crop (Gong et al., 2015; Yao et al., 2017). They also found the shaded intercropped soybean leaves had relatively more chlorophyll *b* to increase the capacity for light harvesting. In contrast to soybean (a C_3 species), maize as a C_4 species is less shade tolerant. A lack of differences in the absorptance values between treatments was found (Fig. 4.5D; Fig. 4.6D), indicating that the light harvesting of maize leaves was hardly increased when shaded in intercropping.

In studies on simultaneous intercrops, where maize and comparatively low stature species were sown simultaneously, the light condition of maize was improved, and maize leaf photosynthetic rate measured at light levels lower than 1800 μmol m^{-2} s^{-1} was higher than in sole maize (Li et al., 2019; Liu et al., 2018; Nasar et al., 2022). In the subtropical conditions of Sichuan province, China, where maize is sown before soybean in a relay intercropping sequence, maize had a higher LNC and higher leaf photosynthetic rate than sole maize (Feng et al., 2020). The study of Feng et al. (2020) was done using an experimental design that is different from ours in several respects: (i) they used an additive design for species density, maintaining the same number of plants per ha in the intercrop as in the sole crop, (ii) they used an additive N input strategy in which the fertilizer input in the intercrop is the sum of the fertilizer inputs in the two component sole crops, and (iii) the relay sequence is different. It is therefore not possible to attribute the difference in photosynthetic response of maize in the Chinese study and our study to any particular difference in experimental conditions. Results suggest that the design principles of our study (replacement design and substitutive N fertilizer strategy) are not conducive to maximal photosynthetic performance of maize; however, the principles used in China, particularly the high N input in intercropping, may not be acceptable in Europe because of environmental policies to reduce N leaching. In addition, after winter in western Europe, C_3 crops are sown before maize, not the other way around.

In our trials, maize overtopped faba bean only after the appearance of the tassel (Fig. S4.17). Thus maize plants in intercrops grew in a shady environment during most of the vegetative growth. Plants that grow in a shady environment invest relatively more assimilates in leaf area and relatively less in root length (Ryser and Eek, 2000). This might result in N deficiency during later growth. The leaf N of intercropped maize could be thus reduced, which would then constrain leaf photosynthetic capacity (Fig. 4.7). The findings suggest that interspecific light competition during early growth of intercropped maize may lead to a cascade of physiological effects that result in suppressed N uptake and ultimately decreases leaf photosynthesis of intercropped maize during later growth.

In maize-wheat intercropping, N acquisition of intercropped maize is constrained as wheat is more competitive for N due to its fine root system and earlier sowing than maize (Gou et al., 2018; Li et al., 2001a; Liu et al., 2015). In the maize-wheat intercrop, the reduced leaf N reflected the effect of N competition with wheat. Despite the N fixation ability of the legume (Bedoussac et al., 2015), light competition with a vigorous legume, like faba bean in our study, can result in a constrained access of maize to fertilizer N. The small amount of fertilizer N (20 kg ha^{-1}) applied in the faba bean strip was most likely used up during its early growth. In contrast to high-input strip intercropping (Li et al., 2011c), in which cereals have extra access to soil N because of N fixation of legumes, the agronomically appropriate low fertilizer input to the legume in our trials means that maize could in this system not benefit from relaxed competition for N. In the experiments conducted in China (Liu et al., 2020; Ma et al., 2020), an extra N application was made at maize tasseling in both relay intercrops and sole crops, to allow additional N uptake. In our trials, such extra application at tasseling could have allowed intercropped maize to increase N uptake and thus better exploit the increased light resource in the late maize growing season.

The high performance of maize in relay intercropping has been related to exploitation by maize of the extra light and nutrient resources that become available after the harvest of the early-sown companion species (Li et al., 2001b; Wang et al., 2023; Zhao et al., 2023). Maize is said to “recover” from competition in this type of relay system, a phenomenon referred to as the competition-recovery principle (Zhang and Li, 2003). Previous studies have indicated that overyielding of intercrops increased with temporal niche differentiation between the two species (Xu et al., 2020; Yu et al., 2015; Zhao et al., 2023). In the experiment of Ma et al. (2020), winter wheat was harvested before maize tasseling, creating a relatively early access for maize to extra resources, increasing leaf photosynthetic rate after the harvest of wheat. However, a comparison with the study of Ma et al. (2020) is difficult to make as it is unclear what light levels they used to obtain leaf photosynthetic rate. In our trials, both wheat and faba bean were harvested after the maize tassel appeared. As no recovery is apparent from our observations it does seem plausible that timing of the release from competition is important for the resulting leaf traits. This may be further analyzed in future research.

An early harvest of the companion species in relay intercrops may be beneficial for maize leaf photosynthetic capacity. This can be achieved by using a winter-sown rather than a spring-sown cereal or legume. Using a late maturing maize variety if the season length allows could also be a recommendation to relax intercropped maize from competition early when maize root system and foliage are growing. However, the window of opportunity in the Netherlands is small due to the relatively cool climate, where the temperature sum may not be sufficient for full maturation of a late maturing maize crop. Use of late maturing varieties is, however, well possible in warmer climates than the Netherlands.

We found increased maize yield per plant in the maize-wheat intercrop compared to sole maize, but no significant overyielding of maize in the maize-faba bean intercrop (Table 4.1). We also found that leaf photosynthetic capacity of intercropped maize was negatively affected by resource competition with both faba bean and wheat. In addition, maize plants in both intercrops did not have increased leaf area per plant as compared to the sole crop during the season (Fig. S4.18). Thus, an enhanced photosynthetic capacity of the intercropped maize canopy would not be expected. We conclude that changed photosynthetic capacity of maize leaves in intercropping is not a plausible factor for the observed higher maize yields under the conditions of this study.

In the same experiments Wang et al. (2023) reported that relay intercrops involving maize had advantages in both land productivity and absolute yield gain compared to sole crops, due to temporal complementarity between component species in intercrops. In relay intercropping, high productivity of intercrops is in many instances associated with an increased accumulated light capture compared to the sole crop, resulting from complementarity in space and time to companion species (Gou et al., 2017a; Yu et al., 2015; Zhang et al., 2008). The observed overyielding of maize in the maize-wheat intercrop could be explained by increased light capture when maize overtopped wheat and after the harvest of wheat, while this may not have been as much the case in the maize-faba bean intercrop as faba bean was taller than wheat and harvested later than wheat. Maize plants intercropped with faba bean experienced a relatively longer period of shading and had less time to capture extra light. Thus those maize plants may have had just sufficient increase in accumulated light capture compared to sole maize to compensate for the earlier reduction in light capture due to faba bean shading. Further analysis could be conducted to quantify the accumulated light capture of maize in intercrops to explain the yield performance, using models of light interception in heterogenous canopies (Gou et al., 2017a; Li et al., 2021; Zhu et al., 2015).

4.5 Conclusion

We compared maize leaf traits related to photosynthesis in sole maize, maize-faba bean, and maize-wheat relay strip intercropping in the Netherlands. Faba bean was taller than wheat causing heavier shading on maize than wheat did. Accordingly, shade responses were stronger in maize intercropped with faba bean than in maize-wheat. These shading responses comprised larger SLA, lower SLN, and lower A_{1800}. Intercropping with wheat or faba bean reduced maize LNC and SLN. Relaxation of competition after harvest of the early-sown species did not result in increased leaf N and increased A_{1800} in maize; thus we did not observe the recovery of leaf photosynthetic capacity that we hypothesized. We conclude that maize leaf photosynthetic capacity was not substantially improved in relay strip intercropping due to competition for light and soil N with the earlier sown companion species. Responses of photosynthetic capacity of maize leaves did therefore not substantially contribute to higher maize yields in the studied intercrops, but overyielding nevertheless did occur in maize-wheat. The results are related to intercropping design choices, such as the use of a replacement design and N fertilization in accordance with each species' density. The results suggest that leaf photosynthetic capacity of maize in relay intercropping could be increased by an earlier relaxation of competition for light and N, e.g., before maize tasseling, or an extra application of N fertilizer to maize during the reproductive stage; however, this may be contrary to the environmental goal of minimizing N leaching after crop harvest. Hence, further research is needed on the optimization of fertilizer application in intercrops with maize.

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Data availability

The data of this study are available at Data Archiving and Networked Services (DANS) at https://doi.org/10.17026/dans-z6p-7ane.

Chapter 5

The acclimation of leaf photosynthetic traits of maize to interspecific competition in intercropping

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This chapter will be submitted for publication.

Abstract

Crop species in intercropping have altered growth environment compared to those in pure stands. Limited information is available on the acclimation of photosynthetic capacity of maize (*Zea mays*) leaves to intercropping. We compared maize leaf photosynthetic parameters in relay strip intercropping with faba bean (*Vicia faba*) or with wheat (*Triticum aestivum*), and in pure maize stands. We determined maize leaf photosynthetic capacity ($A_{\mathrm{g,max}}$), ATP production capacity (J_{atpmax}), and Rubisco carboxylation capacity (V_{cmax}). We analyzed associations between differences in maize leaf photosynthetic parameters and leaf mass per area (LMA), leaf nitrogen concentration (LNC), and specific leaf nitrogen (SLN). During maize vegetative phase, leaf photosynthetic parameters were correlated with LMA. Intercropping with faba bean decreased LMA of the maize next to it, and so as $A_{\mathrm{g,max}}$, J_{atpmax}, and V_{cmax}. Intercropping with wheat decreased maize SLN, while no negative effect on leaf photosynthetic parameters was observed. During maize reproductive phase, leaf photosynthetic parameters were correlated with LNC. Maize in both intercrops had increased $A_{\mathrm{g,max}}$ and J_{atpmax}, while LNC and SLN were not improved. Results illustrate that light competition during maize vegetative phase and constrained access to soil N during its reproductive phase negatively affect leaf photosynthetic capacity and the biochemical parameters in intercropping.

Keywords: Acclimation, Leaf biochemical parameters, Leaf photosynthetic capacity, Leaf traits, Maize-faba bean intercropping, Maize-wheat intercropping.

5.1 Introduction

Intercropping is the planned cultivation of multiple crop species on the same field for at least part of their growing season. Intercropping can give higher yields per unit land area than pure stands (Brooker et al., 2015; Li et al., 2020b, 2023; Xu et al., 2020). The component species in intercropping can use resources differently in time and space, leading to altered acquisition of light, water, and nutrients compared to pure stands (Gou et al., 2017a; Liu et al., 2020; Zhang et al., 2022). For instance in relay intercropping, where component species grow partly simultaneously, the species compete for resources only during the co-growth period (Liu et al., 2015; Yu et al., 2015; Zhu et al., 2014). In such intercrops species grow partly alone either before or after the co-growth period, allowing the plants to capture more resources than they would in pure stands where they compete with conspecifics during the whole growing period (Yu et al., 2015; Zhao et al., 2023; Zhu et al., 2015).

Growth conditions of a species vary with the type of companion species in intercropping. For instance, maize (*Zea mays*) experiences stronger competition for soil nitrogen (N) when intercropped with wheat (*Triticum aestivum*) than it does in pure stands (Gou et al., 2018). This competition is diminished when maize is intercropped with a legume, as legumes can fix atmospheric nitrogen thereby releasing maize from competition for soil N (Li et al., 2001a, 2001b, 2006).

Many intercrops combine maize with an earlier sown C_3 species, e.g., legumes, or also wheat, and usually such relay-mixtures are grown in strips to facilitate sowing, harvesting, and other management (Li et al., 2020b). Relay intercropping results in a dynamic environment for maize plants, which initially suffer from competition for light and soil resources with the early-sown companion species, and later are exposed to gaps where the early-sown species have been harvested, allowing the maize plants to capture more resources than in pure stands (Gou et al., 2017a; Liu et al., 2015, 2020; Ma et al., 2020; Zhu et al., 2014).

Maize is not only a widely used species in intercropping, but also usually responsible for most of the yield gain in the intercrops in which it is involved (Li et al., 2020b, 2023; Wang et al., 2023). There is a need to understand how maize achieves its high performance in intercrops. Photosynthesis is the basis of maize production. While crop productivity depends on the combined photosynthetic rate of all leaves within the canopy (Long et al., 2006), it is important to investigate first how leaf photosynthesis performs in intercropping. However, limited information is available on effects of intercropping on light-saturated gross photosynthetic rate of leaves (Pelech et al., 2022), especially with regard to how this rate acclimates to the changes in the environment typical of relay intercropping.

To date, studies on the acclimation of leaf photosynthesis to intercropping have been done in single species combinations or at a single growth stage. Furthermore, previous studies did not measure both light response and CO_2 response curves (Gong et al., 2015; Gou et al., 2018; Li et al., 2020d; Liu et al., 2018; Nasar et al., 2020, 2022; Pelech et al., 2022; Yang et al., 2017; Yao et al., 2017; Yin et al., 2021). Therefore, they did not allow inferring the capacity of underlying processes of leaf photosynthesis. A comparative analysis could help to understand whether and to which extent acclimation of maize leaves to growing with allospecific neighbors affects the parameters related to leaf photosynthetic capacity.

In photosynthesis, the absorbed light is used to drive electron transport that results in the production of ATP. In C_4 plants like maize, ATP is then partitioned between use for the C_4 cycle and the C_3 cycle. The photosynthetic rate of maize leaves can be predicted as limited by three biochemical parameters: (i) J_{atpmax}, the maximum rate of ATP production; (ii) V_{pmax}, the maximum carboxylation rate of phosphoenolpyruvate carboxylase (PEPc) in the C_4 cycle; (iii) V_{cmax}, the maximum carboxylation rate of Rubisco in the C_3 cycle (von Caemmerer and Furbank, 1999; Yin et al., 2011). It has been reported that shading decreased V_{cmax} of small-statured forb species in grassland mixtures compared to pure stands (Roscher et al., 2011). It is expected that in relay intercropping, responses of biochemical parameters of maize leaves contribute to changes in maize leaf photosynthetic capacity compared to pure stands.

Some studies have reported effects of intercropping on leaf traits, such as leaf mass area (LMA), leaf N concentration (LNC), and specific leaf N (SLN; leaf N content per unit area). Shading by component species in intercropping resulted in leaves that had lower LMA than leaves in pure stands (Gong et al., 2015; Liu et al., 2017; Pelech et al., 2022; Yao et al., 2017). Gou et al. (2018) observed lower LNC and SLN of maize when relay intercropped with wheat than in pure maize stands, due to competition for soil N with the early-sown wheat. By contrast, an increased LNC and SLN were found in maize intercropped with the legumes soybean (*Glycine max*) or alfalfa (*Medicago sativa*) compared to sole maize (Feng et al., 2020; Nasar et al., 2021, 2022). Decreased LMA of shaded leaves can be accompanied with decreased chloroplasts per unit leaf area and decreased photosynthetic components per unit leaf area (Lambers et al., 2008; Sage and McKown, 2006; Tazoe et al., 2006). Analysis of the relationships between leaf photosynthetic parameters and mentioned leaf traits could help to understand whether changes in maize leaf photosynthetic parameters are associated to the effects of intercropping on maize LMA, indicating competing for light as a possible causal pathway for changes in leaf photosynthetic traits, or associated to the effects on LNC, suggesting an effect of competition for soil N.

In this study, we aim to investigate the acclimation of photosynthetic capacity of maize leaves and the associated leaf biochemical parameters to relay strip intercropping with wheat or faba bean at two maize growth stages: (i) when maize and the early-sown wheat or faba bean grow together; (ii) when the early-sown species are harvested and maize grows alone. Here a cereal and legume are used because they exemplify two contrasting types of intercrops with maize: combining with a cereal should result in strong competition for soil N, and with a legume should result in diminished competition for soil N compared to pure maize stands. We distinguished the acclimation of maize leaf photosynthesis in border rows and inner rows in the intercrop strips, as interspecific competition is most strongly expressed in the border rows of strips (Li et al., 2021; Wang et al., 2020).

We first determined the effect of relay intercropping on the photosynthesis-light response curve of selected maize leaves. Light-saturated gross photosynthetic rate ($A_{\mathrm{g,max}}$) was estimated. We then determined the acclimation of maize leaf biochemical parameters (i.e., J_{atpmax}, V_{pmax}, and V_{cmax}) to relay intercropping. We assessed the contribution of those acclimations to changes in maize $A_{\mathrm{g,max}}$. Leaf biochemical parameters were estimated using a biochemical C_4 photosynthesis model with combined gas exchange and chlorophyll fluorescence measurements over a range of CO_2 and light levels in *vivo* maize plants (Yin et al., 2011). We also analyzed relationships between differences in leaf photosynthetic parameters and leaf traits (i.e., LMA, LNC, and SLN), to link the acclimation of leaf photosynthesis with interspecific competition for light and soil N.

The study tested two hypotheses: (i) When maize co-grows with wheat, maize $A_{\mathrm{g,max}}$ is decreased compared to sole maize, due to decreased access to soil N. When maize co-grows with faba bean, maize $A_{g,max}$ is increased compared to sole maize as faba bean can relax competition for soil N in intercrops. (ii) After the early-sown wheat and faba bean have been harvested, maize in both intercrops has increased $A_{g,max}$ compared to sole maize, due to improved access to light and soil N.

5.2 Materials and Methods

5.2.1 Experimental design

A field experiment was conducted at Droevendaal Experimental Farm in Wageningen, the Netherlands (51° 59’ 20” N, 5° 39’ 16” E) in 2018. Spring wheat (*Triticum aestivum* L. cv. Nobless) was sown on 21 March at a density of 383 seeds m^{-2}, and harvested 17 July. Faba bean (*Vicia faba* L. cv. Fanfare) was sown on 21 March at a density of 44 seeds m^{-2}, and harvested 30 July. Maize (*Zea mays* L. cv. LG30.223) was sown on 4 May at a density of 10 seeds m^{-2}, and harvested 10 September (Fig. 5.1). In maize-wheat and maize-faba bean relay strip intercropping, 1.5 m-wide maize strips were alternated with 1.5 m-wide wheat or faba bean strips (Fig. 5.2). Each maize strip comprised three rows. Each wheat or faba bean strip comprised six rows. In both intercrops, the row at each side of the species strip is referred to as border row while the other rows are inner rows. The relative density (density in the intercrop relative to the sole crop) was 0.5 in all species. A randomized complete block design with six replicates was used. The plot size was 9 m in east-west × 11 m in north-south directions. The row orientation was approximately north-south.

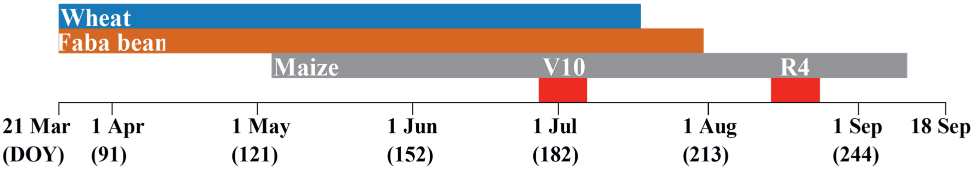

Fig. 5.1 Growing periods of wheat, faba bean, and maize, and the times of measurements in maize. Red bands represent the times of maize gas exchange and chlorophyll fluorescence measurements, first at the ten-leaf stage of maize (V10: Julian calendar day (DOY) 178-180, 183-187) and secondly at the kernel dough stage (R4: DOY 226-229, 232-235). Maize growth stages were defined according to Darby and Lauer (2010).

Soil at the experimental site was sandy with 3.4% organic matter and a pH of 5.7. Before sowing, mineral N in the 0-30 cm soil layer was 22 kg N ha^{-1}. Potassium (as $K_2SO_4 \cdot MgSO_4$; 105 kg K_2O ha^{-1}) and phosphorus (as $Ca(H_2PO_4)_2 \cdot H_2O$; 68 kg P_2O_5 ha^{-1}) were applied homogeneously over the field before sowing. Nitrogen fertilizer ($NH_4NO_3 \cdot CaMg(CO_3)_2$) was applied at a rate of 20 kg N ha^{-1} in faba bean, 125 kg N ha^{-1} in wheat, and 170 kg N ha^{-1} in maize, in accordance with the agronomic requirement of the species (for details see Supplementary Table S5.1). Fertilizer N was applied within species strips in intercrops, which resulted in identical amounts of fertilizer per unit area in the species strip as in the corresponding sole crops. Weeds, diseases, and pests were controlled as needed (Supplementary Table S5.1). Daily air temperature, daily photosynthetically active radiation, and monthly precipitation are shown in Supplementary Figs S5.1 to S5.2. Irrigation was applied to prevent water stress (Supplementary Fig. S5.2).

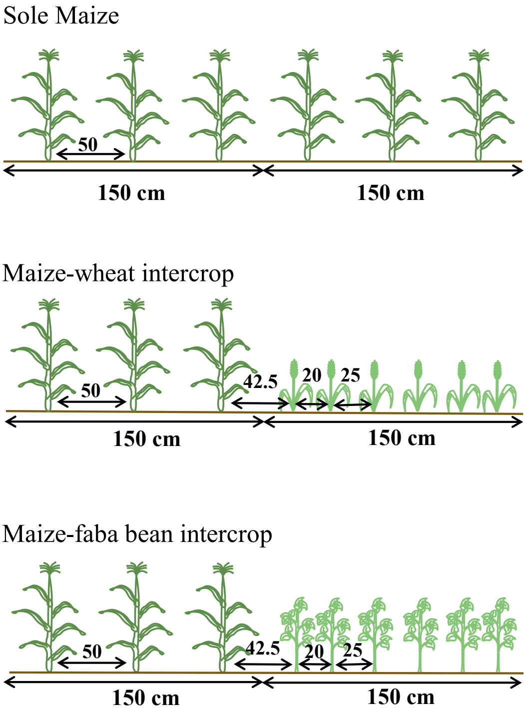

Fig. 5.2 Schematic illustration of row pattern in sole maize, maize-wheat, and maize-faba bean strip intercropping. In both intercrop treatments, 1.5 m maize strips were alternated with 1.5 m strips of the companion species. The outer rows of each 1.5 m species strip are border rows and the other rows are inner rows. The row distance of maize was 50 cm. In each wheat or faba bean strip, the row distance between inner rows was 25 cm, but the distance between the border rows and the neighboring inner rows was reduced to 20 cm to allow space for the wheels of the tractor which had a track of 133 cm. The distance between maize border rows and wheat or faba bean border rows was thus 25 + 17.5 = 42.5 cm, where 25 cm was half the row distance of maize and 17.5 cm was obtained by summing 12.5 cm (half the row distance of wheat or faba bean) and 5 cm (the distance over which the border rows in a wheat or faba bean strip was moved "inward" into the strip).

5.2.2 Gas exchange and chlorophyll fluorescence measurements

We measured leaf photosynthesis of maize in sole maize and the two intercrops at maize ten-leaf stage (V10) and kernel dough stage (R4) (Figs 5.1 and 5.3), and in six blocks at each stage. In each intercrop plot, two maize plants were selected in one maize strip, and one randomly selected plant from one border row and one plant from the adjacent inner maize row. In each sole maize plot, one plant was randomly selected. All selected plants were at least 1 m away from the plot edge.

At each stage, one leaf per plant was measured. At maize V10 stage, leaf 10 was selected, which was the youngest fully developed leaf (Fig. 5.3). At the R4 stage, we selected the ear leaf. The ear leaf was usually leaf 9 or 10 when maize had one cob per plant. When maize had two cobs per plant, leaf 10 was measured. At maize V10 stage, maize leaf 10 was not fully above the canopy of faba bean, while it was completely above wheat canopy (Fig. 5.3). The maize R4 stage was four weeks after the harvest of wheat, and two weeks after the harvest of faba bean. Due to the absence of the companion species, the ear leaf of maize in both intercrops received more light than ear leaves in sole maize.

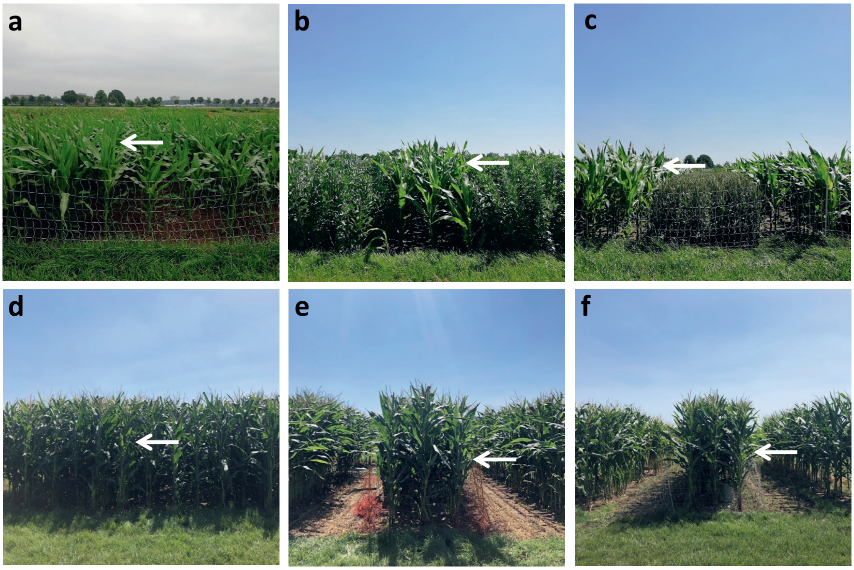

Fig. 5.3 Photos of the experimental setting of sole maize (A, D), maize with faba bean (B, E), and maize with wheat (C, F), at two growth stages of maize: V10 (A-C) and R4 (D-F). White arrows indicate the position of the leaf on which measurements were made. At V10, measurements were made on leaf 10. At R4, measurements were made on the ear leaf.

Gas exchange and chlorophyll fluorescence measurements were conducted simultaneously on each leaf, avoiding the midrib of the leaf. We used two open gas exchange measurement systems: a LI-COR 6400XT (LI-COR Inc., Lincoln, NE, USA) with an integrated 6400-40 fluorometer chamber (center wavelength of blue actinic is 470 nm and red actinic 630 nm), and a LI-COR 6800 (LI-COR Inc., Lincoln, NE, USA) with a 6800-01A fluorometer chamber (center wavelength of blue actinic is 475 nm and red actinic 635 nm). Both LI-COR devices contained LEDs emitting 10% blue and 90% red light. The leaf chamber of both LI-COR devices was 2 cm^2. For each CO_2 response curve, the steps of ambient CO_2 (C_a) were: 400, 250, 100, 50, 400, 400, 500, 800, and 1200 µmol mol^{-1} (3 min per C_a step to reach steady state), while keeping incident light level (I_{inc}) at 1800 µmol m^{-2} s^{-1}. For each I_{inc} response curve, I_{inc} was controlled in a decreasing series of 1800, 1000, 500, 300, 200, 150, 100, and 50 µmol m^{-2} s^{-1} (3 min per I_{inc} step), while keeping C_a at 400 µmol mol^{-1}. In order to obtain the data used for estimating the calibration factor which converts fluorescence chlorophyll measurements into ATP production rate, additional I_{inc} response curves within the electron transport limited range were conducted: the steps of I_{inc} were: 300, 200, 150, 100, and 50 µmol m^{-2} s^{-1} (3 min per I_{inc} step), while keeping C_a at 2000 µmol mol^{-1} to mimic non-photorespiratory conditions. The O_2 concentration of all measurements was 21%. At each step of C_a or I_{inc}, leaf net photosynthetic rate (A_n; µmol CO_2 m^{-2} s^{-1}) was allowed to reach steady state, and the steady-state fluorescence yield (F_s) was recorded. The maximum fluorescence yield (F_m') was obtained from multiphase flashes: (1) the flash intensity of phase 1 was 8000 µmol m^{-2} s^{-1} for 300 ms; (2) in phase 2, flash intensity was attenuated by 40% for 300 ms; (3) in phase 3, the flash intensity returned to 8000 µmol m^{-2} s^{-1} for 300 ms. The F_m' was obtained as the intercept of the linear regression of fluorescence yields against the inverse of the flash intensity during phase 2. Photon use efficiency of Photosystem II electron transport (Φ_2; µmol electron µmol^{-1} photon) was obtained as: $\Phi_2 = (F_m' - F_s)/F_m'$.

Prior to each measurement series, leaves were placed in the leaf chamber cuvette at I_{inc} of 1800 µmol m^{-2} s^{-1} and C_a of 400 µmol mol^{-1} for approx. 30 min adaptation. For the first measurement in the morning, a longer adaptation (approx. 50 min) was used to let stomata open. Measurements were conducted from approx. 8:30 to 15:00. Measurements for each leaf took approx. 3.5 hours, and a maximum of two leaves were measured per day, hence it was not possible to finish all measurements in one block (five leaves) in a half day. To minimize the confounding effects of changes in time of day, leaves measured in the morning (approx. 8:30

to 12:00) or afternoon (approx. 12:00 to 15:00) were randomized over treatments (Supplementary Table S5.2). For each leaf, the order of the three response curves was also randomized (Supplementary Table S5.2). Measurements were conducted block by block, and all measurements in one block were made with the same instrument. Thus, the random variation associated with the time of day or instrument was absorbed in the block effect during analysis. Flow rate was 300 μmol s^{-1} for all measurements. Leaf-to-air vapour pressure difference was controlled within 1.0-1.5 kPa. Leaf temperature was controlled at 25 °C. The leaf temperature control range is ± 6 °C from ambient temperature for LI-COR 6400XT, and ± 10 °C for LI-COR 6800. The air temperature during measurements was lower than 30 °C, which allowed both instruments to control leaf temperature at 25 °C.

A_n and intercellular CO_2 (C_i; μmol mol^{-1}) obtained from all measurements were corrected for leakage of the leaf chamber (Flexas et al., 2007). To do so, the apparent net photosynthetic rate was measured at the same steps of C_a with the same I_{inc} used for measuring normal A_n-C_i curves (see earlier), on five photosynthetically inactive maize leaves (thermally killed by immersion in boiling water for five minutes). A_n was corrected by linear relationship between the apparent net photosynthetic rate and C_a. C_i was then re-calculated using manufacturer's formulae with the corrected A_n as input.

5.2.3 Leaf area, leaf N, and light absorptance

Leaf blades used for gas exchange and chlorophyll fluorescence measurements were harvested from maize plants. The midrib was removed and the area of the remaining blade was measured using a leaf area meter (LI-3100 area meter, Lincoln, USA). For each blade, three leaf discs (2.16 cm^2 per disc) were punched around the position where the gas exchange and chlorophyll fluorescence measurement was made. The transmittance and reflectance of each disc were measured in the spectral range of the light source of the gas exchange measurement, using a Spectrometer (STS-VIS miniature Spectrometer, Ocean Optics, USA). The leaf absorptance (*abs*) was calculated as one minus transmittance minus reflectance. The three leaf discs and the blade excluding midrib were then weighed after drying to constant weight at 70 °C. The three discs were then used to determine leaf N concentration (LNC; mg N g^{-1} leaf), using an element C/N analyser (Flash 2000, Thermo Scientific) based on the Micro-Dumas combustion method. Leaf mass per area (LMA; g leaf m^{-2} leaf) was calculated as dry weight divided by the area of the blade without midrib. Specific leaf N (SLN; g N m^{-2} leaf) was calculated as: $\mathrm{LMA} \times (\mathrm{LNC} \times 10^{-3})$.

5.2.4 Estimation of maize leaf photosynthetic parameters

Parameters describing the main processes underlying photosynthesis were derived by modelling. A model description and methodology for parameter estimation is given in Supplementary Methods S5.1 to S5.2. The list of variables is summarized in Supplementary Table S5.3. An abbreviated description is given below. During the process of estimation, model selection was used to determine whether values of parameters were different among treatments or groups of treatments (see below).

Light-saturated gross photosynthetic rate ($A_{\mathrm{g,max}}$; µmol CO_2 m^{-2} s^{-1}) was estimated by fitting a nonrectangular hyperbola equation (Supplementary Eq. S5.1) to A_{n} over a range of absorbed light levels (I_{abs}) under 400 µmol mol^{-1} C_{a}, using the already estimated photon use efficiency of CO_2 assimilation under limiting light levels (ε; µmol CO_2 µmol^{-1} photon) and day respiration (R_{d}; µmol CO_2 m^{-2} s^{-1}) as inputs.

Based on the model of von Caemmerer and Furbank (1999), Yin et al. (2011) proposed a biochemical C_4 photosynthesis model that predicts A_{n} as the minimum of four possibly limiting rates. They are: (i) A_{EE} (µmol CO_2 m^{-2} s^{-1}), where the rate of the C_4 cycle is limited by the activity of phosphoenolpyruvate carboxylase (PEPc) while that of the C_3 cycle is limited by the activity of RuBP carboxylase (Rubisco); (ii) A_{ET} (µmol CO_2 m^{-2} s^{-1}), where the C_4 cycle is limited by the activity of PEPc while the rate of the C_3 cycle is limited by the rate of ATP production (J_{atp}; µmol ATP m^{-2} s^{-1}); (iii) A_{TE} (µmol CO_2 m^{-2} s^{-1}), where the C_4 cycle is limited by J_{atp} while the C_3 cycle is limited by the Rubisco activity; (iv) A_{TT} (µmol CO_2 m^{-2} s^{-1}), where both the C_4 cycle and the C_3 cycle are determined by J_{atp}. The biochemical C_4 photosynthesis model also estimates bundle sheath conductance (g_{bs}; mol m^{-2} s^{-1}), which determines the rate of CO_2 leakage from the bundle sheath cell to the mesophyll cell. In this study, the biochemical C_4 photosynthesis model was adopted to estimate the maximum activity of PEPc (V_{pmax}; µmol CO_2 m^{-2} s^{-1}), the maximum activity of Rubisco (V_{cmax}; µmol CO_2 m^{-2} s^{-1}), and g_{bs}, fitting to all data of combined gas exchange and chlorophyll fluorescence measurements on light response curves at the two C_{a} levels (400 µmol mol^{-1} and 2000 µmol mol^{-1}) and CO_2 response curves.

At high light levels, ATP production rate reaches its maximum value (J_{atpmax}; μmol ATP m^{-2} s^{-1}). J_{atpmax} was estimated by fitting a nonrectangular hyperbola equation (Supplementary Eq. S5.13) to J_{atp}-I_{abs} curves obtained from chlorophyll fluorescence measurements under 400 μmol mol^{-1} C_{a}. The inputs were the already estimated photon use efficiency of Photosystem II electron transport under limiting light levels ($\Phi_{2\mathrm{LL}}$; μmol electron μmol^{-1} photon), and the calibration factor (s'; μmol ATP μmol^{-1} electron) used to convert chlorophyll fluorescence-based data into J_{atp}.

5.2.5 Model selection

At each maize stage, model selection was adopted to find the model that fits the data best with as few as possible parameters to be estimated.

To do so, first, observations from sole maize (SM), border and inner row maize in maize-faba bean intercropping (MB-Border; MB-Inner), and maize-wheat intercropping (MW-Border; MW-Inner) were grouped in different ways based on the assumption of which parameters differed among which groups of treatments. Thus, each model had multiple versions fitted to different groups of data. For the model with one parameter to be estimated, four model versions were fitted with parameterization on (i) the data of SM, MB-Border, MB-Inner, MW-Border, and MW-Inner separately. In this case, five values, one for each treatment, were estimated; (ii) three groups of data- data of SM, combined data of MB-Border and MB-Inner, and combined data of MW-Border and MW-Inner. In this case, three values were estimated: one for sole maize, and one common value for border and inner row maize in both maize-bean and maize-wheat; (iii) two groups of data- data of SM, and combined data of MB-Border, MB-Inner, MW-Border, and MW-Inner. In this case, two values were estimated: one for sole maize and one common value for maize in all intercrop treatments; (iv) the combined data of all five treatments. In this case, one common value was estimated. For the model with two parameters see Supplementary Methods S5.3.

Then, the *Akaike Information Criterion* (AIC) was used to select models. The model with the lowest AIC value was considered the best fit to the data. Models with AIC values less than 2 units apart (ΔAIC < 2) were considered equivalent, in which condition the simpler model (with fewer parameters) was chosen (Bolker, 2008) (for an example see Supplementary Methods S5.3). To calculate the AIC value of each model version, maximum likelihood estimate of the parameters of each model version was first obtained using mle2's formula interface in the "bbmle" package (Bolker and R Core Team, 2022) of R version 4.3.1 (R Core Team, 2023). All variables were assumed normally distributed. Optimization algorithm "Nelder-Mead" was used when estimating parameters except for g_{bs} and V_{cmax}, which were estimated by algorithm "L-BFGS-B" specifying the lower boundary value of g_{bs} to be 0.01 mmol m^{-2} s^{-1}. The AIC values of all models are given in Supplementary Table S5.4. To evaluate the consistency of findings in model selection, parameters estimated using the data of each treatment at each stage and the standard errors are given in Supplementary Table S5.5.

5.2.6 Relationships between leaf photosynthetic parameters and leaf traits

To explore relationships between leaf photosynthetic parameters and leaf traits (i.e., LMA, LNC, and SLN) across treatments at each stage, leaf photosynthetic parameters were estimated by fitting the model to the data of each replicate in each treatment at each maize stage. When g_{bs} and V_{cmax} were estimated simultaneously, the standard error of V_{cmax} could not be estimated. Thus, V_{cmax} per replicate was estimated with the value of g_{bs} obtained from the results of model selection (AIC) as input. All models were fitted using mle2's formula interface with optimization algorithm "Nelder-Mead". Linear relationships were determined at each stage using the combined data from the five treatments. The R base function lm (R Core Team, 2023) was used.

5.3 Results

5.3.1 Effects of intercropping on light response curves of maize leaf photosynthesis

Light response curves of maize leaves in intercrops differed from those in sole maize mainly in their $A_{g,max}$ (Fig. 5.4; Table 5.1). At the V10 stage, $A_{g,max}$ of leaf 10 was lower in border row maize in the maize-faba bean intercrop than in any of the other treatments, indicating a negative effect of intercropping with faba bean on maize $A_{g,max}$, but no negative effect of wheat. $A_{g,max}$ decreased from V10 to R4 stage in all treatments. At maize R4 stage, the ear leaf in all intercrop treatments (both border rows and inner rows) had higher $A_{g,max}$ than sole maize. R_d was stable among treatments and was greater at maize V10 than R4 stage.

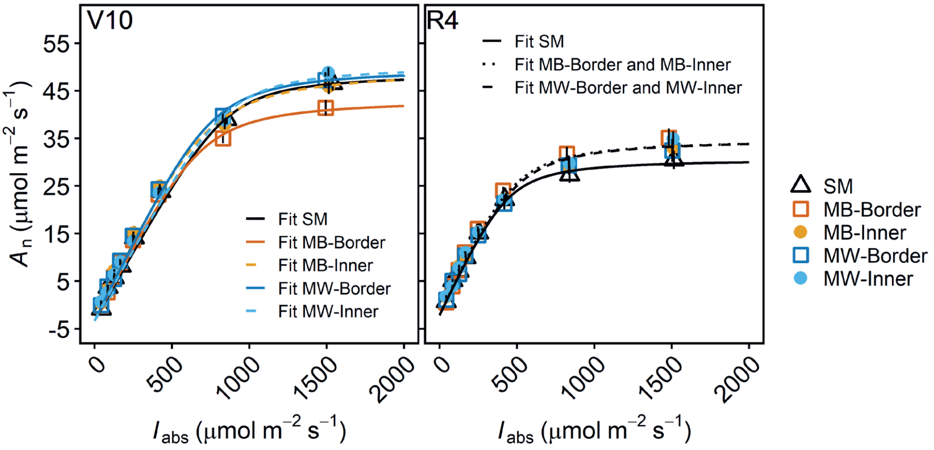

Fig. 5.4 Measured (points) and modelled (curves) light response curves of A_n in different treatments at maize V10 and R4 stages. Error bars represent standard errors of the means of A_n. A_n-I_{abs} curves were drawn using the nonrectangular hyperbola equation (Supplementary Eq. S5.1) with estimated values of ε, R_d, and $A_{g,max}$ (Table 5.1) as inputs. At each maize stage, first, ε and R_d were estimated separately. Then, $A_{g,max}$ was estimated with the already estimated ε and R_d as inputs. According to the results of model selection (AIC), at maize V10 stage, five values of ε, one common value of R_d, and five values of $A_{g,max}$ were estimated. Thus, the five treatments had different photosynthesis light response curves. At maize R4 stage, according to AIC, three values of ε, one common value of R_d, and two values of $A_{g,max}$ were estimated. Thus, three curves were drawn. As $A_{g,max}$ only differed between maize in sole maize and in intercrops, A_n-I_{abs} curves of maize intercropped with faba bean and with wheat overlapped at high light levels.

Table 5.1 Fitted parameter values (standard errors in brackets) for photon use efficiency of CO_2 assimilation under limiting light levels (ε), day respiration (R_d), and light-saturated gross photosynthetic rate ($A_{g,max}$) at maize V10 and R4 stages. Identical values of a parameter across treatments indicate that the parameter was not different among those treatments at that stage according to AIC.

Parameter	Stage	SM	MB-Border	MB-Inner	MW-Border	MW-Inner
ε (mol mol^{-1})	V10	0.058 (0.003)	0.063 (0.003)	0.067 (0.003)	0.066 (0.003)	0.060 (0.003)
	R4	0.068 (0.002)	0.071 (0.001)	0.071 (0.001)	0.068 (0.001)	0.068 (0.001)
R_d (µmol m^{-2} s^{-1})	V10	3.28 (0.25)	3.28 (0.25)	3.28 (0.25)	3.28 (0.25)	3.28 (0.25)
	R4	2.11 (0.17)	2.11 (0.17)	2.11 (0.17)	2.11 (0.17)	2.11 (0.17)
$A_{g,max}$ (µmol m^{-2} s^{-1})	V10	52.1 (1.0)	46.7 (0.9)	53.0 (0.9)	53.3 (0.9)	53.8 (1.0)
	R4	32.9 (0.8)	37.1 (0.4)	37.1 (0.4)	37.1 (0.4)	37.1 (0.4)

5.3.2 Effects of intercropping on maize J_{atp}-I_{abs} curves

The efficiency of converting absorbed light into ATP at low light levels was comparable among treatments and maize stages (Fig. 5.5). However, J_{atpmax} of leaf 10 was lower in the border row maize next to faba bean than in the other four treatments at maize V10 stage (Fig. 5.5; Table 5.2). J_{atpmax} was lower at maize R4 than V10 stage in all treatments. At maize R4 stage, the ear leaf in all intercrop treatments had higher J_{atpmax} than in sole maize.

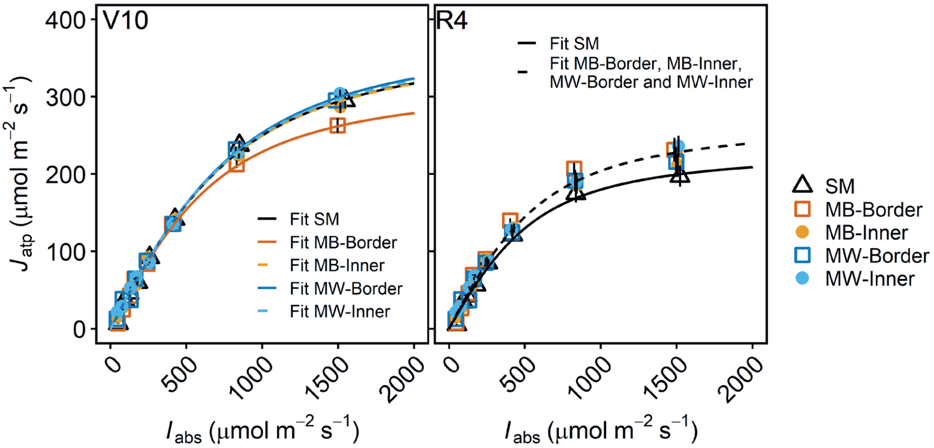

Fig. 5.5 Light response curves of ATP production rate (J_{atp}) based on absorbed light (I_{abs}) in different treatments at maize V10 and R4 stages. Points represent mean values of calculated J_{atp}. Error bars represent standard errors of the means. Curves were drawn using the nonrectangular hyperbola equation (Supplementary Eq. S5.13) with estimated values of Φ_{2LL}, s', and J_{atpmax} (Table 5.2) as inputs. At each maize stage, first, Φ_{2LL} and s' were estimated separately. Then, J_{atpmax} was estimated with the already estimated Φ_{2LL} and s' as inputs. At maize V10 stage, according to AIC, one common value of both Φ_{2LL} and s', and five values of J_{atpmax} were estimated. Thus, five curves were drawn. At maize R4 stage, two values of Φ_{2LL}, one common value of s', and two values of J_{atpmax} were estimated. Thus, two curves were drawn.

Table 5.2 Fitted parameter values (their standard errors in brackets) for photon use efficiency of Photosystem II electron transport under limiting light levels (Φ_{2LL}), the calibration factor converting chlorophyll fluorescence-based data into J_{atp} (s'), and maximum rate of ATP production (J_{atpmax}) at maize V10 and R4 stages. Identical values of a parameter across treatments indicate that the parameter was not different among those treatments at that stage according to AIC.

Parameter	Stage	SM	MB-Border	MB-Inner	MW-Border	MW-Inner
Φ_{2LL} (mol mol^{-1})	V10	0.675 (0.006)	0.675 (0.006)	0.675 (0.006)	0.675 (0.006)	0.675 (0.006)
	R4	0.598 (0.010)	0.650 (0.010)	0.650 (0.010)	0.650 (0.010)	0.650 (0.010)
s' (mol mol^{-1})	V10	0.35 (0.01)	0.35 (0.01)	0.35 (0.01)	0.35 (0.01)	0.35 (0.01)
	R4	0.36 (0.01)	0.36 (0.01)	0.36 (0.01)	0.36 (0.01)	0.36 (0.01)
J_{atpmax} (μmol m^{-2} s^{-1})	V10	382 (5)	325 (5)	381 (5)	392 (6)	387 (6)
	R4	235 (8)	272 (4)	272 (4)	272 (4)	272 (4)

5.3.3 Effects of intercropping on maize V_{pmax}, V_{cmax}, and g_{bs}

At each maize stage, V_{pmax} was comparable among treatments (Table 5.3). At maize V10 stage, V_{cmax} of leaf 10 was lower in the border row maize next to faba bean than in any of the other treatments. However, both border and inner row maize in the maize-wheat intercrop had higher V_{cmax} than sole maize. At maize R4 stage, V_{cmax} of the ear leaf did not differ among treatments.

At both stages and across treatments, the estimated values of g_{bs} were low (Table 5.3), indicating conductance for diffusion of CO_2 from the bundle sheath cell back to the mesophyll cell was low (note that maize g_{bs} at 25 °C was reported to be 1.4-10.3 mmol m^{-2} s^{-1} in Yin et al. (2011)). Our estimated low g_{bs} reflects an efficient CO_2-concentrating mechanism (CCM). According to A_n-C_i curves (Supplementary Fig. S5.3), A_n became CO_2-saturated at low C_i levels (approx. 97 μmol mol^{-1} at V10 and 140 μmol mol^{-1} at R4), which also indicates an efficient CCM.

Table 5.3 Fitted parameter values (standard errors in brackets) for maximum rate of PEP carboxylation (V_{pmax}), maximum rate of Rubisco carboxylation (V_{cmax}), and bundle sheath conductance to CO_2 leakage (g_{bs}) at maize V10 and R4 stages. Identical values of a parameter indicate that the parameter was not different among those treatments at that stage according to AIC.

Parameter	Stage	SM	MB-Border	MB-Inner	MW-Border	MW-Inner
V_{pmax} (μmol m^{-2} s^{-1})	V10	102.2 (3.5)	102.2 (3.5)	102.2 (3.5)	102.2 (3.5)	102.2 (3.5)
	R4	68.7 (1.9)	68.7 (1.9)	68.7 (1.9)	68.7 (1.9)	68.7 (1.9)
V_{cmax} (μmol m^{-2} s^{-1})	V10	54.1 (1.3)	49.0 (1.2)	52.4 (1.2)	55.3 (1.3)	55.1 (1.2)
	R4	41.5 (0.7)	41.5 (0.7)	41.5 (0.7)	41.5 (0.7)	41.5 (0.7)
g_{bs} (mmol m^{-2} s^{-1})	V10	0.11 (0.12)	0.11 (0.12)	0.11 (0.12)	0.11 (0.12)	0.11 (0.12)
	R4	1.30 (0.23)	0.29 (0.15)	0.80 (0.19)	0.77 (0.19)	0.69 (0.18)

5.3.4 The contribution of the acclimation of maize leaf biochemical parameters to $A_{g,max}$

Treatments with lower $A_{\mathrm{g,max}}$ also had decreased J_{atpmax} and/(or) V_{cmax}, indicating the acclimation of maize $A_{\mathrm{g,max}}$ was likely explained by changes in maize J_{atpmax}, or V_{cmax}, or both (for correlations between $A_{\mathrm{g,max}}$ and J_{atpmax}, and between $A_{\mathrm{g,max}}$ and V_{cmax}, see Supplementary Fig. S5.4). To explore which biochemical parameter contributed more to the acclimation of maize $A_{\mathrm{g,max}}$, we investigated which of the four rates (i.e., A_{EE}, A_{ET}, A_{TE}, and A_{TT}) was limiting the net rate of leaf photosynthesis (A_{n}) at high light level (i.e., 1800 μmol m^{-2} s^{-1} I_{inc}) (Fig. 5.6). As R_{d} did not vary among treatments at both maize stages (Table 5.1), the limiting biochemical parameters for A_{n} at high light level could also limit $A_{\mathrm{g,max}}$.

At the V10 stage, A_{n} at high light level was predicted as the rate of A_{EE} in sole maize, border row maize in maize-bean, and both border and inner row maize in maize-wheat (Fig. 5.6A, B, D, and E), indicating that A_{n} at high light level was limited by V_{pmax} and V_{cmax}. Because V_{pmax} was comparable among treatments (Table 5.3), differences in V_{cmax} were the leading factor for the acclimation of maize $A_{\mathrm{g,max}}$. The decreased $A_{\mathrm{g,max}}$ of border row maize in the maize-faba bean intercrop was largely due to the lower V_{cmax}.

At the R4 stage, A_{n} at high light level was predicted as the rate of A_{TE} in sole maize (Fig. 5.6F), indicating that A_{n} at high light level was limited by J_{atpmax} and V_{cmax}. Because V_{cmax} was comparable among treatments at this stage (Table 5.3), the lower $A_{g,max}$ of sole maize as compared to maize in intercrop treatments was caused by the lower J_{atpmax}.

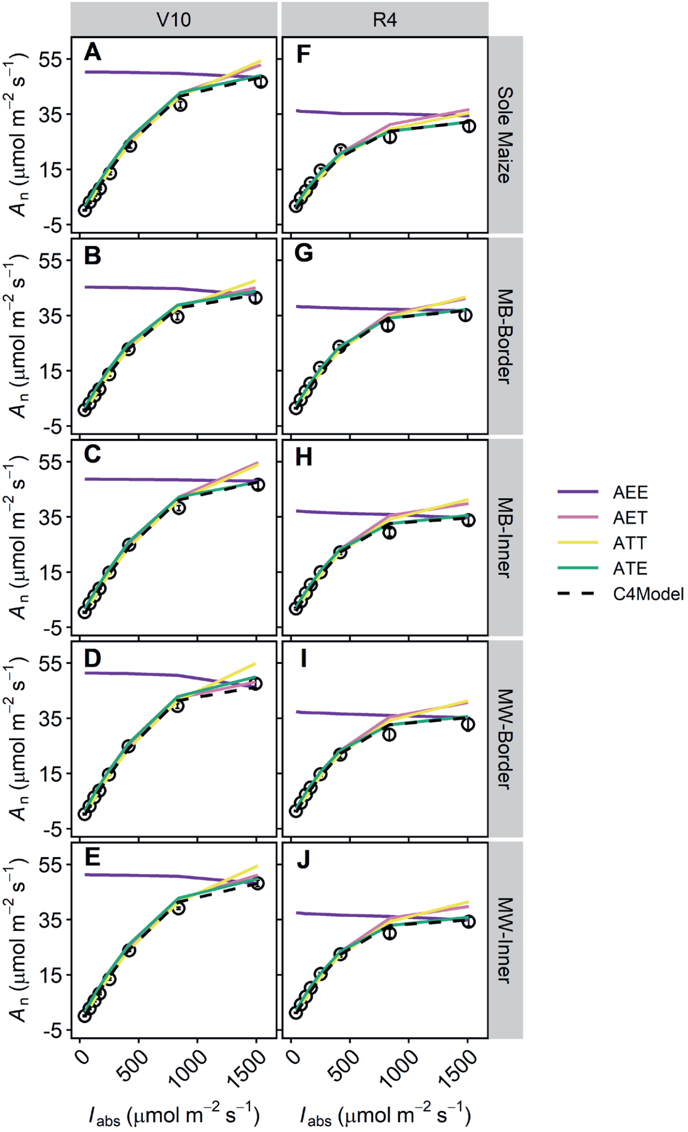

Fig. 5.6 Measured (points) and modelled (curves) A_n-I_{abs} in five treatments at maize V10 (A-E) and R4 (F-J) stages. Error bars represent standard errors of the means. Black dashed curves were drawn using the biochemical C4 photosynthesis model (Supplementary Methods S5.1, with Supplementary Eq. S5.13 instead of Supplementary Eq. S5.11 to calculate J_{atp}) with estimated values of Φ_{2LL}, s', R_d, J_{atpmax}, V_{pmax}, V_{cmax}, and g_{bs} (Tables 5.1 to 5.3) as inputs. Solid curves in color represent A_n-I_{abs} predicted by A_{EE}, A_{ET}, A_{TE}, and A_{TT} separately. The equations for A_{EE}, A_{ET}, A_{TE}, and A_{TT} and their solutions are given in Supplementary Methods S5.1. As the models used the observed I_{abs} and C_i as inputs, the curves are not completely smooth.

5.3.5 Relationships between leaf photosynthetic parameters and leaf traits

We assessed whether the estimated leaf photosynthetic parameter values were associated with leaf morpho-physiological traits (Fig. 5.7). At maize V10 stage, significant correlations of leaf photosynthetic parameters were found with LMA for leaf 10. At this stage, leaf 10 of the border row maize next to faba bean had lower LMA than sole maize and both border and inner row maize in the maize-wheat intercrop (Fig. 5.7; Supplementary Fig. S5.5), indicating strong effects of shading by faba bean on LMA. $A_{\mathrm{g,max}}$, J_{atpmax}, and V_{cmax} of border row maize in maize-faba bean were decreased in leaves with lower LMA.

At maize R4 stage, significant correlations of leaf photosynthetic parameters were found with LNC in the ear leaf, suggesting that access to soil N affected leaf photosynthetic traits. However, the ear leaf in both intercrops did not have higher LNC than sole maize at this stage (Fig. 5.7; Supplementary Fig. S5.5). The significant correlation was likely determined by variation of LNC among replicates in each treatment as data of each treatment were scattered around the regression line.

At both V10 and R4 stages, leaf photosynthetic parameters were correlated with SLN (which integrates LMA and LNC). At maize V10 stage, leaf 10 of border row maize in the maize-faba bean intercrop had lower SLN than sole maize and other intercrop treatments (Fig. 5.7; Supplementary Fig. S5.5). Leaf 10 of border row maize in the maize-wheat intercrop had lower SLN than sole maize, but leaf photosynthetic parameters were not decreased in the border row maize next to wheat. At maize R4 stage, SLN of the ear leaf in both intercrops were not higher than sole maize. Again, the significant correlation was likely due to variation of SLN among replicates in each treatment.

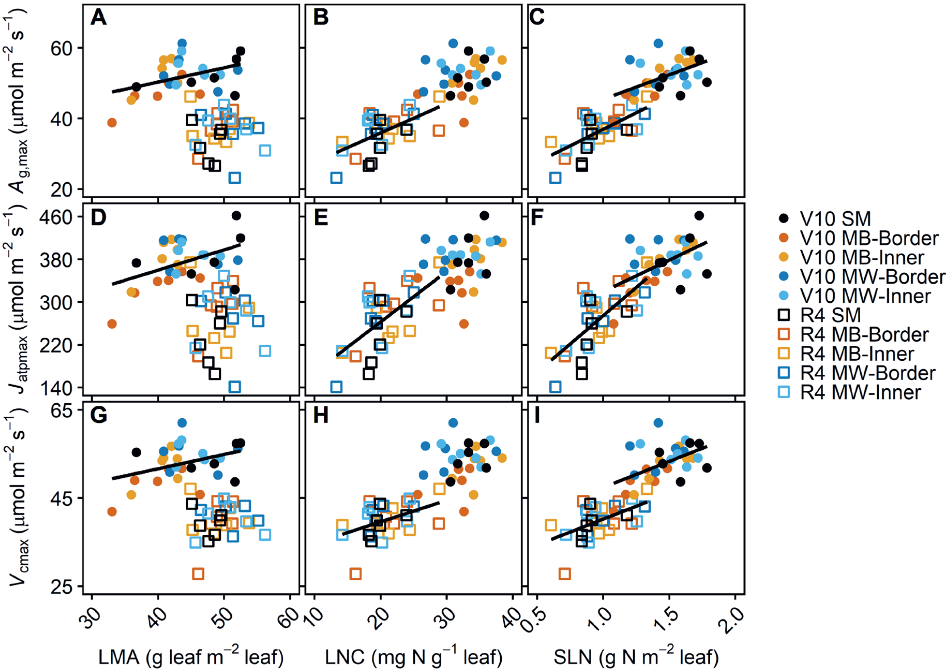

Fig. 5.7 Relationships between leaf photosynthetic parameters (i.e., $A_{g,max}$, J_{atpmax}, V_{cmax}) and leaf mass per area (LMA), leaf N concentration (LNC), and specific leaf N (SLN) at maize V10 (filled circles) and R4 (open squares) stages. At each stage, parameters were estimated by fitting the model to the data of each replicate in each treatment. A linear regression was fit through the combined data from the five treatments at each stage. Only the lines for regressions with $P <$ 0.05 are presented.

5.4 Discussion

In this study, we tested two hypotheses on the acclimation of photosynthetic capacity of maize leaves to relay intercropping with wheat or faba bean. The first hypothesis stated that during the co-growth period, intercropping with faba bean increases maize $A_{g,max}$, while intercropping with wheat decreases maize $A_{g,max}$. However, we found contrasting results. At maize V10 stage, leaf 10 of border row maize in the maize-faba bean intercrop had lower $A_{g,max}$ than leaf 10 of any other treatment (Fig. 5.4; Table 5.1), while there was no effect of intercropping with wheat on maize $A_{g,max}$. We found evidence supporting the second hypothesis that at maize R4 stage, when the early-sown wheat and faba bean had been harvested, maize ear leaf had increased $A_{g,max}$ in both relay intercrops (both border and inner row maize) compared to sole maize (Fig. 5.4; Table 5.1). Thus, during maize vegetative phase, intercropping with faba bean decreased maize leaf photosynthetic capacity, while we observed no negative effect of wheat. Maize recovered to some extent from competition with the early-sown species in both relay intercrops during its reproductive phase.

5.4.1 Interspecific competition for light affects maize leaf photosynthetic parameters during maize vegetative phase

Decreased LMA is a common response to shading (Poorter et al., 2009), and has been observed previously in relay intercropping (Gong et al., 2015; Gou et al., 2018). To minimize light limitation, shaded leaves tend to increase light-harvesting ability and decrease carboxylation capacity (Lambers et al., 2008). Such phenomenon is common in C_3 leaves (Lambers et al., 2008; Sage and McKown, 2006), and has been observed in leaves of soybean (*Glycine max*) seedlings when shaded by maize in maize-soybean intercropping (Gong et al., 2015; Yang et al., 2018). These soybean leaves had relatively more chlorophyll *b* for light harvesting, while light-saturated A_{n} was decreased compared to sole soybean. In our trials, leaves of young maize plants acclimated to shading of faba bean and had decreased $A_{\mathrm{g,max}}$, but the light absorptance was hardly affected by intercropping (Fig. 5.4; Supplementary Fig. S5.5). Thus, maize as a C_4 species, so less shade tolerant than C_3 species, may hardly increase its light harvesting when shaded in intercropping.

The decreased $A_{\mathrm{g,max}}$ in maize-faba bean border was driven by the decreased maximum activity of Rubisco (V_{cmax}) (Fig. 5.6), which was likely associated to the lower Rubisco content per leaf area in those thinner leaves (Pengelly et al., 2010; Tazoe et al., 2006). This finding indicates that light competition with the early-sown faba bean decreased carboxylation capacity of Rubisco in maize leaves during maize vegetative phase.

Competition for soil N is stronger when maize plants are intercropped with wheat than with faba bean or when growing in pure stands, as wheat is more competitive for N (Li et al., 2001a; Liu et al., 2015; Zhang and Li, 2003). Gou et al. (2018) reported decreased LNC and SLN in maize intercropped with wheat as compared to sole maize, but maize leaf A_{n} was higher in intercropped than sole maize. A similar observation was done in our maize-wheat intercrop at maize V10 stage, where border row maize had decreased SLN while $A_{\mathrm{g,max}}$ was not decreased (Figs 5.4 and 5.7). We also found that maximum activity of Rubisco (V_{cmax}) in these border row maize was even higher than in sole maize (Table 5.3). Leaves grown under high light conditions tend to allocate a higher proportion of leaf N to Rubisco (Sage and McKown, 2006; Tazoe et al., 2006). Leaf 10 of border row maize plants was above the canopy of wheat, and so exposed to better light conditions than leaf 10 of sole maize and maize intercropped with faba bean (Fig. 5.3). Such improved light conditions likely increased leaf N investment into Rubisco, which could partly be confirmed by higher leaf N use efficiency for V_{cmax} in maize-wheat border than sole maize (Supplementary Fig. S5.6). As a result, such increased leaf N use efficiency could compensate for the decreased SLN. The findings suggest that in the maize-wheat intercrop, improved light conditions during maize vegetative phase may increase the investment of leaf N to carboxylation capacity, which may compensate for N stress.

5.4.2 Access to soil N determines maize leaf photosynthetic parameters in intercropping during maize reproductive phase

In intercropping, when maize overtops its companion species, maize leaves experience better light conditions compared to pure stands (Li et al., 2019; Liu et al., 2018). In these studies, leaf A_{n} of intercropped maize was higher than that of sole maize. Likewise, after the early-sown faba bean and wheat had been harvested, maize in our trials had higher $A_{\mathrm{g,max}}$ and J_{atpmax} at the R4 stage (Figs 5.4 and 5.5), due to better light conditions compared to sole maize.

At the R4 stage, photosynthetic traits of maize leaves were determined by differences in LNC rather than LMA (Fig. 5.7). However, both LNC and SLN of maize leaves were not increased in both intercrops as compared to sole maize (Fig. 5.7; Supplementary Fig. S5.5). Feng et al. (2020) observed that maize had higher LNC in a relay intercropping sequence where maize was sown before soybean compared to sole maize. Gou et al. (2018) observed lower maize LNC and SLN at maize flowering in maize-wheat relay intercropping than in sole maize, due to N competition with the early-sown wheat. In our trials, the initial disadvantages of shading by faba bean and N competition with wheat likely constrained the N acquisition of maize during later phases. Thus, leaf photosynthetic capacity of maize leaves in both intercrops may be constrained by N deficiency during later phases.

5.4.3 Options to increase photosynthetic capacity of maize leaves in relay intercropping

The key period for the later-sown maize in relay intercropping is after the harvest of the early-sown species, when the maize plants can capture more light and soil resources from the strip where the early-sown species is harvested (Zhao et al., 2023). However, despite maize yield increase in maize-wheat intercropping (Wang et al., 2023), leaf photosynthetic capacity of maize in our trials was not substantially higher than in sole maize (Fig. 5.4). The overyielding of intercropped maize was therefore more likely due to enhanced light capture compared to the sole crop, particularly after the companion crop was harvested (Gou et al., 2017; Zhang et al., 2008).

In the experiment of Ma et al. (2020), winter wheat was mature before maize V10 stage. In their case, competition with wheat was relaxed when maize root system and foliage were growing, allowing intercropped maize to take advantage of early access to extra light and soil N from the wheat strip. Their maize leaf A_{n} kept increasing and was higher in intercropped than sole maize after winter wheat had been harvested. In our trials, the early-sown wheat and faba bean were harvested after maize tassel appearance.

Thus, changes in designs of intercrops could be an option to increase photosynthetic capacity of maize leaves and crop photosynthesis in intercropping. An early harvest of the early-sown species can relax intercropped maize from competition during its vegetative period and increase leaf photosynthetic capacity. Using a winter-sown rather than a spring-sown cereal or legume as companion species could be a recommendation to increase leaf photosynthetic capacity of intercropped maize. Alternatively, a supplementary N fertilization could be given at a late growth stage of maize, to make up for N shortages in the soil. Such late N fertilization in intercrops has been found to boost intercropped maize yields in China (Liu et al., 2020), but such a late fertilization might increase the risk of N losses and might thus not be sustainable.

Breeding crop ideotypes with enhanced traits to increase intercropping advantage could also be an option (Bourke et al., 2021; Haug et al., 2023). We found changes in V_{cmax} and J_{atpmax} were leading for the acclimation of maize leaf photosynthetic capacity (Fig. 5.6). A maize ideotype with increased leaf photosynthetic capacity through increased V_{cmax} and J_{atpmax} could be considered, which could be achieved through an increased content of photosynthetic components (Salesse-Smith et al., 2020). During the maize vegetative period, such an ideotype maize could grow faster and quicker, and earlier become the dominant species in relay intercrops. In such case, intercropped maize may be relieved from competition earlier during the vegetative period and have the ability to capture extra light and soil N resources, which could optimize leaf photosynthetic capacity in our relay intercropping.

Breeding for intercropping systems should also consider interactions between the ideotype required for one species and companion species, because the purpose of breeding for intercropping is to optimize complementarity of the whole system (Brooker et al., 2015). In our relay intercrops, faba bean and wheat started their reproductive stage around the maize four-leaf stage. The ideotype maize may grow quickly and compete for resources during the later reproductive period of faba bean and wheat. In such case the ideotype maize may have little effect on N capture of its companion species, but to which extent the yields of faba bean and wheat may suffer through enhanced shading and thereby affect resource use efficiency of the whole intercropping system would have to be quantified.

5.5 Conclusion

In conclusion, our findings indicate that light competition during maize vegetative phase and constrained access to soil N during maize reproductive phase negatively affected traits associated with the Rubisco carboxylation capacity and ATP production capacity in maize leaves and thereby photosynthetic capacity. This lack of an improved leaf photosynthetic capacity is in contrast to earlier suggestions based on less extensive data and on intercrops with a more limited co-growth period. It indicates the need for further analyses of the relation between light capture and photosynthetic capacity during reproductive growth on the one hand and observed additional yield on the other hand.

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Data availability

The data that support the findings of this study are openly available at Data Archiving and Networked Services (DANS) at https://doi.org/10.17026/dans-xrn-zfxud.

Chapter 6

General Discussion

In this thesis, I studied eco-physiological responses of crop species at multiple levels (field, plant, and leaf) in strip intercropping in conventional agriculture in the Netherlands. Experiments were done with four crop species (maize, wheat, faba bean, and pea) combined in bi-specific strip intercrops in six combinations. The intercrops were arranged in a narrow strip design, in which component species were grown in 1.5 m-wide strips consisting of three rows of maize or six rows of wheat, faba bean, and pea. A strip- and species-specific N fertilization was applied, where each species, within its strips in both intercrops and sole crops, received moderate rates of fertilizer N according to recommendations for arable crop fertilization in the Netherlands for non-organic agriculture (Ministerie van Landbouw, Natuur en Voedselkwaliteit, 2019). Under the temperate climate, intercrops with maize were grown as relay intercrops with a C_3 species, whereby maize was sown and harvested later than the C_3 species.

In Chapter 2, I studied the effects of intercropping on N uptake. Then, I studied the effects of intercropping on plant morphological traits (Chapter 3) and maize leaf photosynthetic traits (Chapters 4 and 5). I focused on wheat and faba bean as two contrasting companion species for relay intercropping with maize, in which wheat, as a cereal, competes for soil N with maize, while such competition is expected to be diminished when grown with faba bean due to its ability for N_2 fixation (Bedoussac et al., 2015; Li et al., 2006).

In this general discussion, I will first summarize the main findings of this thesis by answering the research questions listed in Chapter 1 (Section 6.1). Second, I will connect the multiple-level eco-physiological responses of crop species in intercrops, and elucidate how does this work contribute to an improved understanding of crop performance in intercropping (Sections 6.2 to 6.4). Third, I will discuss the contribution of species-tailored N fertilization in strip intercropping to sustainable agriculture (Section 6.5), and propose recommendations on optimizing intercropping systems (Section 6.6). Finally, I will discuss the future prospects of intercropping research (Section 6.7).

6.1 Research questions answered by this thesis

What are the effects of combining four commonly grown species- maize, wheat, faba bean, and pea- in bi-specific intercrops on N uptake when species receive tailored fertilizer N under Dutch growing conditions?

In Chapter 2, data were presented from a two-year field experiment (2018 and 2019) on above-ground N uptake (kg N ha^{-1} within a 1.5 m-wide species strip) of species in six intercrops, i.e., wheat-maize, faba bean-maize, pea-maize, wheat-faba bean, pea-faba bean, and pea-wheat, and in the four corresponding sole crops. Compared to the sole crop, C$_3$ species had higher N uptake in the relay intercrop with maize, while the maize had increased N uptake only when grown with wheat or pea and only in one year with a larger temporal niche differentiation (TND) (Box 1.1 in Chapter 1). In simultaneous intercrops involving faba bean, N uptake of the companion wheat or pea decreased in comparison with sole crops. When evaluating at the whole intercrop level, values of the land equivalent ratio for N uptake (LER$_N$) (Box 1.1) larger than one mostly occurred in relay intercropping, while values of simultaneous intercropping were close to one. This indicates that relay intercropping required less land to yield the same N uptake as the combined sole crops. It indicates that relay intercrops allow an improved N uptake under the conditions of the study with tailored N application and an oceanic climate, but simultaneous intercrops do not.

The results thus show that under the species-tailored fertilization strategy in the Dutch growing conditions, temporal complementarity was an important factor driving advantages in N uptake in strip intercropping. Combining cereals with faba bean in either relay intercrops or simultaneous intercrops did, however, not result in increased N uptake compared to cereals in the sole crop.

What plastic responses of shoot growth occur in relay intercrops of maize with wheat or faba bean? To what extent is such plasticity related to differences in light signals between intercrops and sole crops?

In Chapter 3, data on shoot traits, red to far-red ratio (R:FR) and transmitted photosynthetically active radiation (PAR) at the shoot base were presented from field experiments in wheat-maize intercrop, faba bean-maize intercrop, and sole crops of wheat, faba bean, and maize. Wheat plants in border rows of the species strips in intercrops had more tillers per plant than plants in the sole crop. The border row faba bean in intercrops had shorter internodes and more branches per plant than sole faba bean. Plasticity in these border rows of wheat or faba bean was related to a higher R:FR perceived in the radiation from the direction of neighboring maize rows, as well as a higher PAR within the canopy, compared to respective light signals perceived in sole crops. The maize had longer sheaths in the lower canopy in both intercrops than in the sole crop, with the longest sheaths observed in the intercrop with faba bean. This observation coincided with a lower R:FR perceived by maize plants in the radiation from the direction of neighboring bean rows and a lower PAR in the faba bean-maize intercrop than in sole maize and the wheat-maize intercrop.

Thus, the earlier sown species responded to weaker signals for light competition in relay intercrops than in sole crops during early growth. The later sown maize showed shade avoidance traits in response to stronger shade signals in intercrops than in sole crops during its early growth, and interspecific competition for light was stronger when intercropping with faba bean than with wheat.

What are the responses of maize leaf traits and leaf photosynthetic capacity to relay intercropping with wheat or faba bean?

In Chapter 4, I investigated the effects of interspecific competition on maize leaf photosynthetic capacity (A_{1800}) (Box 1.1) and leaf traits, i.e., specific leaf area (SLA; cm^2 leaf g^{-1} leaf), leaf N concentration (LNC; mg N g^{-1} leaf), and specific leaf N (SLN; g N m^{-2} leaf). The distribution of PAR within canopies in the three crop systems- wheat-maize intercrop, faba bean-maize intercrop, and sole maize- were measured to indicate the light conditions to which maize leaves were exposed. During the vegetative stage, maize in both wheat-maize and faba bean-maize intercrops responded to the shading by the early-sown species, showing thinner leaves (larger SLA) with lower SLN and lower A_{1800} than maize leaves in the sole crop, and the responses were strongest in maize plants next to faba bean. During the reproductive stage of maize, when the early-sown wheat and faba bean had been harvested, maize leaves in both intercrops were exposed to better light conditions than leaves in the sole maize; however, this did not result in increased LNC, SLN, and A_{1800} in intercropped maize compared with sole maize.

Chapter 4 showed that during the vegetative stage of maize, competition for light with the early-sown species decreased maize leaf photosynthetic capacity in both relay intercrops compared to the sole crop. This negative effect was stronger when maize was intercropped with faba bean than with wheat. During the reproductive stage, lower leaf N in maize in both intercrops than in sole crops negatively affected leaf photosynthetic capacity of intercropped maize, despite the gradual improved light conditions.

How do maize leaf photosynthetic parameters acclimate to relay intercropping with wheat or faba bean? To what extent are changes in value of the photosynthetic parameters related to altered leaf thickness or leaf N?

In Chapter 5, I made measurements on gas exchange and chlorophyll fluorescence over a range of CO_2 and light levels on selected maize leaves in *vivo*. The measurements were made in intercrops of maize with wheat or faba bean and in sole maize. Light-saturated gross photosynthetic rate ($A_{g,max}$), the maximum rate of ATP production (J_{atpmax}), and the maximum carboxylation rate of Rubisco (V_{cmax}) were estimated. Photosynthesis-light response curves of maize leaves in both relay intercrops differed from those in sole crops mainly in their $A_{g,max}$. During the vegetative stage, values of maize leaf photosynthetic parameters ($A_{g,max}$, J_{atpmax}, and V_{cmax}) increased with leaf mass area (LMA, g leaf m^{-2} leaf). These parameters were lower in thinner leaves in maize grown next to faba bean than those in other crop systems. During the reproductive stage, leaf photosynthetic parameters were correlated with LNC rather than LMA. In this stage, although higher values of $A_{g,max}$ and J_{atpmax} were observed in maize leaves in both intercrops than in sole crops, both LNC and SLN of intercropped maize were not improved compared to sole maize.

The study indicated that during the maize vegetative stage, interspecific competition for light played a role in the acclimation of maize leaf photosynthetic parameters to relay intercropping. In this stage, intercropping with faba bean, but not with wheat, decreased Rubisco carboxylation capacity, ATP production capacity, and thereby $A_{g,max}$ in maize leaves compared to leaves in the sole crop. During the reproductive stage, changes in maize leaf photosynthetic parameters were related to altered leaf N concentration, rather than leaf thickness. However, maize leaf N did not benefit from intercropping with either wheat or faba bean in this late stage.

6.2 Temporal complementarity contributes to enhanced intercropping performance

Combining crop species that grow and use resources (light, water, and nutrients) during different periods of the season in an intercrop allows reduced competition compared to sole crops, in which the plants experience competition throughout the entire season (Gou et al., 2017a; Li et al., 2020d; Liu et al., 2015, 2020; Ma et al., 2020). This phenomenon is known as temporal complementarity in intercrops, which is quantified by the "temporal niche differentiation" index (TND, Box 1.1; Yu et al., 2015). In intercrops combining C_3 and C_4 species, temporal complementarity is reported to drive intercropping advantages in both land productivity and absolute yield gain compared to sole crops (Li et al., 2020b; Xu et al., 2020; Yu et al., 2015).

I found a positive relationship between LER_N and TND across all species combinations (Chapter 2), indicating that temporal complementarity was a key factor driving intercropping advantages in N uptake over corresponding sole crops. Relay intercrops involving maize achieved LER_N values greater than one (except for the pea-maize intercrop in 2019), while simultaneous intercrops had LER_N values mostly close to one. In contrast, advantages in N uptake of cereal/legume simultaneous intercropping (i.e., $LER_N > 1$) were observed in an alternate-row design with zero fertilizer input (Bedoussac and Justes, 2010). Such a system allows a high degree of rhizosphere interactions between cereals and legumes, boosting N_2 fixation by legumes and leaving extra stored soil N for the neighboring cereal plants compared to sole cereals. In the cereal/legume simultaneous intercrops studied, complementarity for N uptake was diminished, on the one hand, by the strip design, and on the other hand, by the N fertilization strategy, which was sufficient for wheat growth (Chapter 2). I conclude that complementary N uptake in strip intercropping was caused by temporal complementarity, and was not due to complementarity in N uptake between cereals and legumes through the biological N fixation of legumes.

Consistent patterns in terms of advantages in land productivity of the intercrops studied were reported in Wang et al. (2023), in which relay intercrops had values of the land equivalent ratio (LER) (Box 6.1) greater than one (with one exception: pea-maize intercrop in 2019), while the LER of simultaneous intercrops tended to be lower than one (Fig. 6.1). Thus, the studied relay intercrops achieved a land use advantage compared to sole crops. In addition, positive absolute yield gains, i.e., values of the net effect (NE) greater than zero (Box 6.1), were found in the relay intercrops, but not in the simultaneous intercrops (Wang et al., 2023; also in Fig. 6.1). The unstable performance of the pea-maize relay intercrop was likely due to the severe lodging of pea in this system in 2019, due to heavy rainfall during the summer. Pea has weak stems that failed to be supported by the neighboring young maize plants in the intercrop, while they intertwined and supported each other in sole crops and they received support from faba bean or wheat in the simultaneous intercrops (Chapter 2). The severe lodging of pea makes it not a suitable crop species in a relay strip intercropping. Pea is more suited for intercropping with firm-stemmed species in full mixtures (Barillot et al., 2012).

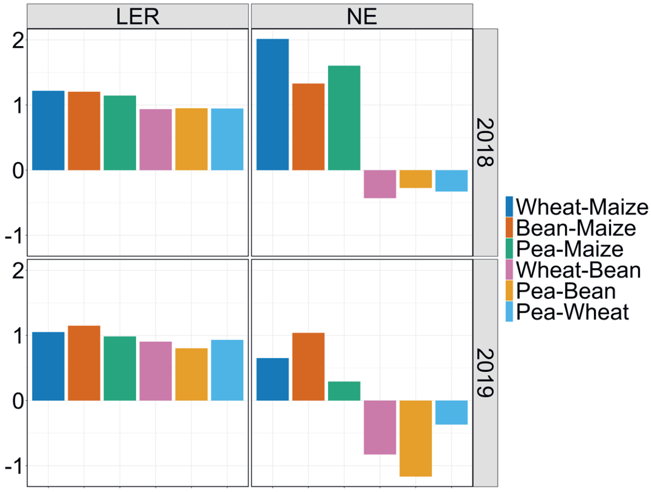

Fig. 6.1 Land equivalent ratio for grain yield (LER) and net effect (NE) of the intercrops studied in year 2018 and 2019 (data retrieved from *https://dx.doi.org/10.17026/dans-266-ws85*). Each bar indicates mean values of each species combination in each year.

I conclude that combining maize with a firm-stemmed C_3 cereal or legume in relay strip intercropping efficiently utilizes land for both productivity and N uptake, and achieves positive yield gains, making it a promising system for western-European growing conditions. In the remaining part of this general discussion, I focus on the eco-physiological responses of crop species in faba bean-maize and wheat-maize relay strip intercropping, aiming to elucidate contributions to agronomic practices aligning with “Good Agricultural Practices”. In the following sections (Section 6.3 and 6.4), I discuss the link between observations on N uptake and shoot plasticity of earlier sown species in the two relay intercrops. Furthermore, I discuss relationships between results on N uptake, shoot plasticity, and leaf photosynthetic traits in the later sown maize.

6.3 Earlier sown species benefit from an improved light environment and improved access to soil N in relay strip intercropping

In relay intercrops, the early-sown species has a better starting position than the same species grown as a sole crop. They are exposed to extra light resources from the strips of companion late-sown species in intercrops during the phase when the companion species is absent and smaller than the earlier sown species (Gou et al., 2017a; Wang et al., 2015; Zhu et al., 2015). Such better light conditions could stimulate biomass accumulation, leading to higher above-ground biomass and N uptake at maturity in the early-sown C_3 species (wheat, faba bean, and pea) in the relay intercrops than in sole crops (Chapter 2). Relay-intercropped wheat had a higher N concentration in the biomass than sole wheat (Chapter 2). This could have resulted from better access to soil N compared to the sole crop. Therefore, the intercropped wheat likely benefited from both improved light conditions and the fertilization strategy, resulting in larger root systems than sole wheat, allowing it to forage for N from the neighboring maize strips as previously reported (Li et al., 2006).

In strip intercropping, border rows of the species strips experience a larger degree of interspecific complementarity than the adjacent inner rows (Wang et al., 2020). Accordingly, plants in different row positions in a species strip exhibit various trait responses in strip intercrops (Li et al., 2020c; Li et al., 2021; Zhu et al., 2016). For instance, in a wheat-maize relay strip intercropping, wheat plants in border rows had more tillers per plant than inner rows and plants in the sole crop (Zhu et al., 2016). A similar phenomenon was observed in the wheat-maize intercrop studied, where border row wheat plants had more tillers than sole wheat (Chapter 3). Increases in branch number per plant were observed in border row faba bean in the relay intercrop with maize, compared to sole faba bean. Plasticity in these border row plants could contribute to increased accumulated light capture in intercrops compared to sole crops (Zhu et al., 2015), playing a role in increased N uptake of earlier sown species in relay intercrops compared to sole crops (Chapter 2).

However, assessing the contribution of shoot plasticity to complementarity in light capture in an intercrop through field experiments is challenging. Complementarity in light capture in intercrops results from, on the one hand, the non-uniformity of intercrop canopies in space and time due to the inherent differences in plant architecture of component species (i.e., differences in genotype). On the other hand, it arises from shoot plasticity (i.e., changes in phenotype of a genotype) (Zhu et al., 2015). These two factors occur at the same time under the field conditions. A functional-structure plant (FSP) model, considering plasticity in plant architecture, physiological processes, and environmental factors, has been adopted to assess the contribution of shoot plasticity to complementarity in light capture in wheat-maize relay strip intercropping (Zhu et al., 2015) and in maize-soybean simultaneous strip intercropping (Li et al., 2021). Thus, modeling could serve as a tool to understand how resource capture in intercrops depends on plasticity in plant traits. Further analysis with observations obtained from western-European growing conditions is needed to provide suggestions for trait selection and breeding to optimize intercropping systems.

Above-ground light competition signals, i.e., reduced R:FR detected in shoots, not only regulate plasticity in shoot traits, but also plasticity in root traits (Gottlieb and Gruntman, 2022; van Gelderen et al., 2018), such as inhibiting the emergence of lateral roots in *Arabidopsis thaliana* (van Gelderen et al., 2018). In a simultaneous intercrop of maize and soybean, a lower R:FR was found in the intercropped soybean canopy than in the sole crop (Yang et al., 2014). The authors reported reduced total root biomass and a lower ratio of root to shoot biomass in intercropped soybean compared to the sole crop. The findings indicate the link between light signals detected in shoots and plasticity in root traits in intercropping. In the relay intercrops studied, faba bean, being an earlier sown species, perceived an increased R:FR in border row plants compared to sole faba bean, preventing shade-avoidance responses and likely triggering a larger allocation of substrates to roots. As a result, these border row plants of faba bean exhibited shorter internodes than the sole crop plants (Chapter 3), and may have had larger root systems leading to a substantial increase in N uptake compared to the sole crop (Chapter 2). It does seem plausible that R:FR detected in shoots is important for regulating plasticity in shoot and root traits, affecting soil resource capture in intercropping.

6.4 Later sown maize responds to interspecific competition for light and N in relay strip intercropping

Previous studies conducted under conventional high-input agriculture have proposed a "competition-recovery principle" in relay intercropping (Zhang and Li, 2003). This principle indicates that during the period when component species grow together in relay intercrops, interspecific competition decreases the growth and resource capture of the later sown species, while they have a recovery after the earlier sown species has been harvested, resulting in similar or even higher yields in intercrops than in sole crops. A substantial recovery in maize above-ground biomass was observed in the relay intercrops with wheat or pea in both years, where the intercropped maize had higher biomass than the sole crop at maturity (Chapter 2). However, such high performance was not observed in maize intercropped with faba bean in either year.

In relay intercropping, high productivity of intercrops often correlates with an enhanced accumulated light capture compared to the sole crop, as a result of complementarity in space and time with companion species (Gou et al., 2017a; Zhang et al., 2008). The overyielding of late-sown maize in relay intercrops compared to sole crops has been reported to increase with TND (Zhao et al., 2023). A larger TND, i.e., a greater temporal complementarity, allows for less overlap in resource competition between component species and a longer period for maize to exploit the extra light coming from the empty neighboring strip where the earlier-sown species has been harvested (Yu et al., 2015). Compared to 2018, the cool and wet spring of 2019 led to a two-week delay in sowing C_3 species (wheat, faba bean, and pea), resulting in an extended co-growth period and a reduced temporal complementarity with maize. The maize in the relay intercrop with wheat or pea benefited from a sufficient temporal complementarity, overyielding in N uptake compared to maize in the sole crop in 2018 but less so in 2019 (Chapter 2).

The tall-statured faba bean cultivar “Fanfare” has a rapidly developing and dense canopy (Andersen et al., 2020), and is taller than wheat (cultivar “Nobless”) and pea (cultivar “Astronaute”). This faba bean cultivar also has a longer growing season than the planted cultivars of the other two C_3 species. In addition, the better starting position for faba bean in the relay intercrop resulted in more branches in border row plants than in sole crops (Chapter 3). Consequently, maize experienced a longer period of strong shading while a shorter recovery period when relay intercropped with faba bean compared to being intercropped with wheat or pea. Thus, intercropping maize with faba bean resulted in a substantial decrease in above-ground biomass and N uptake compared to sole maize during its vegetative stage (Chapter 2). Later on, the maize intercropped with faba bean hardly recovered from early competition due to the restricted period of growing alone in the field. Therefore, the lack of sufficient complementarity in light capture in the faba bean-maize relay intercrop negatively affected maize above-ground biomass and N uptake. However, this negative effect was not observed in the earlier sown faba bean. In fact, the advantages of such a system in N uptake compared to sole crops (i.e., $LER_N > 1$) were attributed to the good performance of faba bean.

6.4.1 Assessing light competition: impacts on maize performance in intercropping with faba bean or wheat during the vegetative stage

Zhu et al. (2014, 2015) indicated that plasticity in maize shoot traits, in response to early competition for light, has a cascading effect on whole-plant development in a wheat-maize relay intercropping. In such an intercrop, the slow early development of maize caused by shading from wheat decreased maize final leaf area index. In my experiments, the strong light signals (i.e., lower R:FR and lower PAR) perceived by the maize next to faba bean likely led to a lower leaf area index than the sole maize (Chapter 3). During the later phase of maize growth, when the companion faba bean had been harvested, the accumulated light capture by the border row maize hardly compensated for the earlier reduction in light capture due to faba bean shading. At maturity, N uptake by these border row maize was lower than that of the sole crop (Chapter 2). This suggests that early competition for light with faba bean likely leads to a cascade of decreasing light capture during later growth, resulting in decreased N uptake by the intercropped maize.

In addition, the strong signal for light competition perceived by maize plants in the faba bean-maize intercrop likely triggered more resources to traits for light-capturing capabilities, i.e., longer sheaths (Chapter 3), at the expense of traits associated with leaf photosynthetic capacity, such as leaf thickness (larger SLA observed in Chapter 4). Thinner leaves is a common response to shading (Poorter et al., 2009), which has been observed in late-sown soybean in a maize-soybean intercrop (Gong et al., 2015) and late-sown maize in a wheat-maize intercrop (Gou et al., 2018). During the maize vegetative stage, maize leaf photosynthetic capacity (A_{1800}), as well as dynamic parameters of leaf photosynthesis ($A_{g,max}$, J_{atpmax}, and V_{cmax}), were correlated with leaf thickness (Chapters 4 and 5). Therefore, in relay strip intercropping, early competition for light determined maize leaf photosynthesis, and the negative effects were stronger for maize intercropped with faba bean than with wheat.

Compared to intercropping with faba bean, maize intercropped with wheat may experience stronger competition for soil N (Li et al., 2006). In a wheat-maize relay intercrop, interspecific competition for N negatively affected maize LNC and SLN compared to maize in its sole crop (Gou et al., 2018). In this study, as well, competing for N with wheat likely decreased access to soil N in intercropped maize compared to sole maize during the vegetative stage. This is supported by the observation that intercropped maize had lower N concentration in shoots than sole maize (Chapter 2). Consequently, maize leaves in border rows of the strip next to wheat had decreased LNC and SLN compared to leaves in sole crops (Chapters 4 and 5). However, A_{1800}, $A_{\mathrm{g,max}}$, and V_{cmax} in these border row maize were not negatively affected. Maize overtopped wheat during its vegetative stage (Chapter 3), so became the dominant species and experienced better light conditions compared to maize intercropped with faba bean and the sole crop. Such improved light conditions likely increased leaf N investment in Rubisco, which is to some extent supported by the higher leaf N use efficiency for V_{cmax} in wheat-maize border than in sole maize (Chapter 5). Therefore, the acclimation of maize leaf photosynthesis to improved light conditions compensates for the negative effect of competition for soil N during the vegetative stage in relay intercropping.

6.4.2 The moderate fertilizer inputs constrained access to soil N in intercropped maize during the reproductive stage

Competition with earlier sown species decreased maize access to soil N in the relay intercrops compared to maize in the sole crop (Chapter 2). This did not cause N deficiency in intercropped maize during the vegetative stage, as the plant N was sufficient for maize, due to a second topdressing applied at the onset of maize stem elongation. However, when the maize entered its grain-filling stage, N deficiency in intercropped maize occurred, as evidenced by N concentration in maize shoots in all intercrops dropping below the critical N dilution curve. Also in an earlier wheat-maize relay intercrop experiment conducted with locally conventional N inputs in the Netherlands, N deficiency occurred in intercropped maize at flowering (Gou et al., 2018). In contrast to high-input relay strip intercropping often reported form China, in which the late-sown maize has extra access to soil N remaining in the strip where the earlier sown species has been harvested (Li et al., 2001a, 2001b; Li et al., 2011a; Xing et al., 2023), in our case, the total available mineral N was fully taken up by the earlier sown species in the relay intercrops (Chapter 2), leaving little for its neighboring maize. The observed higher N uptake in maize intercropped with wheat or pea than sole maize in Chapter 2 was likely due to increases in above-ground biomass, a result of complementary light capture.

The N deficiency in intercropped maize had a negative effect on the leaf N during the reproductive stage. In this stage, maize leaves in both faba bean-maize and wheat-maize intercrops were exposed to better light conditions than the leaves in the sole crop (Chapters 4 and 5). However, both LNC and SLN were not improved in intercropped maize compared to sole maize. In studies conducted in subtropical climates in China, maize was sown before soybean in a relay intercrop (Du et al., 2018; Feng et al., 2020; Yang et al., 2017). The authors applied an additive design for the intercrop regarding plant density and N fertilization, with the fertilizer input in the intercrop being equal to the sum of the fertilizer inputs in the two respective sole crops, with the aim of maximizing intercropping advantages in productivity compared to sole crops. In such a system, a higher LNC and a higher leaf photosynthetic rate were observed in intercropped maize compared to sole maize (Feng et al., 2020). A comparison of the findings of Feng et al. (2020) with observations of maize LNC in the relay intercrops studied suggests that a higher leaf N concentration in intercropped maize compared to sole maize could be achieved by improved light conditions during maize vegetative stage in combination with higher fertilizer input.

However, such a high-input fertilization is not aligned with “Good Agricultural Practices”. High N inputs not only discourages N_2 fixation of legumes (Coskun et al., 2017) but also may lead to N leaching from the soil, as legumes only require limited N fertilization (Wang et al., 2022). In this study, maize leaf photosynthetic capacity (A_{1800}) and leaf photosynthetic parameters ($A_{\mathrm{g,max}}$, J_{atpmax}, V_{cmax}) were found to correlate with LNC rather than leaf thickness during maize reproductive stage (Chapters 4 and 5). The N deficiency in the intercropped maize could constrain its leaf photosynthetic capacity and the dynamic parameters, preventing improvement even when experiencing better light conditions than maize in the sole crop during later phases. Therefore, the responses of maize leaf photosynthetic traits in relay intercropping hardly contributed to the observed higher maize above-ground biomass or grain yield (Chapter 4) in the intercrop with wheat compared to sole maize. The overyielding of maize in the relay intercrops studied was therefore likely due to complementarity in light capture, as previously reported (Gou et al., 2017a; Zhang et al., 2008).

Explaining crop performance through results obtained from individual leaves is challenging because a crop canopy consists of a population of individual plants. Moreover, radiation conversion efficiency of a crop species at maturity is determined by leaf photosynthetic capacity of all leaves within the canopy and during the entire growing season (Long et al., 2006). Contradictions between observations at the leaf level and the crop level were found in an earlier wheat-maize intercrop experiment, in which the intercropped maize had higher leaf photosynthetic capacity at flowering but lower radiation conversion efficiency at maturity than sole maize (Gou et al., 2018). In an intercrop, light distribution within the canopy differs from that in a pure stand over time (shown in Chapter 4) due to heterogeneity in canopy structure, which could be considered when scaling up from the photosynthetic capacity of individual leaves to canopy photosynthesis in intercrops. An FSP model could serve as a tool in this domain (Evers et al., 2019), investigating how radiation conversion efficiency of intercrops depends on the responses of leaf photosynthetic capacity.

In conclusion, the earlier sown species in relay strip intercropping responded to an improved light environment and had higher above-ground biomass and N uptake than the corresponding sole crops. On the contrary, competition for light with the early-sown species during the maize reproductive stage led to decreases in its above-ground biomass, N uptake, leaf photosynthetic capacity, and traits associated with Rubisco carboxylation capacity and ATP production capacity, compared to maize in the sole crop. During the maize reproductive stage, N deficiency in intercropped maize negatively affected maize leaf photosynthetic capacity and constrained maize recovery in N uptake. This raises two questions: i) whether species-tailored fertilization in strip intercropping is recommended for sustainable agriculture (Section 6.5); ii) how to increase maize leaf photosynthetic capacity in relay strip intercropping, making it a contributing factor to the overyielding of intercropped maize (see in Section 6.6).

6.5 Relay strip intercropping combined with species-tailored fertilization is advisable for "Good Agricultural Practices"

In this study, the intercrop received the weighted average N inputs of in the sole crops, based on the proportions of the area of each species in the intercrop. The "starter" fertilizer N (20 kg ha^{-1}) applied within the strips of faba bean or pea in all intercrop treatments, as well as in their sole crops, allowed the two legumes to exploit N_2 fixation. This is evidenced by their final N uptake that far exceeded the applied amount (Chapter 2). The two cereals (wheat and maize) in the relay intercrops captured almost all mineral N available in the soil (Chapter 2). The results suggest that relay intercropping, coupled with the applied N fertilization strategy, boosts N_2 fixation by legumes, fully takes up available mineral N in the soil, and reduces environmental costs.

However, such a thorough capture of available mineral N did not occur in wheat in the simultaneous intercrop with a legume (Chapter 2). In the studied cereal/legume simultaneous intercrops, wheat did not benefit from intercropping with a legume (faba bean or pea) in both above-ground biomass and N uptake compared to the sole crop. In contrast to cereal/legume mixed intercropping (with no distinct row arrangement) or alternate row intercropping conducted with low-input fertilization, where N uptake by the cereal is increased compared to the sole crop (Bedoussac and Justes, 2010; Ghaley et al., 2005), the strip design in this study likely diminished rhizosphere interactions (Jensen et al., 2020), resulting in a lack of complementarity in N capture between cereals and legumes. Moreover, strong shading by faba bean decreased wheat biomass in simultaneous intercrops (Chapter 2), preventing the wheat from fully capturing the total available mineral N in the soil. When comparing wheat in the simultaneous intercrop to wheat in the relay intercrop, a more thorough capture of available N by the cereal is achieved through complementarity in light capture.

The fertilizer nitrogen equivalent ratio (FNER) was developed to answer the question whether intercropping saves fertilizer resources required for a given production (van der Werf et al., 2021; Xu et al., 2020) (Box 6.1). An FNER greater than one indicates that sole crops need more fertilizer N available than the intercrop to obtain the same production, so the intercrop uses fertilizer N more efficiently than sole crops do. In this study, values of FNER in the relay intercrops tended to be larger than one, while those in the simultaneous intercrops tended to be smaller than one (Chapter 2), indicating that relay intercrops, but not simultaneous intercrops, had advantages in saving fertilizer N. Given the observations mentioned on LER_N (Chapter 2), LER, and NE (Fig. 6.1) in this chapter, it is suggested that relay intercropping maize with a firm-stemmed species, when combined with strip- and species-tailored N fertilization in accordance with “Good Agricultural Practices”, could be a potential solution to several sustainability issues in agriculture, including food security, biodiversity loss, environmental degradation, and climate change (Hossain et al., 2020).

Despite the strong shading from faba bean, which had a negative effect on maize above-ground biomass and N uptake compared with sole maize, the presence of faba bean contributed to a significant decrease in total fertilizer inputs in the intercrop. Additionally, the increases in biomass and N uptake in the intercropped faba bean compared to its sole crop align with the needs for diversification of food systems and the production of more plant-based protein for human diets (van Zanten et al., 2023). Given that maize, being a C_4 species, has a higher temperature requirement and a longer growing period than the C_3 species, it allows the presence of temporal complementarity when intercropped with a C_3 species under temperate climates. Faba bean-maize relay strip intercropping is still recommended when aiming to achieve increased production of faba bean and comparable production of maize while simultaneously using less land and reduced fertilizer inputs compared to sole crops. Nevertheless, there may be options to enhance maize performance in relay strip intercropping through both agronomic practices and breeding (see in Section 6.6).

6.6 Practical recommendation: how to let maize thrive in relay strip intercropping?

In this study, N deficiency in intercropped maize during the recovery period negatively affected its leaf photosynthetic capacity. As mentioned, the overyielding of the late-sown maize has been reported to increase with TND (Zhao et al., 2023). An option to enhance maize performance in relay strip intercropping is to reduce the period of competition with the earlier sown companion species, i.e., the co-growth period, and to increase the period available for maize growth after the earlier sown species has been harvested, i.e., the recovery period. An early harvest of the companion species in relay intercrops could increase maize leaf photosynthetic capacity and biomass. Relay strip intercropping with a winter-sown C_3 species, rather than a spring-sown one, would be a recommendation. Additionally, using a late-maturing maize variety, if the season length allows, could also alleviate competition during its vegetative stage.

Recently, there has been increasing interest in breeding for intercropping (Brooker et al., 2015; Haug et al., 2023). On the one hand, intercropping serves as a potential solution to address sustainability in agriculture, and there is still room to increase its yield potential and stability (Gou et al., 2017b). On the other hand, the crop species used in intercropping have, in fact, been bred in the context of sole cropping. The ideotype of a crop species required in intercrops, however, would be different from that in sole crops. This difference arises not only because the growth environments are altered by species interactions in intercrops, but also because breeding for intercropping should optimize the complementarity of the whole system.

In this study, changes in V_{cmax} and J_{atpmax} were found to contribute to the acclimation of maize leaf photosynthetic capacity in the relay intercrops (Chapter 5). A maize ideotype with enhanced Rubisco carboxylation capacity and ATP production capacity may be considered, which could be achieved by increasing the content of leaf photosynthetic components (Salesse-Smith et al., 2020). Such an ideotype maize may grow fast and become the dominant species in relay intercrops during its vegetative stage, capturing more light and soil resources than when they are growing in sole crops. However, there is a concern about whether such an ideotype maize would have a negative impact on the productivity of the companion species due to enhanced shading, as well as its impact on the resource use efficiency of the whole system. Modeling might be an interesting tool prior to engaging into such novel breeding routs (Evers et al., 2019) and to weigh the relative advantages of changing intercropping designs versus changing the genotypes.

In experiments conducted in conventional high-input agriculture (with fertilizer input exceeding 300 kg N ha^{-1}) in China, a third topdressing has been applied to maize during the tasseling stage in both wheat-maize relay intercrop and sole maize plots (Liu et al., 2015, 2020). This extra application provided sufficient available soil N for maize, allowing the intercropped maize to have a sufficient N concentration and to exploit better light conditions experienced in intercrops compared to sole crops. An extra N application at maize tasseling may also be an option to have increased leaf photosynthetic capacity in intercropped maize compared to sole maize. However, the implementation of such a recommendation should consider both environmental policies on N emissions and consider as well the purpose of conducting intercropping: maximizing intercropping advantages over sole crops or taking environmental impacts into account.

Postponing fertilizer N topdressing to maize reproductive stage was reported to lead to a substantial N recovery in maize in a pea-maize relay strip intercrop (Xu et al., 2023). In the study of Xu et al. (2023), the intercropped maize had already received high amounts of fertilizer N (> 250 kg ha^{-1}) before it received the postponed fertilizer N. In this study, postponing the second application, which was applied during maize vegetative stage at a rate of 90 kg ha^{-1}, to maize reproductive stage may be considered. However, there is a concern on N deficiency that may occur in intercropped maize before it receives the postponed topdressing, as the maize only received 80 kg ha^{-1} at sowing. There is a need for more experimentation on the amount and timing of fertilizer N application to optimize maize performance while still aligning with the logic of the current EU "Good Agricultural Practices".

6.7 Concluding remarks and thoughts for future research

In this thesis, I studied the eco-physiological responses of crop species in strip intercropping. I aimed to observe results that are consistent with "Good Agricultural Practices", through investigating mechanisms of species interactions in strip intercropping applied with strip- and species-tailored fertilization strategy. Temporal complementarity between species, rather than complementarity in N uptake mechanisms associated with the ability of N_2 fixation by legumes, contributed to improved N uptake in intercrops compared to sole crops. The earlier sown species benefited from better light environments and improved access to soil N compared to when they were in sole crops. For the later sown maize, the observed variation in N uptake and the negative effects on leaf photosynthetic capacity were attributed to factors such as interspecific competition for light during the maize vegetative stage, N deficiency during its reproductive stage, and the length of the period when maize is co-growing with earlier sown species and when maize is recovering from early competition.

Recommendations are given for releasing interspecific competition during the maize vegetative stage, such as selecting species cultivars or breeding ideotype maize, as well as for preventing N shortage in the soil through applying extra fertilizer N or postponing topdressing after maize tasseling. In this thesis, I focused on the effects of strip intercropping on species' morphological traits at the plant level and physiological traits at the leaf level. However, it remains uncertain how the observed responses at the leaf or plant level contribute to the resource use efficiency of a population of plants in an intercrop stand. Further analysis on upscaling through modeling, such as FSP modeling, could be considered in this domain. Additionally, this work could offer options for selecting traits in breeding routes for intercropping.

Box 6.1 Metrics used in Chapter 6

Land equivalent ratio (LER)

The land equivalent ratio (LER) is used to assess the relative land productivity compared to sole crops (Willey and Rao, 1980):

$$\mathrm{LER} = \frac{Y_{\mathrm{I},1}}{Y_{\mathrm{M},1}} + \frac{Y_{\mathrm{I},2}}{Y_{\mathrm{M},2}} \tag{1}$$

where $Y_{\mathrm{I},i}$ is the grain yield of species i in an intercrop, expressed per unit of total area of the intercrop, and $Y_{\mathrm{M},i}$ is the grain yield of species i in its sole crop, expressed per unit area of the sole crop. An LER greater than one indicates that the combined sole crops need more land to achieve the same production as the intercrop.

Net effect (NE)

The net effect (NE) is used to assess the absolute yield gain compared to sole crops (Loreau and Hector, 2001):

$$\mathrm{NE} = \left(Y_{\mathrm{I},1} + Y_{\mathrm{I},2}\right) - \left(Y_{\mathrm{M},1} \times \mathrm{LS}_1 + Y_{\mathrm{M},2} \times \mathrm{LS}_2\right) \tag{2}$$

where $Y_{\mathrm{M},i} \times \mathrm{LS}_i$ is the expected yield of species i, calculating as the grain yield (per unit area of the sole crop) of species i in the respective sole crop ($Y_{\mathrm{M},i}$) and the land sharing of species i (LS_i) in intercropping. In this study, land sharing of all species is 0.5. An NE greater than zero indicates that intercropping achieves a positive absolute yield gain compared to sole crops, that is: the total production is greater than expected.

Fertilizer nitrogen equivalent ratio (FNER)

The fertilizer nitrogen equivalent ratio (FNER) is used to assess the relative fertilizer savings of intercrops for productivity compared to sole crops (Xu et al., 2020):

$$\mathrm{FNER} = \left(\frac{Y_{\mathrm{I},1}}{Y_{\mathrm{M},1}} \times \frac{F_{\mathrm{M},1}}{F_{\mathrm{IC}}}\right) + \left(\frac{Y_{\mathrm{I},2}}{Y_{\mathrm{M},2}} \times \frac{F_{\mathrm{M},2}}{F_{\mathrm{IC}}}\right) \tag{3}$$

where $F_{\mathrm{M},i}$ is the amount of fertilizer N applied within strips of species i in the respective sole crop, and F_{IC} is the fertilizer N input per unit area of the intercrop. In this study, all species received tailored fertilizer inputs applied within the strips in both intercrops and respective sole crops. As the intercrops comprised 50% area of both species, the N input per unit area of the intercrop was equal to the average of the input in the sole crop of the component species. An FNER greater than one indicates that intercropping saves fertilizer N inputs for the same product output as sole crops.

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Temporal complementarity drives N uptake in crop species mixtures with or without a legume

Supplementary Information for Chapter 2:

Table S2.1 AIC values of all models and the fitted parameter values of the selected model

Table S2.2 AIC values of all models used to quantify the relationships between LER_N and TND

Table S2.3 Comparisons of above-ground N uptake per species strip

Table S2.4 Comparisons of above-ground N uptake in each row for each species

Table S2.5 Comparisons of above-ground N concentration per species strip

Table S2.6 Comparisons of above-ground biomass and N uptake per species strip during intermediate harvests and at final harvest in 2019

Fig. S2.1 Above-ground N concentration per species strip versus above-ground biomass per species strip for faba bean and pea in 2019

Table S2.1 AIC values of all models used to estimate N dilution curves, and the fitted parameter values of the selected model for maize (i) and wheat (ii). The AIC value of each model version was calculated based on negative log-likelihood and a penalty term for the number of parameters: $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The **bold** AIC value represents the selected model. ΔAIC is the difference of a model compared to the selected model.

(i) Maize. AIC values of all models:

Model	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
1	Monocrop, Wheat-Maize, Bean-Maize, Pea-Maize	a and b	9	-5.16	12.8	3.84
2		a	6	0.82	12.8	3.81
3		b	6	3.99	16.0	6.98
4	(Monocrop, Wheat-Maize), Bean-Maize, Pea-Maize	a and b	7	3.35	17.3	8.35
5		a	5	6.96	17.0	7.96
6		b	5	5.31	15.3	6.30
7	(Monocrop, Bean-Maize), Wheat-Maize, Pea-Maize	a and b	7	7.19	21.2	12.2
8		a	5	9.28	19.3	10.3
9		b	5	10.9	20.9	11.9
10	(Monocrop, Pea-Maize), Wheat-Maize, Bean-Maize	a and b	7	6.18	20.2	11.2
11		a	5	9.69	19.7	10.7
12		b	5	8.09	18.1	9.08
13	(Wheat-Maize, Bean-Maize), Monocrop, Pea-Maize	a and b	7	-1.26	12.7	3.74
14		a	5	0.85	10.9	1.85
15		b	5	5.15	15.1	6.14
16	(Wheat-Maize, Pea-Maize), Monocrop, Bean-Maize	a and b	7	-4.53	9.47	0.46
17		a	5	1.00	11.0	2.00
18		b	5	4.40	14.4	5.39
19	(Bean-Maize, Pea-Maize), Monocrop, Wheat-Maize	a and b	7	-2.51	11.5	2.49
20		a	5	0.89	10.9	1.89
21		b	5	4.20	14.2	5.19
22	(Wheat-Maize, Bean-Maize, Pea-Maize), Monocrop	a and b	5	-0.14	9.86	0.86
23		a	4	1.00	**9.00**	0.00
24		b	4	5.15	13.1	4.14
25	(Monocrop, Bean-Maize, Pea-Maize), Wheat-Maize	a and b	5	10.4	20.4	11.4
26		a	4	11.5	19.5	10.5
27		b	4	11.6	19.6	10.6
28	(Monocrop, Wheat-Maize, Pea-Maize), Bean-Maize	a and b	5	6.99	17.0	7.99
29		a	4	10.6	18.6	9.56
30		b	4	8.24	16.2	7.24
31	(Monocrop, Wheat-Maize, Bean-Maize), Pea-Maize	a and b	5	9.68	19.7	10.7
32		a	4	10.0	18.0	9.03
33		b	4	10.9	18.9	9.88
34	All data combined	None	3	11.7	17.7	8.67

Fitted parameter values (standard errors in brackets) of the selected model (i.e., Model 23). Identical values of a parameter across treatments indicates that the parameter was not different among those treatments.

Parameter	Monocrop	Wheat-Maize	Bean-Maize	Pea-Maize
a_{m}	4.43 (0.14)	3.81 (0.25)	3.81 (0.25)	3.81 (0.25)
b_{m}	0.37 (0.03)	0.37 (0.03)	0.37 (0.03)	0.37 (0.03)

(ii) Wheat. The AIC values of all models:

Model	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
1	Monocrop, Wheat-Maize, Wheat-Bean, Pea-Wheat	a and b	9	-13.9	4.06	3.27
2		a	6	-2.58	9.42	8.64
3		b	6	-4.78	7.22	6.44
4	(Monocrop, Wheat-Maize), Wheat-Bean, Pea-Wheat	a and b	7	-6.83	7.17	6.38
5		a	5	-0.30	9.70	8.91
6		b	5	0.91	10.9	10.1
7	(Monocrop, Wheat-Bean), Wheat-Maize, Pea-Wheat	a and b	7	-2.62	11.4	10.6
8		a	5	-2.56	7.44	6.66
9		b	5	-1.61	8.39	7.60
10	(Monocrop, Pea-Wheat), Wheat-Maize, Wheat-Bean	a and b	7	-8.40	5.60	4.81
11		a	5	-1.67	8.33	7.54
12		b	5	-1.30	8.70	7.91
13	(Wheat-Maize, Wheat-Bean), Monocrop, Pea-Wheat	a and b	7	-8.63	5.37	4.59
14		a	5	0.83	10.8	10.0
15		b	5	-4.34	5.66	4.88
16	(Wheat-Maize, Pea-Wheat), Monocrop, Wheat-Bean	a and b	7	-13.9	0.09	-0.69
17		a	5	-2.19	7.81	7.02
18		b	5	-4.41	5.59	4.80
19	(Wheat-Bean, Pea-Wheat), Monocrop, Wheat-Maize	a and b	7	-10.8	3.24	2.45
20		a	5	-1.07	8.93	8.14
21		b	5	-4.77	5.23	4.44
22	(Wheat-Maize, Wheat-Bean, Pea-Wheat), Monocrop	a and b	5	-9.21	**0.79**	0.00
23		a	4	1.04	9.04	8.25
24		b	4	-4.25	3.75	2.96
25	(Monocrop, Wheat-Bean, Pea-Wheat), Wheat-Maize	a and b	5	-1.01	8.99	8.20
26		a	4	-0.91	7.09	6.31
27		b	4	-0.36	7.64	6.86
28	(Monocrop, Wheat-Maize, Pea-Wheat), Wheat-Bean	a and b	5	-5.31	4.69	3.90
29		a	4	-0.28	7.72	6.93
30		b	4	1.53	9.53	8.74
31	(Monocrop, Wheat-Maize, Wheat-Bean), Pea-Wheat	a and b	5	1.26	11.3	10.5
32		a	4	1.27	9.27	8.48
33		b	4	1.40	9.40	8.61
34	All data combined	None	3	1.74	7.74	6.95

Fitted parameter values (standard errors in brackets) of the selected model (i.e., Model 22). Identical values of a parameter across treatments indicates that the parameter was not different among those treatments.

Parameter	Monocrop	Wheat-Maize	Wheat-Bean	Pea-Wheat
a_{W}	6.30 (0.26)	5.69 (0.10)	5.69 (0.10)	5.69 (0.10)
b_{W}	0.65 (0.03)	0.55 (0.01)	0.55 (0.01)	0.55 (0.01)

Table S2.2 AIC values of all models used to quantify the relationships between $\mathrm{LER}_{\mathrm{N}}$ and TND. The bold AIC value represents the selected model. ΔAIC is the difference of a model compared to the selected model.

Model	Degrees of freedom	log-likelihood	AIC	ΔAIC
1	4	46.253	-84.5	10.3
2	5	46.664	-83.3	11.5
3	5	46.254	-82.5	12.3
4	6	47.087	-82.2	12.7
5	6	51.543	-91.1	3.8
6	9	56.428	**-94.9**	0.0
7	14	60.578	-93.2	1.7

Table S2.3 Comparisons of above-ground N uptake per species (kg ha^{-1}) for maize, wheat, faba bean, and pea at final harvest. Values of means and their 95% confidence intervals in brackets are presented for each focal species when it was with conspecific in monocrops and with other species in intercrops. The 95% confidence interval was obtained separately for each year, with the treatment as the fixed effect and the block as the random effect. Shared letters denote non-significant differences between treatments within each year and focal species according to Fisher's LSD test at $P < 0.05$. To allow a direct comparison between sole crops and intercrops, N uptake in intercrops is expressed per unit area (kg ha^{-1}) of the 1.5 m-wide strip of a species.

Year	Species	Companion		Species	Companion	
2018	Maize	Maize	265 [235, 294] b	Wheat	Wheat	172 [163, 181] b
		Wheat	307 [277, 336] a		Maize	224 [215, 233] a
		Faba bean	251 [222, 281] b		Faba bean	165 [156, 174] b
		Pea	307 [278, 337] a		Pea	166 [157, 175] b
2019		Maize	269 [248, 289] ab		Wheat	183 [162, 204] bc
		Wheat	286 [266, 307] a		Maize	212 [191, 233] a
		Faba bean	248 [227, 268] b		Faba bean	158 [136, 179] c
		Pea	271 [250, 292] ab		Pea	206 [185, 227] ab
2018	Faba bean	Faba bean	310 [279, 341] c	Pea	Pea	242 [229, 255] b
		Maize	405 [374, 436] a		Maize	269 [256, 282] a
		Wheat	342 [311, 373] bc		Wheat	222 [209, 235] c
		Pea	356 [325, 387] b		Faba bean	201 [188, 214] d
2019		Faba bean	359 [287, 432] b		Pea	265 [227, 302] a
		Maize	479 [407, 552] a		Maize	245 [207, 282] a
		Wheat	426 [353, 498] ab		Wheat	232 [195, 270] a
		Pea	384 [311, 457] ab		Faba bean	168 [131, 206] b

Table S2.4 Comparisons of above-ground N uptake in each row (g m^{-2}) for maize (i), wheat (ii), faba bean (iii), and pea (iv) at final harvest. Values of means and their 95% confidence intervals in brackets are presented for each focal species. The 95% confidence interval was obtained in each year, with Species Combination_ Row as the fixed effect and block as the random effect. Shared letters denote non-significant differences between treatments within each year according to Fisher's LSD test at *P* < 0.05.

(i) Maize

Species	Year	Companion	Row	
Maize	2018	Maize	-	26.5 [23.6, 29.4] bc
		Wheat	Border	30.4 [27.0, 33.7] ab
			Inner	31.3 [26.8, 35.7] ab
		Faba bean	Border	24.6 [21.2, 27.9] c
			Inner	26.2 [21.7, 30.7] bc
		Pea	Border	32.5 [29.1, 35.8] a
			Inner	27.2 [22.7, 31.7] bc
	2019	Maize	-	26.9 [24.8, 28.9] a
		Wheat	Border	29.8 [27.3, 32.3] a
			Inner	26.3 [22.7, 29.8] a
		Faba bean	Border	21.8 [19.3, 24.3] b
			Inner	30.6 [27.1, 34.2] a
		Pea	Border	27.5 [25.0, 30.0] a
			Inner	26.3 [22.8, 29.8] a

(ii) Wheat

Species	Year	Companion	Row	
Wheat	2018	Wheat	-	17.2 [16.4, 18.0] cd
		Maize	Border	30.2 [29.0, 31.5] a
			Inner I	18.3 [17.0, 19.5] bc
			Inner II	18.7 [17.4, 19.9] b
		Faba bean	Border	15.6 [14.3, 16.9] e
			Inner I	16.5 [15.2, 17.7] de
			Inner II	17.5 [16.3, 18.8] bcd
		Pea	Border	17.4 [16.1, 18.6] bcd
			Inner I	16.3 [15.1, 17.6] de
			Inner II	16.2 [14.9, 17.5] de
	2019	Wheat	-	18.3 [16.6, 20.0] b
		Maize	Border	27.8 [25.7, 30.0] a
			Inner I	17.3 [15.2, 19.5] b
			Inner II	18.5 [16.3, 20.6] b
		Faba bean	Border	16.3 [14.2, 18.5] bc
			Inner I	14.4 [12.2, 16.6] c
			Inner II	16.6 [14.5, 18.8] bc
		Pea	Border	25.7 [23.5, 27.8] a
			Inner I	17.4 [15.2, 19.5] b
			Inner II	18.7 [16.6, 20.9] b

(iii) Faba bean

Species	Year	Companion	Row	
Faba bean	2018	Faba bean	-	31.0 [28.8, 33.2] f
		Maize	Border	44.4 [41.1, 47.7] a
			Inner I	41.2 [37.9, 44.5] ab
			Inner II	35.9 [32.6, 39.1] cd
		Wheat	Border	34.8 [31.6, 38.1] de
			Inner I	33.6 [30.4, 36.9] def
			Inner II	34.1 [30.8, 37.4] def
		Pea	Border	39.3 [36.0, 42.5] bc
			Inner I	35.9 [32.7, 39.2] cd
			Inner II	31.6 [28.3, 34.8] ef
	2019	Faba bean	-	35.9 [31.1, 40.8] cd
		Maize	Border	56.4 [49.1, 63.6] a
			Inner I	44.5 [37.3, 51.8] b
			Inner II	42.9 [35.6, 50.1] bc
		Wheat	Border	58.3 [51.1, 65.5] a
			Inner I	38.1 [30.9, 45.4] bcd
			Inner II	31.3 [24.1, 38.5] d
		Pea	Border	45.7 [38.5, 53.0] b
			Inner I	37.9 [30.7, 45.2] bcd
			Inner II	31.5 [24.3, 38.8] d

(iv) Pea

Species	Year	Companion	Row	
Pea	2018	Pea	-	24.2 [22.6, 25.8] bc
		Maize	Border	32.1 [29.4, 34.8] a
			Inner I	26.1 [23.3, 28.8] b
			Inner II	22.6 [19.9, 25.3] bcd
		Wheat	Border	21.4 [18.6, 24.1] cde
			Inner I	23.0 [20.3, 25.7] bcd
			Inner II	22.2 [19.5, 25.0] bcd
		Faba bean	Border	18.3 [15.6, 21.0] e
			Inner I	20.0 [17.3, 22.7] de
			Inner II	22.0 [19.2, 24.7] cde
	2019	Pea	-	26.5 [23.4, 29.6] b
		Maize	Border	36.2 [31.1, 41.4] a
			Inner I	19.2 [14.1, 24.3] cde
			Inner II	18.0 [12.8, 23.1] de
		Wheat	Border	25.2 [20.1, 30.4] bc
			Inner I	21.8 [16.7, 26.9] bcde
			Inner II	22.7 [17.6, 27.8] bcd
		Faba bean	Border	15.4 [10.3, 20.5] e
			Inner I	16.8 [11.6, 21.9] de
			Inner II	18.3 [13.2, 23.5] cde

Table S2.5 Comparisons of above-ground N concentration per species (mg g^{-1}) for maize, wheat, faba bean, and pea at final harvest. Values of means and their 95% confidence intervals in brackets are presented for each focal species when it was with a conspecific in monocrops and with other species in intercrops. The 95% confidence interval was obtained in each year, with the treatment as the fixed effect and the block as the random effect. Shared letters denote non-significant differences between treatments within each year according to Fisher's LSD test at $P < 0.05$.

Year	Species	Companion		Species	Companion	
2018	Maize	Maize	10.1 [9.71, 10.5] a	Wheat	Wheat	13.4 [12.9, 13.9] bc
		Wheat	9.48 [9.08, 9.88] b		Maize	13.9 [13.4, 14.4] ab
		Bean	9.47 [9.07, 9.87] b		Bean	14.7 [14.2, 15.2] a
		Pea	9.30 [8.90, 9.70] b		Pea	12.9 [12.3, 13.4] c
2019		Maize	10.8 [9.91, 11.7] a		Wheat	11.6 [10.5, 12.7] c
		Wheat	9.47 [8.57, 10.4] b		Maize	13.6 [12.5, 14.7] ab
		Bean	10.1 [9.14, 11.0] ab		Bean	14.3 [13.2, 15.3] a
		Pea	9.00 [8.09, 9.91] b		Pea	12.5 [11.5, 13.6] bc
2018	Faba bean	Bean	28.8 [27.4, 30.2] a	Pea	Pea	21.7 [21.1, 22.3] b
		Maize	29.4 [28.0, 30.8] a		Maize	23.4 [22.8, 24.0] a
		Wheat	28.6 [27.2, 30.0] a		Wheat	22.1 [21.5, 22.7] b
		Pea	28.5 [27.1, 29.8] a		Bean	21.7 [21.1, 22.3] b
2019		Bean	26.0 [24.4, 27.6] b		Pea	22.4 [21.2, 23.6] a
		Maize	28.1 [26.5, 29.7] a		Maize	22.7 [21.5, 23.9] a
		Wheat	26.6 [25.0, 28.2] b		Wheat	23.0 [21.8, 24.2] a
		Pea	25.8 [24.2, 27.4] b		Bean	22.7 [21.5, 23.9] a

Table S2.6 Comparisons of above-ground biomass and N uptake per species strip for maize (i), wheat (ii), faba bean (iii), and pea (iv) in 2019. Values of means and their 95% confident intervals in brackets are presented for each focal species. The 95% confidence interval was obtained in each individual harvest, with the treatment as the fixed effect and the block as the random effect. Shared letters denote non-significant differences between treatments within an individual harvest according to Fisher's LSD test at $P < 0.05$.

(i) Maize. DOY 261 was the final harvest.

Species	DOY	Companion	Biomass (Mg ha^{-1})	N uptake (kg ha^{-1})
Maize	168	Maize	0.632 [0.440, 0.825] a	25.6 [18.3, 32.8] a
		Wheat	0.356 [0.164, 0.548] b	14.1 [6.84, 21.4] b
		Faba bean	0.401 [0.209, 0.594] b	16.7 [9.47, 24.0] b
		Pea	0.392 [0.200, 0.584] b	15.3 [8.05, 22.6] b
	182	Maize	3.830 [2.690, 4.970] a	105 [77.6, 132] a
		Wheat	2.89 [1.75, 4.03] ab	76.9 [49.8, 104] ab
		Faba bean	2.11 [0.97, 3.25] b	61.3 [34.2, 88.4] b
		Pea	3.00 [1.86, 4.14] ab	76.8 [49.7, 104] ab
	196	Maize	8.45 [7.60, 9.30] a	177 [163, 192] a
		Wheat	6.75 [5.66, 7.92] b	144 [121, 165] b

		Faba bean	4.70 [3.84, 5.55] c	107 [92.5, 122] c
		Pea	7.19 [6.33, 8.04] b	139 [124, 153] b
	234	Maize	20.4 [17.6, 23.3] ab	249 [213, 284] a
		Wheat	23.9 [20.5, 27.3] a	239 [196, 281] ab
		Faba bean	17.6 [14.8, 20.4] b	193 [158, 228] b
		Pea	23.6 [20.7, 26.4] a	224 [189, 260] ab
	261	Maize	24.8 [23.3, 26.4] b	269 [248, 289] ab
		Wheat	30.3 [28.7, 31.8] a	286 [266, 307] a
		Faba bean	24.8 [23.2, 26.4] b	248 [227, 268] b
		Pea	30.1 [28.6, 31.7] a	271 [250, 292] ab

(ii) Wheat. DOY 220 was the final harvest.

Species	DOY	Companion	Biomass (Mg ha^{-1})	N uptake (kg ha^{-1})
Wheat	133	Wheat	1.24 [0.73, 1.75] a	59.7 [36.8, 82.6] a
		Maize	1.31 [0.81, 1.82] a	64.0 [41.1, 86.9] a
		Faba bean	1.30 [0.79, 1.80] a	62.4 [39.5, 85.2] a
		Pea	1.28 [0.77, 1.79] a	62.5 [39.6, 85.4] a
	147	Wheat	3.42 [2.88, 3.96] b	91.9 [76.0, 108] a
		Maize	3.97 [3.43, 4.50] a	110 [94.0, 126] a
		Faba bean	3.62 [3.08, 4.16] ab	108 [92.5, 124] a
		Pea	3.55 [3.01, 4.09] ab	102 [85.7, 117] a
	168	Wheat	8.53 [6.96, 10.1] a	140 [114, 166] a
		Maize	8.44 [6.88, 10.0] a	141 [115, 167] a
		Faba bean	7.45 [5.89, 9.01] a	147 [121, 173] a
		Pea	8.27 [6.70, 9.83] a	140 [114, 165] a
	182	Wheat	12.4 [10.8, 14.1] a	160 [119, 201] a
		Maize	12.7 [11.0, 14.3] a	176 [143, 228] a
		Faba bean	9.65 [7.99, 11.3] b	142 [106, 199] a
		Pea	12.4 [10.8, 14.1] a	179 [138, 220] a
	196	Wheat	14.5 [12.5, 16.5] a	155 [122, 189] b
		Maize	16.8 [13.5, 18.8] a	220 [166, 269] a
		Faba bean	11.7 [9.72, 13.7] b	162 [129, 195] ab
		Pea	15.6 [13.6, 17.6] a	192 [159, 226] ab
	220	Wheat	15.7 [14.4, 17.1] a	183 [162, 204] bc
		Maize	15.6 [14.3, 16.9] a	212 [191, 233] a
		Faba bean	11.1 [9.80, 12.5] b	158 [136, 179] c
		Pea	16.4 [15.1, 17.7] a	206 [185, 227] ab

(iii) Faba bean. DOY 226 was the final harvest.

Species	DOY	Companion	Biomass (Mg ha^{-1})	N uptake (kg ha^{-1})
Faba bean	133	Faba bean	0.534 [0.450, 0.617] a	22.9 [18.6, 27.3] a
		Maize	0.501 [0.418, 0.584] a	21.7 [17.4, 26.1] a
		Wheat	0.548 [0.465, 0.631] a	23.6 [19.2, 27.9] a
		Pea	0.490 [0.407, 0.573] a	21.0 [16.6, 25.4] a
	147	Faba bean	1.93 [1.48, 2.39] a	59.7 [45.0, 74.5] a
		Maize	2.00 [1.55, 2.46] a	65.5 [50.8, 80.2] a
		Wheat	1.63 [1.17, 2.09] a	52.0 [37.3, 66.8] a
		Pea	1.78 [1.33, 2.24] a	55.8 [41.1, 70.5] a
	168	Faba bean	5.62 [4.06, 7.17] b	146 [107, 185] b
		Maize	7.91 [6.35, 9.46] a	229 [190, 268] a
		Wheat	6.10 [4.55, 7.65] b	158 [119, 197] b
		Pea	6.14 [4.59, 7.70] b	150 [111, 190] b
	182	Faba bean	9.29 [7.25, 11.3] b	195 [148, 241] c
		Maize	12.2 [10.1, 14.2] a	291 [244, 337] a
		Wheat	10.5 [8.50, 12.6] ab	234 [187, 280] bc
		Pea	11.2 [9.12, 13.2] ab	257 [210, 303] ab
	196	Faba bean	12.6 [10.1, 15.1] a	273 [213, 333] b
		Maize	16.0 [13.5, 18.5] a	366 [307, 426] a
		Wheat	15.2 [12.7, 17.7] a	299 [239, 359] ab
		Pea	16.2 [13.7, 18.7] a	375 [315, 434] a
	226	Faba bean	13.8 [11.6, 16.0] a	359 [287, 432] b
		Maize	17.0 [14.8, 19.2] a	479 [407, 552] a
		Wheat	15.9 [13.7, 18.2] a	426 [353, 498] ab
		Pea	14.9 [12.7, 17.1] a	384 [311, 457] ab

(iv) Pea. DOY 210 was the final harvest.

Species	DOY	Companion	Biomass (Mg ha^{-1})	N uptake (kg ha^{-1})
Pea	133	Pea	0.703 [0.593, 0.812] a	32.2 [25.6, 38.9] a
		Maize	0.695 [0.586, 0.805] a	32.2 [25.6, 38.8] a
		Wheat	0.659 [0.550, 0.768] a	30.1 [23.5, 36.8] a
		Faba bean	0.666 [0.557, 0.775] a	30.8 [24.2, 37.5] a
	147	Pea	2.41 [2.02, 2.80] a	84.1 [70.4, 97.9] a
		Maize	2.54 [2.15, 2.93] a	89.0 [75.3, 103] a
		Wheat	2.50 [2.11, 2.89] a	87.1 [73.4, 101] a
		Faba bean	2.39 [2.00, 2.78] a	83.7 [69.9, 97.4] a
	168	Pea	6.18 [5.37, 6.98] bc	152 [134, 169] b
		Maize	7.42 [6.61, 8.22] a	197 [179, 214] a
		Wheat	6.49 [5.68, 7.29] b	162 [145, 180] b
		Faba bean	5.83 [5.03, 6.64] c	156 [139, 174] b
	182	Pea	10.3 [9.35, 11.2] b	225 [204, 247] b
		Maize	12.2 [11.3, 13.1] a	264 [243, 285] a

	Wheat	9.72 [8.79, 10.7] b	213 [192, 235] b
	Faba bean	7.44 [6.31, 8.56] c	163 [137, 189] c
196	Pea	12.3 [9.25, 14.5] a	280 [216, 331] a
	Maize	13.2 [10.8, 15.6] a	296 [244, 348] a
	Wheat	13.9 [11.5, 16.3] a	291 [239, 343] a
	Faba bean	7.86 [5.46, 10.3] b	197 [145, 249] b
210	Pea	11.8 [9.93, 13.7] a	265 [227, 302] a
	Maize	10.8 [8.91, 12.7] a	245 [207, 282] a
	Wheat	10.2 [8.33, 12.1] ab	232 [195, 270] a
	Faba bean	7.48 [5.60, 9.36] b	168 [131, 206] b

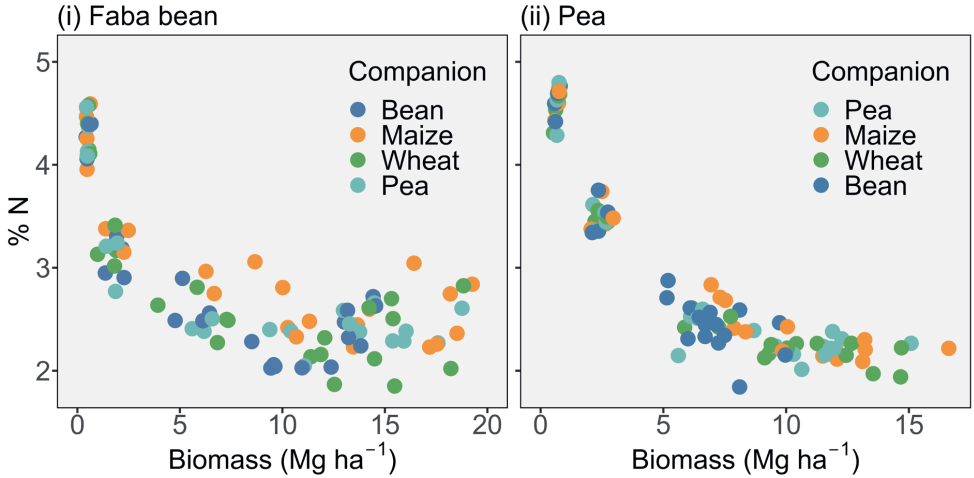


Fig. S2.1 Above-ground N concentration (%N) per species strip versus above-ground biomass per species strip for faba bean (i) and pea (ii) in 2019. Colors within each panel represent %N and biomass of the focal species when it was with different companion species: maize (orange), wheat (green), faba bean (blue), and pea (cyan). Points are the measurements from different replicates.

Shoot plasticity and light signals in relay strip intercropping

Supplementary Information for Chapter 3:

Methods S3.1 The process of model selection for the model with three parameters

Methods S3.2 Calculation of weighting factors of fraction of transmitted PAR (f_{trans}) of border rows in intercropping

Table S3.1 The number of germinated wheat plant per meter

Table S3.2 The AIC values of models used to describe the profile of internode or sheath length along the wheat main stem

Table S3.3 The results of model selection of GAM

Table S3.4 The AIC values of models used to describe the profile of sheath or internode length along the maize stem

Table S3.5 Fitted parameter values and their standard errors

Table S3.6 Comparisons of red to far-red ratio (R: FR) among treatments at each time in each species

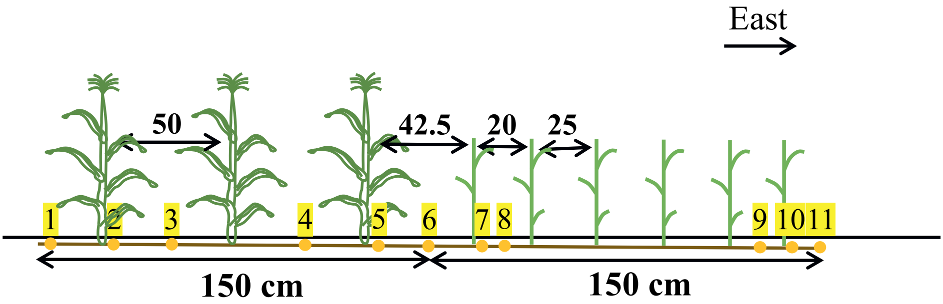
Table S3.7 Comparisons of fraction of transmitted PAR (f_{trans}) among treatments at each time in each species

Methods S3.1 Model versions for the model with three parameters

In wheat and faba bean (Eq. 3.3 and Eq. 3.4), there were two types of model version (as mentioned in the main text): (i) separate parameterization for the data of sole crops and intercrops; (ii) the model was fitted to the combined data of all treatments, in which one common value for each of the three parameters was estimated. For type i, there were three cases: (a) all the three parameters were different between sole crops and intercrops; (b) two of the three parameters were different between groups, while the rest one was the common value. This case had three different model versions according to the combination of the three parameters; (c) one of the three parameters was different between sole crops and intercrops, while the rest two were the common value. This case also had three different model versions. In total, there were eight model versions (see Tables S3.2 and S3.3).

In maize (Eq. 3.5 and Eq. 3.6), there were five types of model version (as mentioned in the main text): separate parameterization for the data of (i) sole maize, wheat-maize, and faba bean-maize; (ii) wheat-maize, and combined data of sole maize and faba bean-maize; (iii) faba bean-maize, and combined data of sole maize and wheat-maize; (iv) sole maize, and combined data of wheat-maize and faba bean-maize; and (v) the model was fitted to the combined data of all treatments. For each of type i, ii, iii, and iv, there were three cases: (a) all the three parameters were different among groups; (b) two of the three parameters were different among groups, while the rest one was the common value. Each type had three different model versions according to the combination of the three parameters; (c) one of the three parameters was different, while the rest two were common. Each type also had three different model versions. In total, there were 29 model versions (See Table S3.4).

Methods S3.2 Calculation of weighting factors of fraction of transmitted PAR (f_{trans}) of border rows in intercropping



Border row maize in intercrops

The space occupied by the maize in a border row is 50 cm. The PAR measurements of positions 1 to 3 are responsible for the western border row maize, and positions 4 to 6 are responsible for the eastern border row maize. The mean f_{trans} values of positions 1 and 2 represents the 25 cm between wheat and maize rows, same for positions 5 and 6. The mean f_{trans} values of positions 2 and 3 represents the 25 cm from the maize row to the middle of two maize rows, same for positions 4 and 5. So, for the western border row maize, the weighting factors for positions 1, 2, and 3 are 0.25, 0.5, and 0.25 separately. For the eastern border row maize, the weighting factors for positions 4, 5, and 6 are 0.25, 0.5, and 0.25 separately.

Border row wheat or faba bean in intercrops

The space occupied by the border row wheat or faba bean is 17.5 + 10 = 27.5 cm. The PAR measurements of positions 6 to 8 are responsible for the western border row plants, and positions 9 to 11 are for the eastern border row plants. The mean f_{trans} values of positions 6 and 7 represents the 17.5 cm between maize and wheat or faba bean rows, same for positions 10 and 11. The mean f_{trans} values of positions 7 and 8 represents the 10 cm from the wheat or faba bean row to the middle of two wheat or faba bean rows, same for positions 9 and 10. So, for the western border row plants, the weighting factors for positions 6, 7, and 8 are 0.32, 0.5, and 0.18 separately. For the eastern border row plants, the weighting factors for positions 9, 10, and 11 are 0.18, 0.5, and 0.32 separately.

Table S3.1 The number of germinated wheat plant per meter. Germinated wheat plants were counted 23 days after wheat sowing. In each plot, measurements were made in three species strips. In each strip, plants within a meter length of two random rows were counted. The average plant number per meter obtained from each plot (or block, see below) was used to calculate wheat tiller number per plant in that plot.

Block	Treatment	Plant number per meter
1	Wheat-maize	96
1	Sole wheat	86
2	Wheat-maize	97
2	Sole wheat	92
3	Sole wheat	91
3	Wheat-maize	93
4	Sole wheat	96
4	Wheat-maize	97
5	Wheat-maize	87
5	Sole wheat	92
6	Wheat-maize	90
6	Sole wheat	87

Table S3.2 The AIC values of models used to describe the profile of internode or sheath length along the wheat main stem. The AIC value of each model version was calculated based on negative log-likelihood and a penalty term for the number of parameters: $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The **bold** AIC value represents the selected model. ΔAIC is the difference compared to the selected model.

The profile of internode length along the main stem: $L_{\mathrm{I,w}} = a \times e^{(-b\times N'_{\mathrm{w}})}$

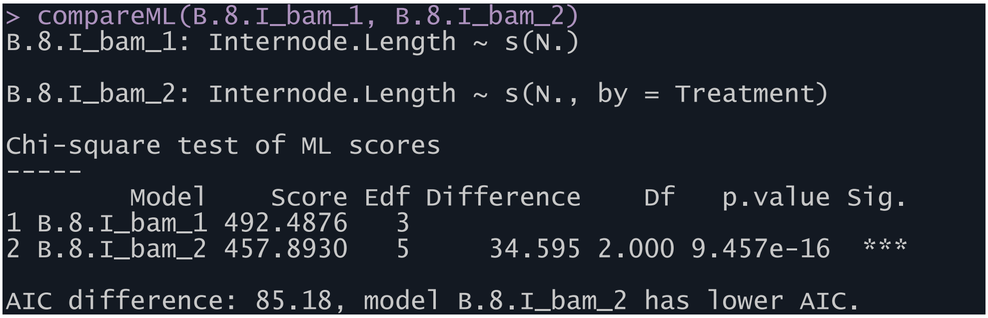
Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Two groups: sole crops and intercrops	a, b	5	220	230	3
M2		b	4	220	228	1
M3		a	4	218	226	-1
M4	One group: all data combined	None	3	220	**226**	0

The profile of sheath length along the main stem: $L_{\mathrm{S,w}} = M_{\mathrm{S,w}} - \frac{M_{\mathrm{S,w}}}{1+e^{-c\times(N'_{\mathrm{w}}-d)}}$

Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Two groups: sole crops and intercrops	$M_{\mathrm{S,w}}$, c, d	7	138	152	2
M2		c, d	6	139	151	1
M3		$M_{\mathrm{S,w}}$, d	6	139	151	1
M4		$M_{\mathrm{S,w}}$, c	6	141	153	3
M5		d	5	139	149	-1
M6		c	5	141	151	1
M7		$M_{\mathrm{S,w}}$	5	142	152	1
M8	One group: all data combined	None	4	143	**151**	0

Table S3.3 The results of model selection of GAM used to describe the profile of internode length along the faba bean main stem (a), and the AIC values of models used to describe the profile of petiole length along the faba bean main stem (b).

(a) The function compareML was used to compare GAM fitted to different groups of data (see below): "B. 8. I_bam_1" represents the model was fitted to all data combined, and "B. 8. I_bam_2" represents the model was fitted to two groups of data- sole crops and intercrops. The result indicates that model "B. 8. I_bam_2" is preferred as it has lower AIC value. Thus, the profile of internode length along the main stem was different between sole bean and border row bean in bean-maize intercrops.



```
> compareML(B.8.I_bam_1, B.8.I_bam_2)
B.8.I_bam_1: Internode.Length ~ s(N.)

B.8.I_bam_2: Internode.Length ~ s(N., by = Treatment)

Chi-square test of ML scores
-----
        Model    Score Edf Difference    Df   p.value Sig.
1 B.8.I_bam_1 492.4876   3
2 B.8.I_bam_2 457.8930   5     34.595 2.000 9.457e-16  ***

AIC difference: 85.18, model B.8.I_bam_2 has lower AIC.
```

(b) The AIC value of each model version was calculated based on negative log-likelihood and a penalty term for the number of parameters: $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The **bold** AIC value represents the selected model. ΔAIC is the difference compared to the selected model.

The profile of petiole length along the main stem: $L_{\mathrm{P}} = \frac{M_{\mathrm{P}}}{1+e^{-f\times(N'_{\mathrm{b}}-g)}}$

Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Two groups: sole crops and intercrops	M_{P}, f, g	7	43	57	2
M2		f, g	6	43	55	0
M3		M_{P}, g	6	42	54	-1
M4		M_{P}, f	6	43	55	0
M5		g	5	43	53	-2
M6		f	5	43	53	-2
M7		M_{P}	5	43	53	-2
M8	One group: all data combined	None	4	43	**51**	-4

Table S3.4 The AIC values of models used to describe the profile of sheath or internode length along the maize stem. The AIC value of each model version was calculated based on negative log-likelihood and a penalty term for the number of parameters: $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The **bold** AIC value represents the selected model. ΔAIC is the difference compared to the selected model.

The profile of sheath length along the stem for $N' <= 6$: $L_{\mathrm{S,m}} = \frac{M_{\mathrm{S,m}}}{1+(\frac{N'_{\mathrm{m}}-j}{k})^2}$.

Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Three groups: sole maize, wheat-maize intercrops, and faba bean-maize intercrops	$M_{\mathrm{S,m}}$, k, j	10	356	376	8
M2		k, j	8	357	373	5
M3		$M_{\mathrm{S,m}}$, j	8	356	372	4
M4		$M_{\mathrm{S,m}}$, k	8	359	375	7
M5		j	6	358	370	2
M6		k	6	360	372	4
M7		$M_{\mathrm{S,m}}$	6	367	379	11
M8	Two groups: wheat-maize intercrops, and combined data of sole maize and faba bean-maize intercrops	$M_{\mathrm{S,m}}$, k, j	7	358	372	4
M9		k, j	6	358	370	2
M10		$M_{\mathrm{S,m}}$, j	6	358	370	2
M11		$M_{\mathrm{S,m}}$, k	6	360	372	4
M12		j	5	358	**368**	0
M13		k	5	360	370	2
M14		$M_{\mathrm{S,m}}$	5	368	378	10

M15	Two groups: faba bean-maize intercrops, and combined data of sole maize and wheat-maize intercrops	$M_{\mathrm{S,m}}$, k, j	7	367	381	13
M16		k, j	6	368	380	12
M17		$M_{\mathrm{S,m}}$, j	6	368	380	12
M18		$M_{\mathrm{S,m}}$, k	6	370	382	14
M19		j	5	369	379	11
M20		k	5	370	380	12
M21		$M_{\mathrm{S,m}}$	5	375	385	17
M22	Two groups: sole maize, and combined data of wheat-maize and faba bean-maize intercrops	$M_{\mathrm{S,m}}$, k, j	7	372	386	18
M23		k, j	6	373	385	17
M24		$M_{\mathrm{S,m}}$, j	6	372	384	16
M25		$M_{\mathrm{S,m}}$, k	6	372	384	16
M26		j	5	373	383	15
M27		k	5	373	383	15
M28		$M_{\mathrm{S,m}}$	5	372	382	14
M29	One group: all data combined	None	4	376	384	16

The profile of sheath length along the stem for $N' >= 7$: $L_{\mathrm{I,m}} = \frac{M_{\mathrm{I,m}}}{1+(\frac{N'_{\mathrm{m}}-h}{i})^2}$.

Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Three groups: sole maize, wheat-maize intercrops, and faba bean-maize intercrops	$M_{\mathrm{S,m}}$, k, j	10	304	324	3
M2		k, j	8	309	325	4
M3		$M_{\mathrm{S,m}}$, j	8	305	**321**	0
M4		$M_{\mathrm{S,m}}$, k	8	309	325	4
M5		j	6	323	335	14
M6		k	6	327	339	17
M7		$M_{\mathrm{S,m}}$	6	319	331	10
M8	Two groups: wheat-maize intercrops, and combined data of sole maize and faba bean-maize intercrops	$M_{\mathrm{S,m}}$, k, j	7	313	327	6
M9		k, j	6	318	330	9
M10		$M_{\mathrm{S,m}}$, j	6	316	328	7
M11		$M_{\mathrm{S,m}}$, k	6	324	336	15
M12		j	5	324	334	12
M13		k	5	327	337	16
M14		$M_{\mathrm{S,m}}$	5	330	340	19
M15	Two groups: faba bean-maize intercrops, and combined data of sole maize and wheat-maize intercrops	$M_{\mathrm{S,m}}$, k, j	7	320	334	13
M16		k, j	6	321	333	11
M17		$M_{\mathrm{S,m}}$, j	6	323	335	14
M18		$M_{\mathrm{S,m}}$, k	6	323	335	14
M19		j	5	324	334	13
M20		k	5	327	337	16
M21		$M_{\mathrm{S,m}}$	5	323	333	12
M22	Two groups: sole	$M_{\mathrm{S,m}}$, k, j	7	313	327	5

M23	maize, and combined data of wheat-maize and faba bean-maize intercrops	*k, j*	6	317	329	8
M24		$M_{\mathrm{S,m}}$, *j*	6	313	325	3
M25		$M_{\mathrm{S,m}}$, *k*	6	314	326	5
M26		*j*	5	330	340	19
M27		*k*	5	330	340	19
M28		$M_{\mathrm{S,m}}$	5	322	332	11
M29	One group: all data combined	None	4	330	338	17

The profile of internode length along the stem: $L_{\mathrm{I,m}} = \frac{M_{\mathrm{I,m}}}{1+(\frac{N'_{\mathrm{m}}-h}{i})^2}$

Model version	Groups of data used to fit model	Parameter differing among groups	Parameter number (k)	-2log(L)	AIC	ΔAIC
M1	Three groups: sole maize, wheat-maize intercrops, and faba bean-maize intercrops	$M_{\mathrm{I,m}}$, *i, h*	10	1079	1099	91
M2		*i, h*	8	1082	1098	90
M3		$M_{\mathrm{I,m}}$, *h*	8	992	**1008**	0
M4		$M_{\mathrm{I,m}}$, *i*	8	1013	1029	21
M5		*h*	6	1020	1032	24
M6		*i*	6	1022	1034	26
M7		$M_{\mathrm{I,m}}$	6	1014	1026	17
M8	Two groups: wheat-maize intercrops, and combined data of sole maize and faba bean-maize intercrops	$M_{\mathrm{I,m}}$, *i, h*	7	1060	1074	66
M9		*i, h*	6	1090	1102	94
M10		$M_{\mathrm{I,m}}$, *h*	6	1009	1021	12
M11		$M_{\mathrm{I,m}}$, *i*	6	1026	1038	30
M12		*h*	5	1020	1030	22
M13		*i*	5	1029	1039	31
M14		$M_{\mathrm{I,m}}$	5	1027	1037	29
M15	Two groups: faba bean-maize intercrops, and combined data of sole maize and wheat-maize intercrops	$M_{\mathrm{I,m}}$, *i, h*	7	1102	1116	108
M16		*i, h*	6	1108	1120	112
M17		$M_{\mathrm{I,m}}$, *h*	6	1035	1047	39
M18		$M_{\mathrm{I,m}}$, *i*	6	1041	1053	44
M19		*h*	5	1035	1045	37
M20		*i*	5	1041	1051	43
M21		$M_{\mathrm{I,m}}$	5	1041	1051	43
M22	Two groups: sole maize, and combined data of wheat-maize and faba bean-maize intercrops	$M_{\mathrm{I,m}}$, *i, h*	7	1068	1082	74
M23		*i, h*	6	1070	1082	73
M24		$M_{\mathrm{I,m}}$, *h*	6	1012	1024	15
M25		$M_{\mathrm{I,m}}$, *i*	6	1017	1029	20
M26		*h*	5	1036	1046	37
M27		*i*	5	1026	1036	28
M28		$M_{\mathrm{I,m}}$	5	1017	1027	19
M29	One group: all data combined	None	4	1041	1049	41

Table S3.5 Fitted parameter values and their standard errors (SE) in wheat (a), faba bean (b), and maize (c). Identical values of a parameter across treatments indicate that the parameter was not different among those treatments based on AIC.

(a) Wheat

Species	Plant traits	Parameter	Sole wheat		Border wheat in WM	
			Mean	SE	Mean	SE
Wheat	Internode length	a	49.8	2.2	49.8	2.2
		b	0.41	0.02	0.41	0.02
	Sheath length	$M_{S,w}$	17.3	0.8	17.3	0.8
		c	0.82	0.17	0.82	0.17
		d	5.01	0.14	5.01	0.14

(b) Faba bean

Species	Plant traits	Parameter	Sole bean		Border bean in BM	
			Mean	SE	Mean	SE
Faba bean	Petiole length	M_P	2.56	0.15	2.56	0.15
		f	0.30	0.07	0.30	0.07
		g	5.31	0.92	5.31	0.92

(c) Maize

Species	Plant traits	Parameter	Sole Maize		Border maize in BM		Border maize in WM	
			Mean	SE	Mean	SE	Mean	SE
Maize	Sheath length (N' <= 6)	$M_{S,m}$	16.5	0.3	16.5	0.3	16.5	0.3
		k	7.90	1.12	7.90	1.12	7.90	1.12
		j	4.70	0.47	4.70	0.47	6.51	0.60
	Sheath length (N' >= 7)	$M_{S,m}$	19.8	0.4	21.8	0.6	22.1	0.4
		k	7.75	0.76	7.75	0.76	7.75	0.76
		j	10.7	0.4	11.5	0.5	12.3	0.5
	Internode length	$M_{I,m}$	25.9	1.1	21.1	0.8	19.5	0.8
		i	3.97	0.20	3.97	0.20	3.97	0.20
		h	7.19	0.22	7.26	0.19	8.37	0.19

Table S3.6 Comparisons of red to far-red ratio (R: FR) among treatments at each time in each species. DAS: days after sowing. Mean values of R: FR and their standard errors in brackets are presented. Different letters denote significant differences (Fisher's LSD test, $P < 0.05$).

Species	DAS wheat	Treatment	R: FR
Wheat	35	WW	0.79 (0.02) b
	35	WM_ facing wheat	0.83 (0.03) b
	35	WM_ facing maize	0.94 (0.05) a
	47	WW	0.17 (0.02) b
	47	WM_ facing wheat	0.29 (0.07) ab

	47	WM_ facing maize	0.45 (0.13) a
	54	WW	0.13 (0.02) b
	54	WM_ facing wheat	0.24 (0.13) b
	54	WM_ facing maize	0.66 (0.07) a
	62	WW	0.11 (0.01) b
	62	WM_ facing wheat	0.08 (0.01) b
	62	WM_ facing maize	0.45 (0.06) a
	83	WW	0.18 (0.01) b
	83	WM_ facing wheat	0.16 (0.01) b
	83	WM_ facing maize	0.37 (0.05) a
Species	DAS bean	Treatment	R: FR
faba bean	35	BB	0.96 (0.01) a
	35	BM_ facing bean	0.97 (0.02) a
	35	BM_ facing maize	0.87 (0.16) a
	47	BB	0.65 (0.08) a
	47	BM_ facing bean	0.48 (0.11) a
	47	BM_ facing maize	0.77 (0.08) a
	54	BB	0.35 (0.06) b
	54	BM_ facing bean	0.35 (0.04) b
	54	BM_ facing maize	0.61 (0.11) a
	62	BB	0.21 (0.05) b
	62	BM_ facing bean	0.23 (0.04) b
	62	BM_ facing maize	0.60 (0.11) a
	83	BB	0.10 (0.01) b
	83	BM_ facing bean	0.10 (0.01) b
	83	BM_ facing maize	0.25 (0.05) a
	99	BB	0.16 (0.07) a
	99	BM_ facing bean	0.11 (0.03) a
	99	BM_ facing maize	0.08 (0.01) a
	112	BB	0.37 (0.02) a
	112	BM_ facing bean	0.23 (0.03) b
	112	BM_ facing maize	0.14 (0.01) c
Species	DAS wheat/bean	Treatment	R: FR
Maize	83	MM	0.49 (0.02) a
	83	WM_ facing maize	0.49 (0.03) a
	83	WM_ facing wheat	0.41 (0.01) b
	83	BM_ facing maize	0.39 (0.02) b
	83	BM_ facing bean	0.18 (0.01) c
	91	MM	0.37 (0.01) b
	91	WM_ facing maize	0.42 (0.02) a
	91	WM_ facing wheat	0.38 (0.03) ab
	91	BM_ facing maize	0.31 (0.02) c

91	BM_ facing bean	0.12 (0.01) d
99	MM	0.17 (0.02) a
99	WM_ facing maize	0.26 (0.04) a
99	WM_ facing wheat	0.27 (0.06) a
99	BM_ facing maize	0.19 (0.07) a
99	BM_ facing bean	0.19 (0.05) a
105	MM	0.18 (0.02) b
105	WM_ facing maize	0.30 (0.03) a
105	WM_ facing wheat	0.27 (0.03) a
105	BM_ facing maize	0.17 (0.03) b
105	BM_ facing bean	0.17 (0.03) b
112	MM	0.21 (0.01) c
112	WM_ facing maize	0.42 (0.07) a
112	WM_ facing wheat	0.40 (0.04) a
112	BM_ facing maize	0.29 (0.03) b
112	BM_ facing bean	0.23 (0.02) bc

Table S3.7 Comparisons of fraction of transmitted PAR (f_{trans}) among treatments at each time in each species. DAS: days after sowing. Mean values of f_{trans} and their standard errors in brackets are presented. Different letters denote significant differences (Fisher's LSD test, $P < 0.05$).

Species	DAS wheat	Treatment	f_{trans}
Wheat	28	WW	0.89 (0.03) a
	28	WM	0.93 (0.02) a
	33	WW	0.72 (0.03) b
	33	WM	0.80 (0.02) a
	43	WW	0.36 (0.03) a
	43	WM	0.49 (0.05) a
	48	WW	0.33 (0.08) b
	48	WM	0.48 (0.05) a
	55	WW	0.15 (0.05) a
	55	WM	0.36 (0.09) a
	65	WW	0.05 (0.02) b
	65	WM	0.21 (0.03) a
	78	WW	0.01 (0.00) a
	78	WM	0.13 (0.03) a
	91	WW	0.03 (0.01) a
	91	WM	0.07 (0.03) a
Species	DAS bean	Treatment	f_{trans}
Faba bean	28	BB	0.88 (0.04) a
	28	BM	0.97 (0.03) a
	33	BB	0.78 (0.03) a
	33	BM	0.84 (0.03) a
	43	BB	0.59 (0.07) a

	43	BM	0.65 (0.06) a
	48	BB	0.55 (0.06) a
	48	BM	0.65 (0.08) a
	55	BB	0.38 (0.03) a
	55	BM	0.53 (0.07) a
	65	BB	0.07 (0.02) a
	65	BM	0.20 (0.05) a
	78	BB	0.01 (0.00) a
	78	BM	0.13 (0.07) a
	91	BB	0.01 (0.00) a
	91	BM	0.03 (0.01) a
	105	BB	0.14 (0.02) a
	105	BM	0.05 (0.02) b
	120	BB	0.38 (0.03) a
	120	BM	0.12 (0.02) b
Species	DAS wheat/bean	Treatment	f_{trans}
Maize	55	MM	0.98 (0.01) a
	55	WM	0.91 (0.04) a
	55	BM	0.91 (0.05) a
	65	MM	0.92 (0.02) a
	65	WM	0.65 (0.08) b
	65	BM	0.57 (0.07) b
	78	MM	0.54 (0.05) a
	78	WM	0.36 (0.07) a
	78	BM	0.26 (0.09) a
	91	MM	0.18 (0.03) ab
	91	WM	0.20 (0.05) a
	91	BM	0.07 (0.02) b
	99	MM	0.12 (0.02) a
	99	WM	0.14 (0.01) a
	99	BM	0.06 (0.01) b
	105	MM	0.07 (0.02) b
	105	WM	0.17 (0.02) a
	105	BM	0.06 (0.01) b
	112	MM	0.06 (0.01) b
	112	WM	0.10 (0.01) a
	112	BM	0.06 (0.01) b

Competition for light and nitrogen with an earlier-sown species negatively affects leaf traits and leaf photosynthetic capacity of maize in relay intercropping

Supplementary Information for Chapter 4:

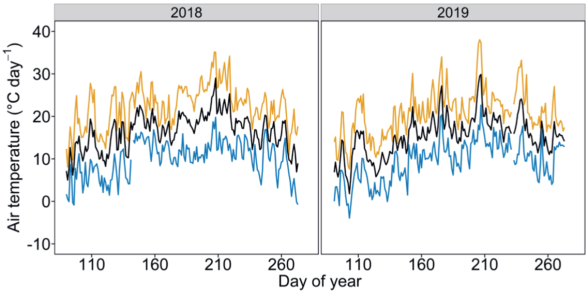


Fig. S4.1 Daily mean (black curve), maximum (yellow curve), and minimum (blue curve) air temperature (°C) during the growing seasons of 2018 and 2019. Data was recorded at weather station De Veenkampen at 2.5 km distance from the experimental site.

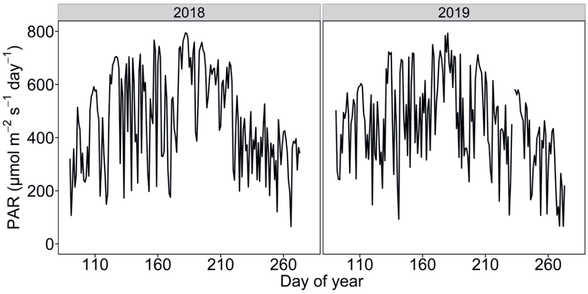


Fig. S4.2 Daily photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) during the growing seasons of 2018 and 2019. Data of daily global radiation (W m^{-2}) was recorded at weather station De Veenkampen at 2.5 km distance from the experimental site. Daily PAR was calculated as: daily global radiation × 4.6 × 50%.

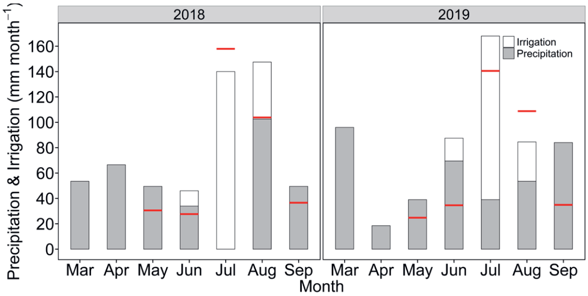


Fig. S4.3 Monthly precipitation (mm), irrigation (mm), and estimated maize evapotranspiration (mm) during the growing seasons of 2018 and 2019. Monthly precipitation and irrigation were recorded at the experimental site. Red lines indicate monthly maize evapotranspiration. Monthly maize evapotranspiration was calculated by averaging daily maize evapotranspiration ET_c (mm day^{-1}) throughout the month. ET_c was calculated as: $ET_c = ET_0 \times K_c$. ET_0 (mm day^{-1}) is daily reference crop evapotranspiration, which was calculated using the FAO Penman-Monteith equation (Allen et al., 1998) with meteorological data from weather station De Veenkampen (2.5 km west of the experimental site). K_c is crop coefficient of maize, which is 0.3 during the maize early-season (May and June), 1.2 during the maize mid-season (July and August), and 0.6 during the maize late-season (September) (Allen et al., 1998).

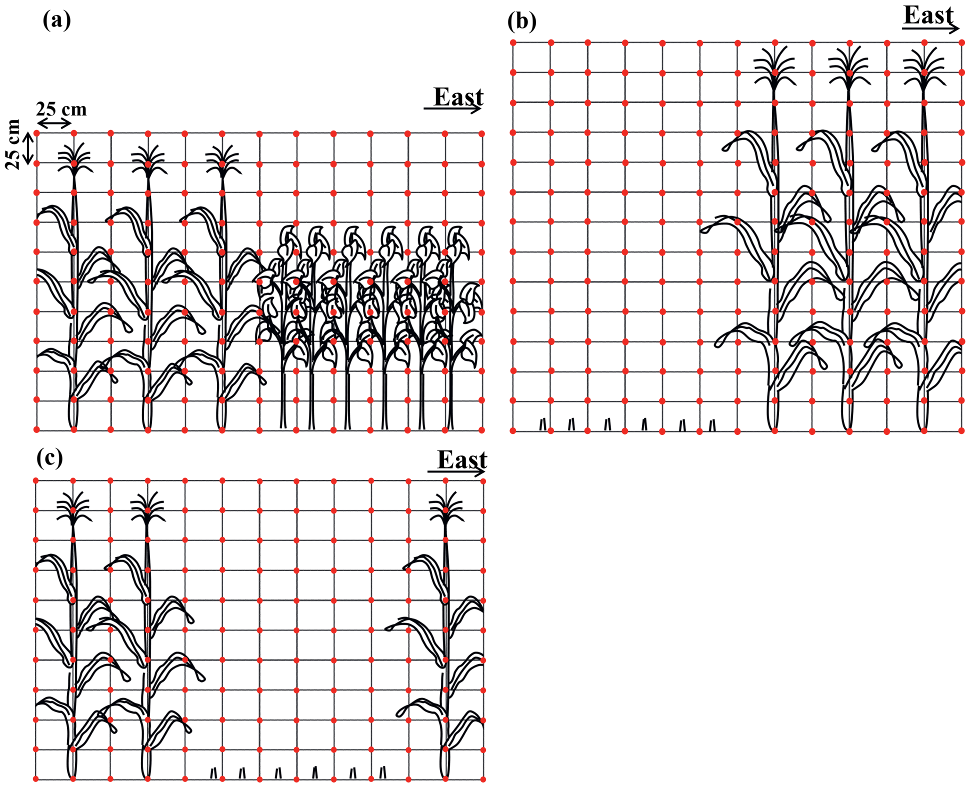


Fig. S4.4 Schematic illustration of the light distribution measurements in (a) maize-faba bean intercrop at maize silking (R1), (b) grain-filling (R4) in 2019, and (c) grain-filling (R4) stage in 2018.

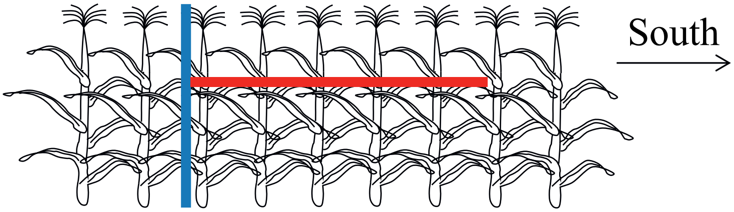


Fig. S4.5 Schematic illustration (side view of the rows) of the light distribution measurements in the maize canopy at maize V6 stage in 2019. The row orientation was approximately north-south. The red bar represents the SunScan probe, which was parallel to the row. The blue bar represents a stick supporting the probe. The probe was fixed at a 90° angle to the stick and can be moved vertically along the stick.

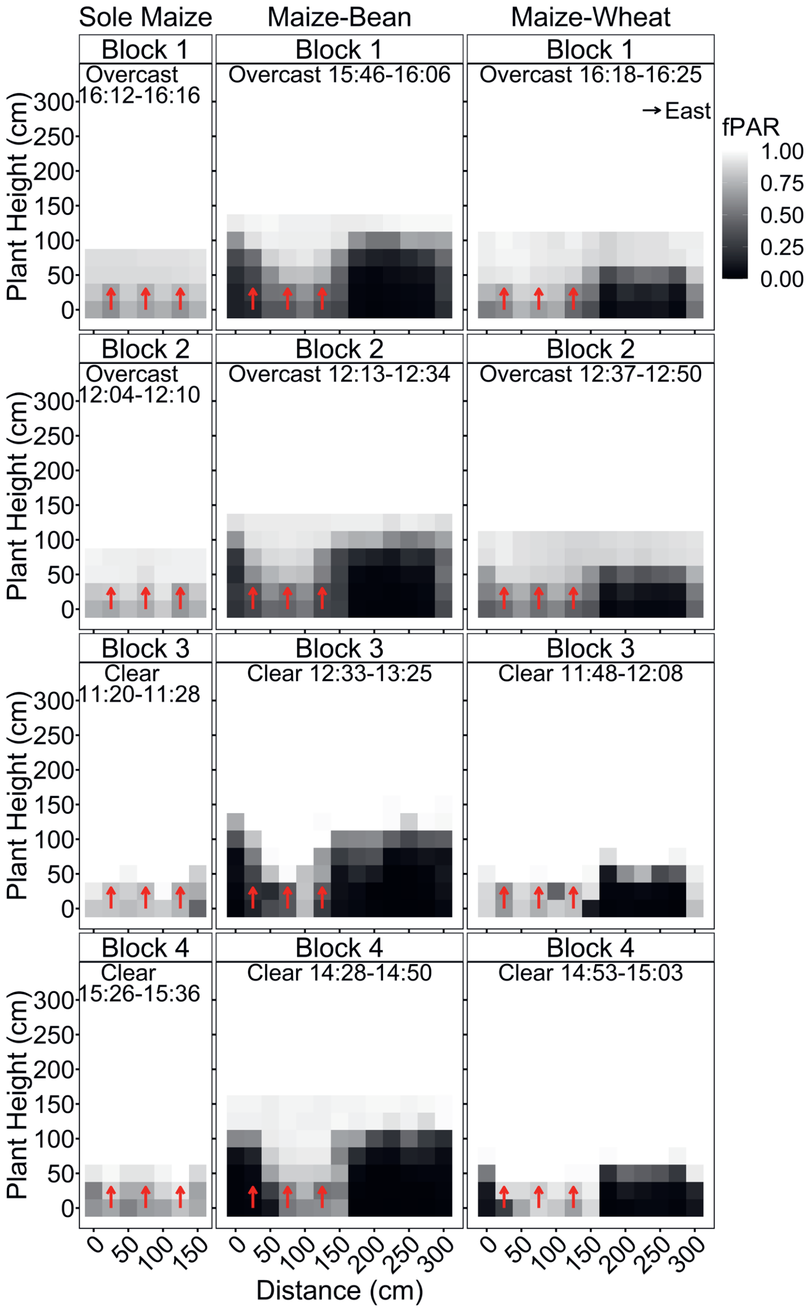


Fig. S4.6 Light distribution in each block at maize six-leaf stage (V6) in 2019. In each plot, measurements were conducted with either clear sky (Clear) or steady overcast sky (Overcast). The time of the day when the measurements were taken is presented in each panel. Red arrows indicate the position of maize rows.

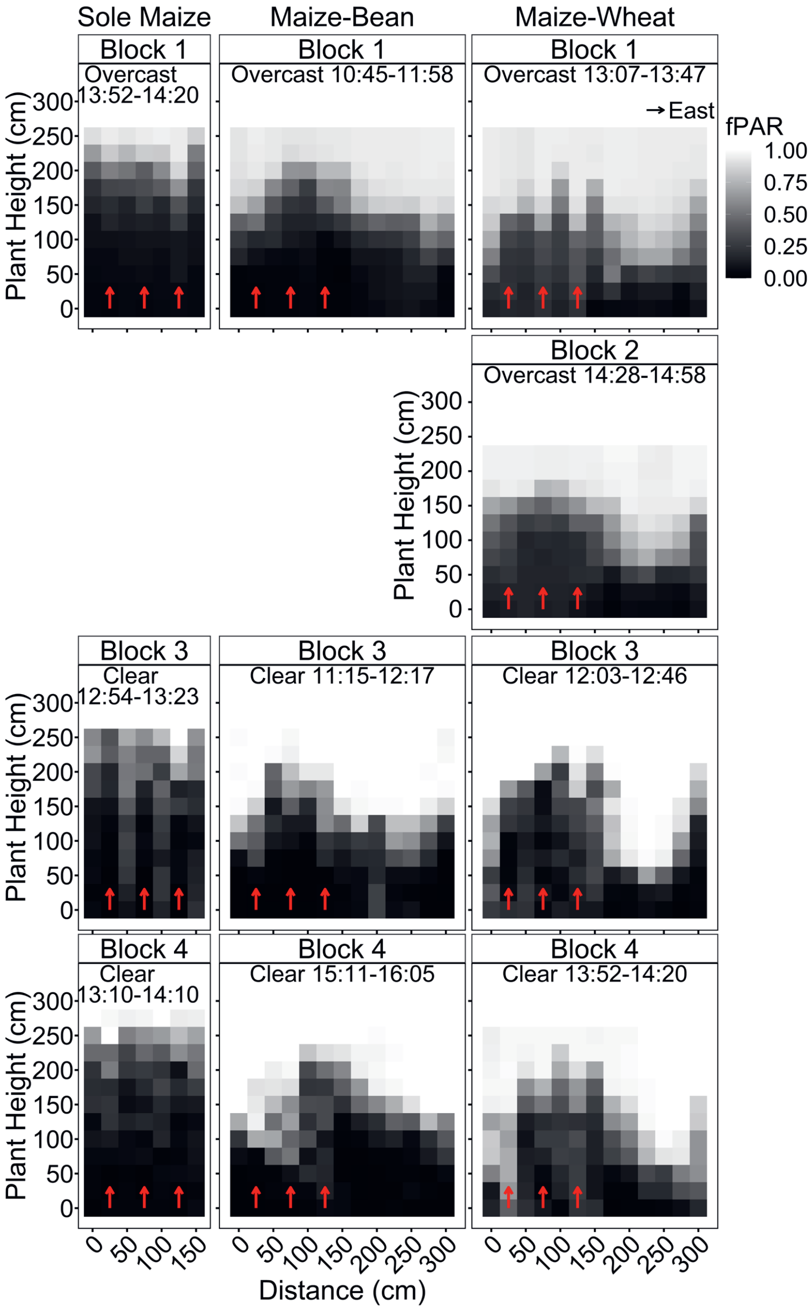


Fig. S4.7 Light distribution in each block at maize R1 stage in 2019. In each plot, measurements were conducted with either clear sky (Clear) or steady overcast sky (Overcast). The time of the day when the measurements were taken is presented in each panel. Red arrows indicate the position of maize rows.

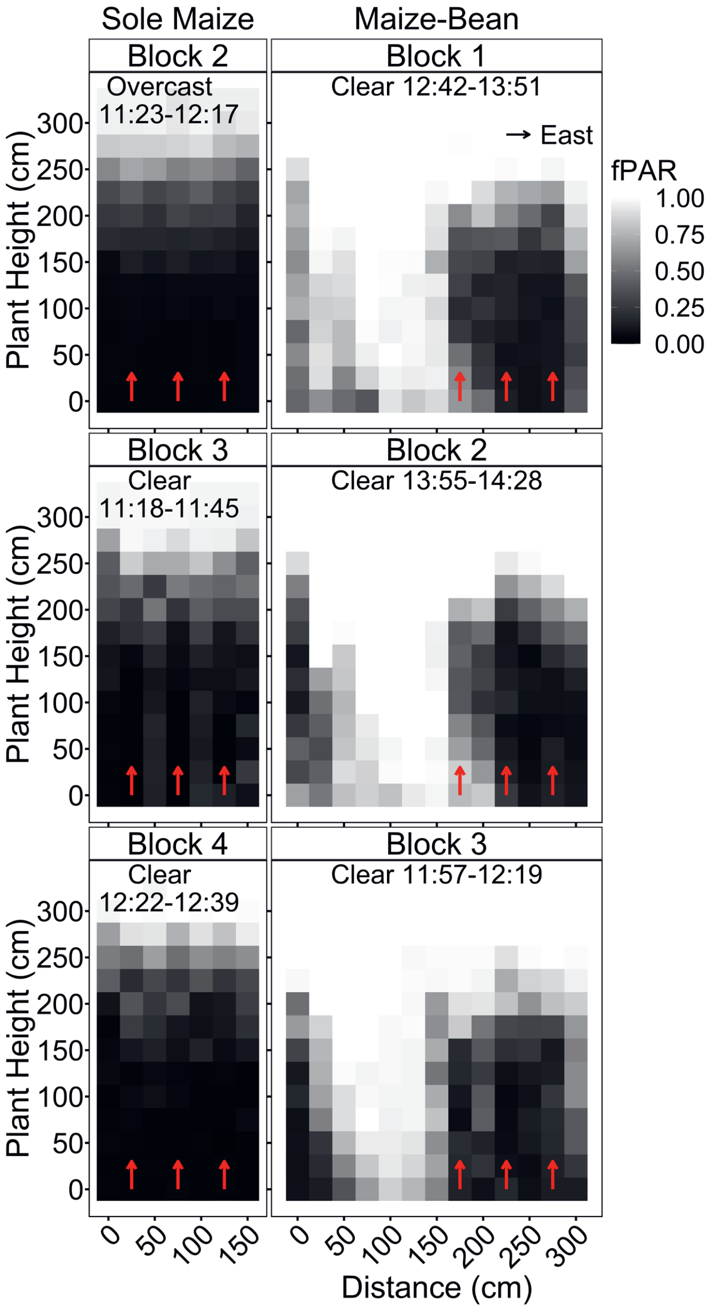


Fig. S4.8 Light distribution in each block at maize R4 stage in 2019. In each plot, measurements were conducted with either clear sky (Clear) or steady overcast sky (Overcast). The time of the day when the measurements were taken is presented in each panel. Red arrows indicate the position of maize rows.

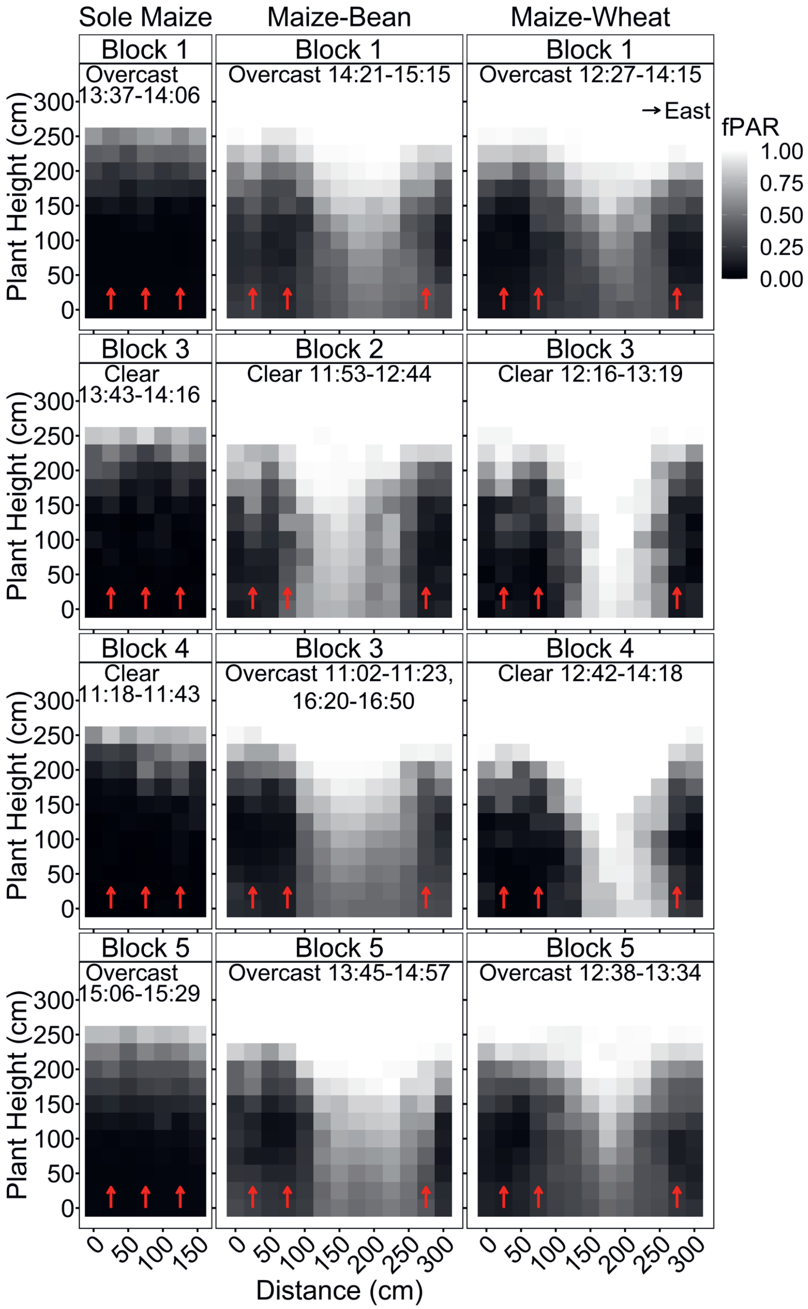


Fig. S4.9 Light distribution in each block at maize R4 stage in 2018. In each plot, measurements were conducted with either clear sky (Clear) or steady overcast sky (Overcast). The time of the day when the measurements were taken is presented in each panel. Red arrows indicate the position of maize rows.

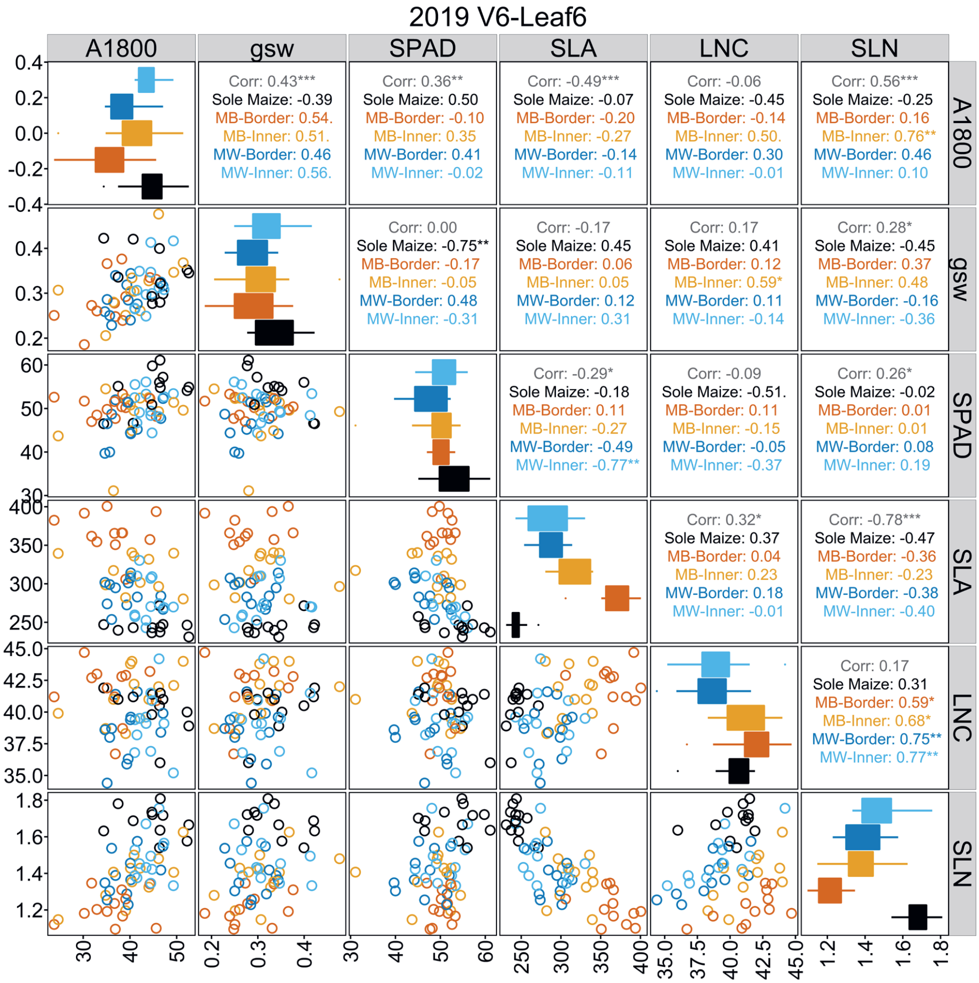


Fig. S4.10 Scatter plot matrices of leaf traits for leaf 6 at maize V6 stage (V6-Leaf 6) in year 2019. A_{1800} (µmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; SLA (cm^2 leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. The matrix was generated using the ggpairs function from the "GGally" package (Barret et al., 2021) in R (R Core Team, 2023). In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

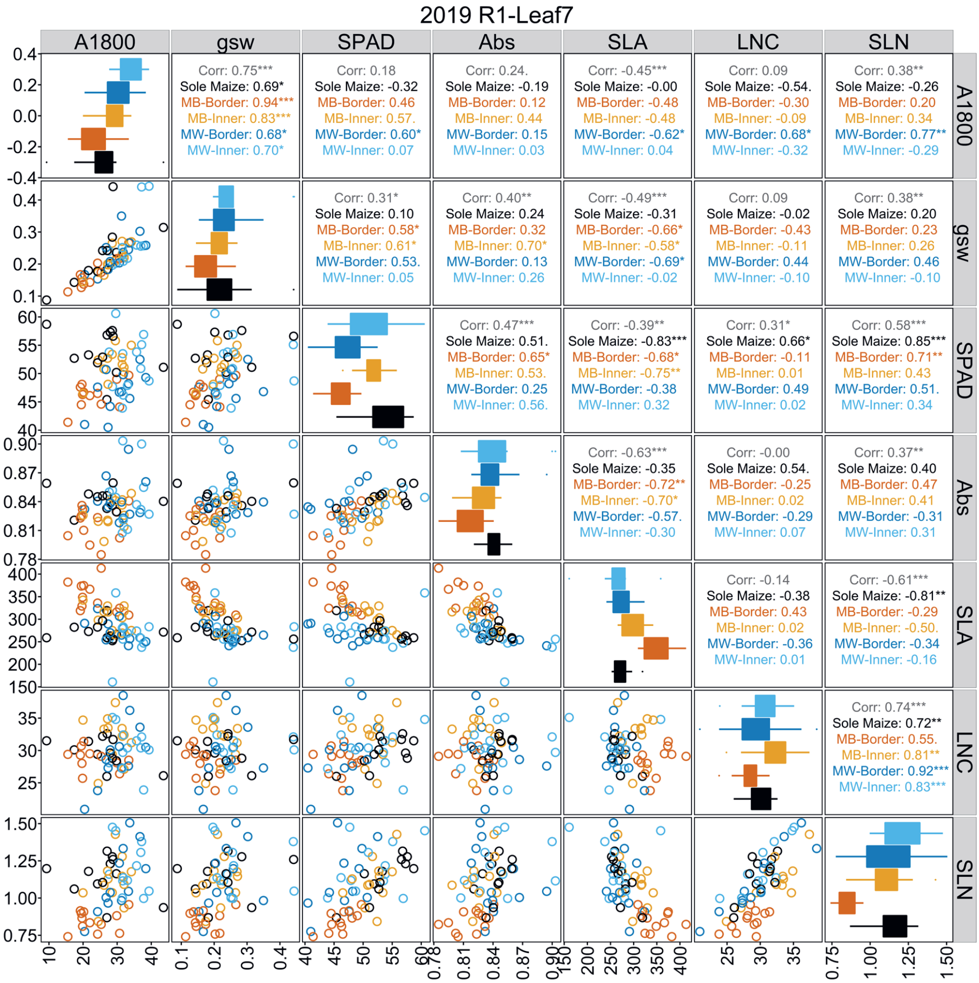


Fig. S4.11 Scatter plot matrices of leaf traits for leaf 7 at maize R1 stage (R1-Leaf 7) in year 2019. A_{1800} (µmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^2 leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

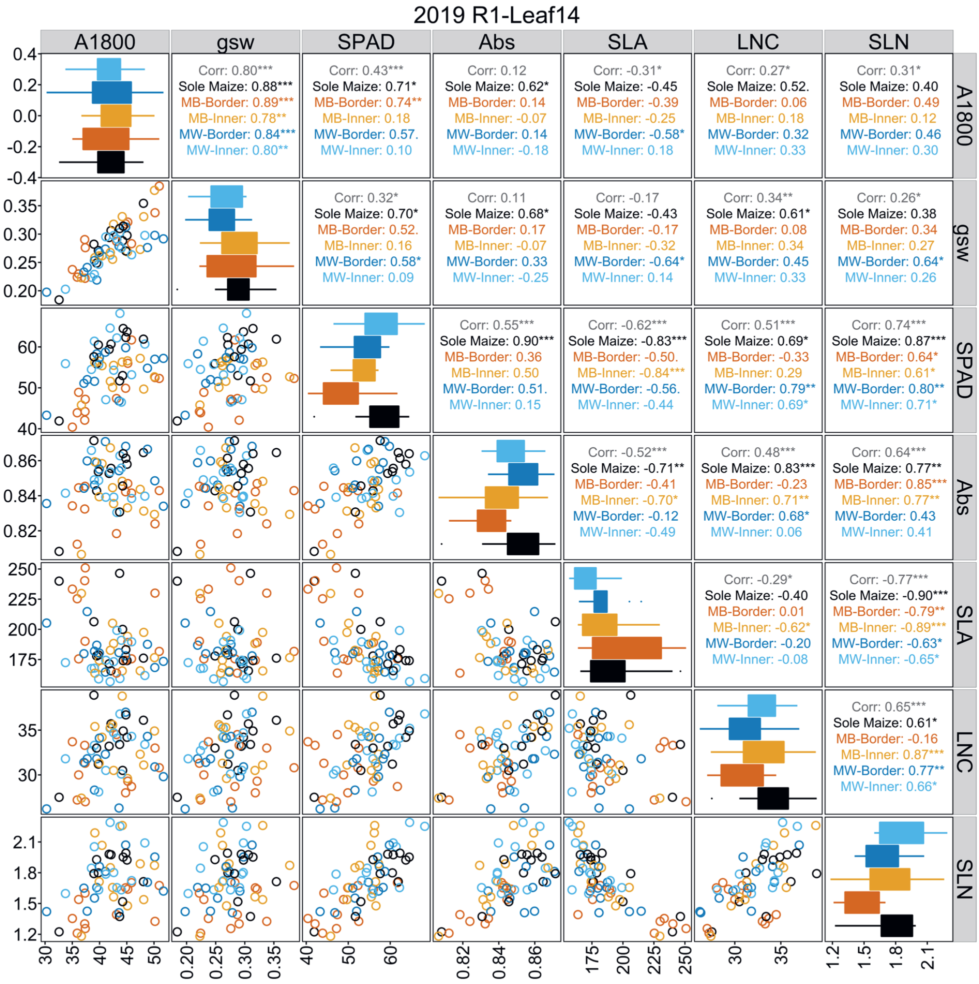


Fig. S4.12 Scatter plot matrices of leaf traits for leaf 14 at maize R1 stage (R1-Leaf 14) in year 2019. A_{1800} (μmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^{2} leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

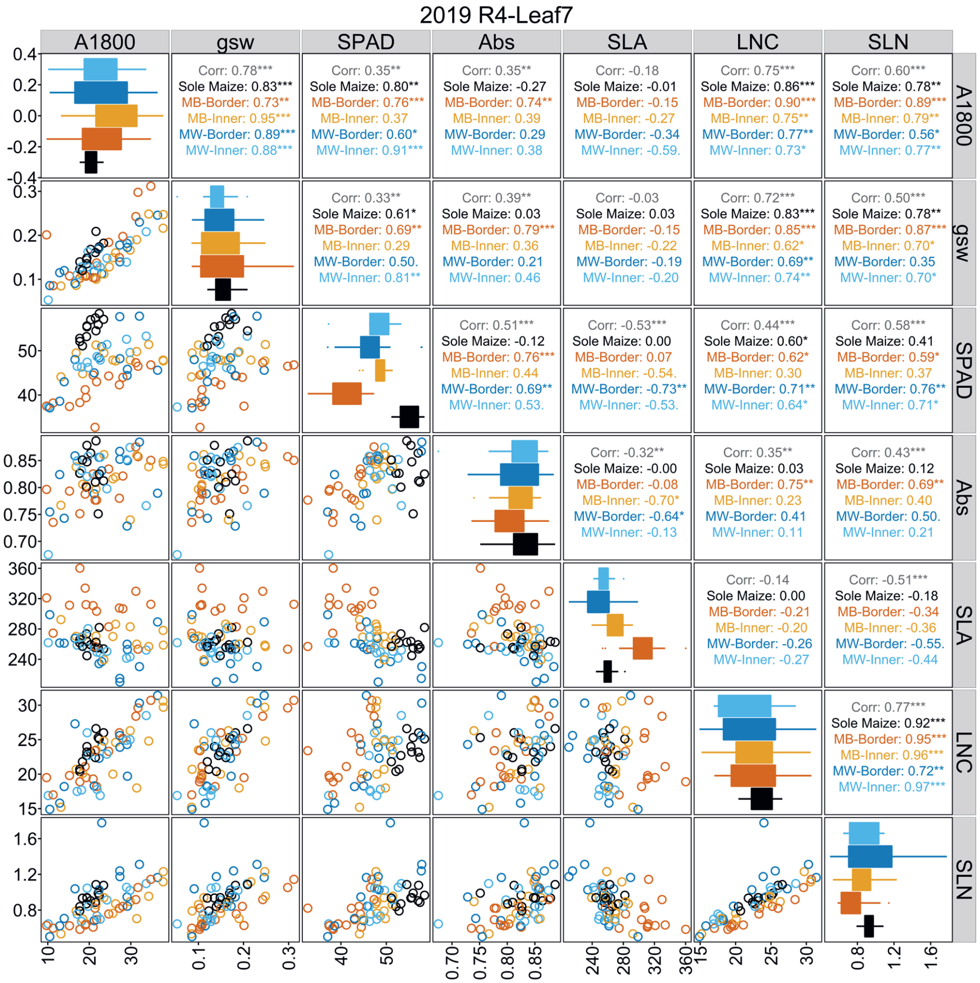


Fig. S4.13 Scatter plot matrices of leaf traits for leaf 7 at maize R4 stage (R4-Leaf 7) in year 2019. A_{1800} (µmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^{2} leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

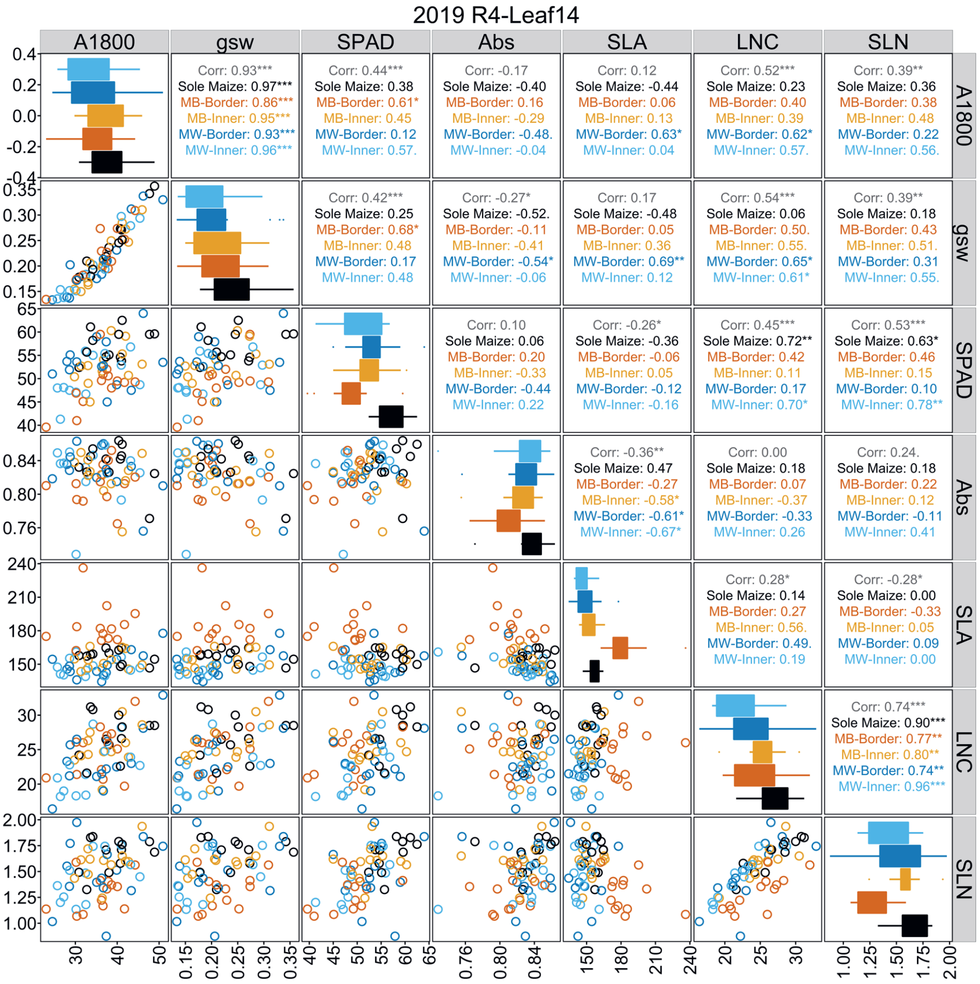


Fig. S4.14 Scatter plot matrices of leaf traits for leaf 14 at maize R4 stage (R4-Leaf 14) in year 2019. A_{1800} (µmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^2 leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

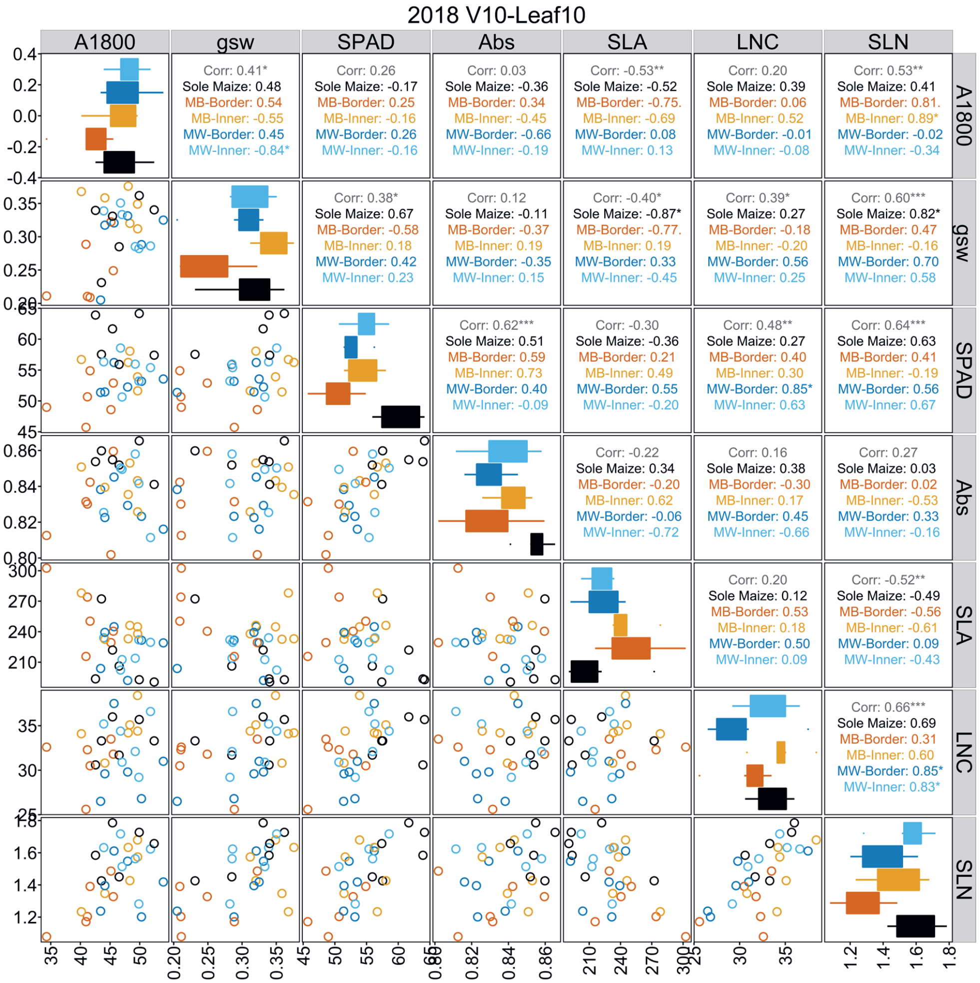


Fig. S4.15 Scatter plot matrices of leaf traits for leaf 10 at maize ten-leaf stage (V10-Leaf 10) in year 2018. A_{1800} (μmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^2 leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

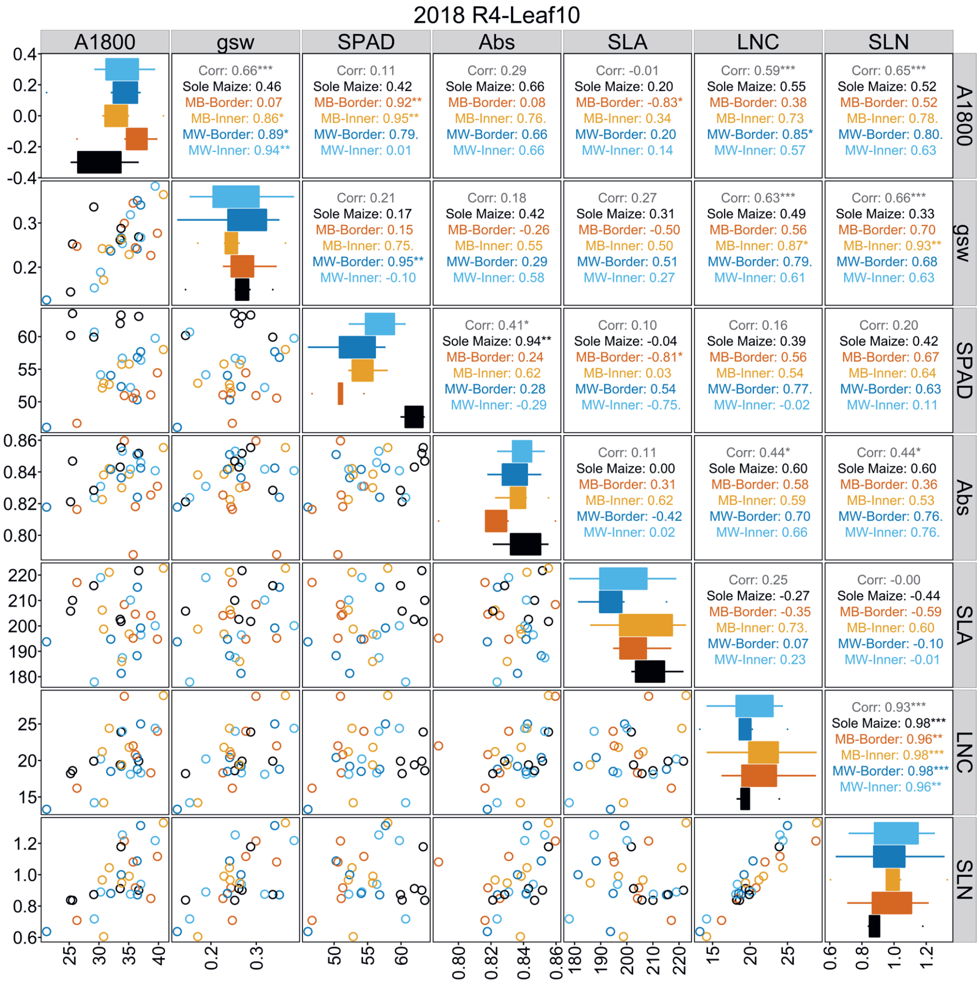


Fig. S4.16 Scatter plot matrices of leaf traits for leaf 10 at maize R4 stage (R4-Leaf 10) in year 2018. A_{1800} (μmol m^{-2} s^{-1}): leaf photosynthetic capacity; g_{sw} (mol m^{-2} s^{-1}): stomatal conductance for water; SPAD: a proxy for chlorophyll content; *Abs*: light absorptance; SLA (cm^2 leaf g^{-1} leaf): specific leaf area; LNC (mg N g^{-1} leaf): leaf nitrogen concentration; SLN (g N m^{-2} leaf): specific leaf nitrogen. In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf traits. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

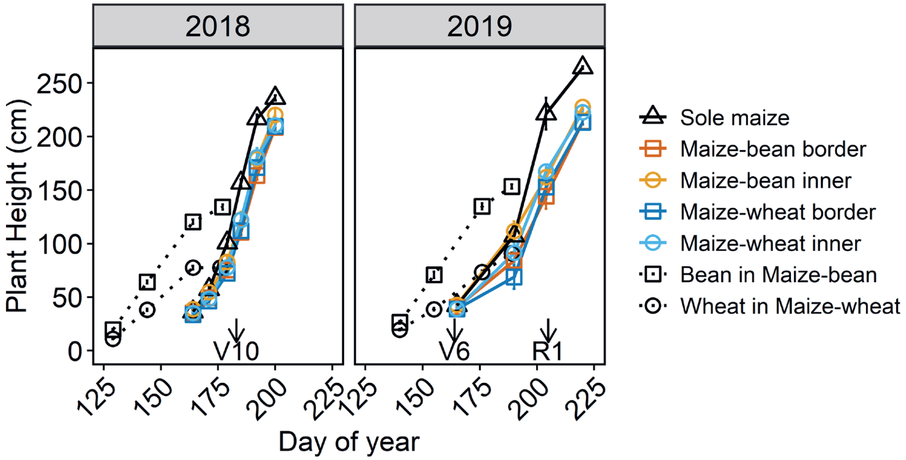


Fig. S4.17 Plant height of maize, wheat, and faba bean in various (inter)cropping systems. Plant height was defined as the length of the main stem, from the ground to the base of the last fully developed leaf. Plant height of maize was measured in sole maize and in border and inner rows of maize strips in maize-faba bean and maize-wheat intercropping. Plant heights of faba bean and wheat were measured in each row of species strips in the intercrop with maize. In 2018, measurements on plant height of maize were made in six blocks. For wheat and faba bean, plant height was measured in six blocks during the first three times and in three blocks in the fourth time. In 2019, measurements were made in four blocks for the three species. Error bars represent standard errors of means. Black arrows indicate when light distribution and (or) maize leaf traits were measured during the co-growth period (maize V10 stage in 2018, maize V6 and R1 stages in 2019).

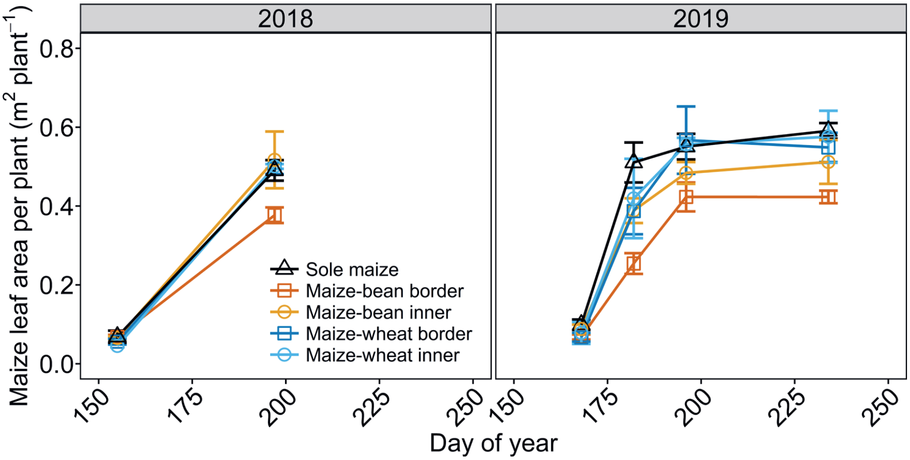


Fig. S4.18 Maize leaf area per plant in different treatments in 2018 and 2019. Total green leaf area of maize was determined two times in 2018 (on Julian calendar day (DOY) 155 and 197), and four times in 2019 (on DOY 168, 182, 196, and 234). In each plot, plants from each row in one strip and at least 1 m away from the edge of the plot were harvested over a 1.5 m row segment. Total green leaf area was determined using a leaf area meter (LI-3100 area meter, USA). Error bars represent standard errors of means.

Table S4.1 Details on application of fertilizer and pesticides

Year	Date	Fertilizer / pesticides	Quantity
Fertilizer application (kg ha^{-1}) (nutrient content)			
2018	28/Feb	K_2SO_4+$MgSO_4$, $Ca(H_2PO_4)_2 \cdot H_2O$	350 (105 kg K_2O ha^{-1}), 150 (67.5 kg P_2O_5 ha^{-1})
	11/Apr	$NH_4NO_3 \cdot CaMg(NO_3)_2$	296 (80 kg N ha^{-1} for wheat), 74 (20 kg N ha-1 for faba bean)
	4/May	$NH_4NO_3 \cdot CaMg(NO_3)_3$	167 (45 kg N ha^{-1} for wheat), 296 (80 kg N ha^{-1} for maize)
	11/Jun	$NH_4NO_3 \cdot CaMg(NO_3)_4$	333 (90 kg N ha^{-1} for maize)
2019	25/Feb	K_2SO_4+$MgSO_4$, $Ca(H_2PO_4)_2 \cdot H_2O$	350 (105 kg K_2O ha^{-1}), 175 (78.75 kg P_2O_5 ha^{-1})
	15/Apr	$NH_4NO_3 \cdot CaMg(NO_3)_2$	296 (80 kg N ha^{-1} for wheat), 74 (20 kg N ha-1 for faba bean)
	6/May	$NH_4NO_3 \cdot CaMg(NO_3)_3$	167 (45 kg N ha^{-1} for wheat), 296 (80 kg N ha^{-1} for maize)
	14/Jun	$NH_4NO_3 \cdot CaMg(NO_3)_4$	333 (90 kg N ha^{-1} for maize)
Chemical pesticides, herbicides, and fungicides (g ha^{-1})			
2018	23/Mar	Pendimethalin	800
	13/Apr	Bentazon	480
	25/Apr	Bentazon	720
	7/May	Bentazon	720
	31/May	Tebuconazole, Prothioconazole, Pirimicarb	125, 125, 250
2019	5/Apr	Pendimethalin	800
	26/Apr	Bentazon	600
	3/May	Bentazon	840
	13/May	Bentazon	720
	16/May	Pirimicarb	250
	3/Jun	Tebuconazole, Prothioconazole	125, 125
	11/Jun	Deltamethrin	6.25
	25/Jun	Tebuconazole, Prothioconazole Cyproconazole, Trifloxystrobin	98.8, 98.8, 26.4, 61.8

Table S4.2 Comparisons of maize leaf traits among treatments in each maize stage and leaf position in 2019. A_{1800}: leaf photosynthetic capacity; g_{sw}: stomatal conductance for water; SPAD: a proxy of chlorophyll content; *Abs*: light absorptance; SLA: specific leaf area; LNC: leaf nitrogen concentration; SLN: specific leaf nitrogen. Mean values of leaf traits and their standard errors in brackets are presented. Different letters denote significant differences (Fisher's LSD test, $P < 0.05$).

Stage-leaf position	Treatment	A_{1800} μmol m^{-2} s^{-1}	g_{sw} mol m^{-2} s^{-1}	SPAD	*Abs*[a]	SLA cm^2 leaf g^{-1} leaf	LNC mg N g^{-1} leaf	SLN g N m^{-2} leaf
V6-Leaf6	Sole Maize	43.5 (2.0) a	0.39 (0.05) a	53.5 (1.5) a	-	245 (3) d	40.4 (0.5) ab	1.68 (0.02) a
	Maize-bean border	36.1 (1.7) c	0.29 (0.02) b	50.3 (0.6) abc	-	366 (7) a	41.7 (0.6) a	1.21 (0.03) d
	Maize-bean inner	40.8 (2.0) ab	0.31 (0.02) ab	48.5 (1.8) bc	-	315 (7) b	41.4 (0.6) a	1.38 (0.04) c
	Maize-wheat border	39.4 (1.2) bc	0.29 (0.01) b	47.0 (1.3) c	-	288 (5) c	38.5 (0.6) c	1.39 (0.03) c
	Maize-wheat inner	44.1 (0.7) a	0.32 (0.02) ab	50.8 (1.0) ab	-	279 (8) c	39.2 (0.7) bc	1.48 (0.04) b
R1-Leaf7	Sole Maize	25.9 (2.3) bc	0.23 (0.03) a	53.7 (1.1) a	0.84 (0.00) ab	273 (6) c	29.8 (0.6) ab	1.14 (0.04) a
	Maize-bean border	23.3 (1.6) c	0.17 (0.01) b	46.2 (0.7) b	0.82 (0.01) c	349 (9) a	28.3 (0.6) b	0.85 (0.02) b
	Maize-bean inner	28.7 (1.1) b	0.21 (0.01) ab	51.7 (0.8) a	0.83 (0.00) bc	303 (8) b	32.0 (1.0) a	1.11 (0.04) a
	Maize-wheat border	30.2 (1.5) ab	0.24 (0.02) a	46.9 (1.1) b	0.84 (0.01) ab	274 (7) c	29.6 (1.4) b	1.12 (0.06) a
	Maize-wheat inner	33.4 (1.1) a	0.27 (0.02) a	51.6 (1.4) a	0.84 (0.01) a	264 (13) c	30.5 (0.9) ab	1.20 (0.05) a
R1-Leaf14	Sole Maize	42.1 (1.2) a	0.29 (0.01) a	57.8 (1.8) a	0.85 (0.00) ab	191 (8) ab	33.8 (0.9) a	1.76 (0.07) ab
	Maize-bean border	41.6 (1.7) a	0.28 (0.01) a	48.9 (1.8) c	0.83 (0.00) c	203 (9) a	30.6 (0.8) c	1.47 (0.05) c
	Maize-bean inner	43.2 (1.1) a	0.29 (0.01) a	53.6 (1.0) b	0.84 (0.00) bc	184 (6) bc	33.0 (0.9) ab	1.77 (0.09) ab
	Maize-wheat border	42.0 (1.8) a	0.26 (0.01) a	54.0 (1.5) ab	0.85 (0.00) a	183 (4) bc	31.2 (0.9) bc	1.68 (0.06) b
	Maize-wheat inner	41.8 (1.1) a	0.27 (0.01) a	57.0 (1.9) ab	0.85 (0.00) ab	173 (4) c	32.9 (0.7) abc	1.86 (0.07) a
R4-Leaf7	Sole Maize	20.3 (0.6) b	0.16 (0.01) a	55.0 (0.7) a	0.83 (0.01) a	261 (3) bc	23.5 (0.6) a	0.93 (0.03) a
	Maize-bean border	22.3 (1.9) ab	0.16 (0.02) a	41.6 (1.1) c	0.80 (0.01) a	305 (7) a	22.7 (1.1) a	0.76 (0.04) b
	Maize-bean inner	26.3 (2.2) a	0.15 (0.02) a	48.3 (0.6) b	0.82 (0.01) a	270 (4) b	23.2 (1.3) a	0.86 (0.05) ab
	Maize-wheat border	23.2 (2.2) ab	0.15 (0.01) a	47.0 (1.7) b	0.82 (0.01) a	252 (7) c	22.8 (1.4) a	1.00 (0.10) a
	Maize-wheat inner	22.3 (2.0) ab	0.14 (0.01) a	47.9 (1.3) b	0.82 (0.02) a	256 (3) bc	21.5 (1.3) a	0.86 (0.05) ab
R4-Leaf14	Sole Maize	38.9 (1.6) a	0.25 (0.02) a	57.2 (0.9) a	0.84 (0.01) a	157 (2) b	27.0 (0.9) a	1.67 (0.05) a
	Maize-bean border	35.7 (1.6) ab	0.22 (0.01) ab	48.6 (1.4) c	0.81 (0.01) b	184 (5) a	24.8 (1.0) ab	1.30 (0.05) c
	Maize-bean inner	37.0 (1.5) ab	0.22 (0.02) ab	52.8 (1.2) b	0.82 (0.01) ab	153 (2) bc	25.3 (0.8) ab	1.59 (0.05) ab
	Maize-wheat border	36.0 (2.0) ab	0.21 (0.02) ab	53.3 (1.3) b	0.83 (0.01) ab	149 (3) bc	23.5 (1.1) bc	1.49 (0.08) b
	Maize-wheat inner	33.7 (1.9) b	0.20 (0.02) b	50.3 (1.4) bc	0.83 (0.01) ab	147 (2) c	22.3 (1.1) c	1.45 (0.07) b

[a] Measurements of light absorptance (Abs) were conducted at maize R1 and R4 stage in 2019.

Table S4.3 Comparisons of maize leaf traits of leaf 10 among treatments in each maize stage in 2018. A_{1800}: leaf photosynthetic capacity; g_{sw}: stomatal conductance for water; SPAD: a proxy of chlorophyll content; *Abs*: light absorptance; SLA: specific leaf area; LNC: leaf nitrogen concentration; SLN: specific leaf nitrogen. Mean values of leaf traits and their standard errors in brackets are presented. Different letters denote significant differences (Fisher's LSD test, $P < 0.05$).

Stage-leaf position	Treatment	A_{1800}	g_{sw}	SPAD	*Abs*	SLA	LNC	SLN
		μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}			cm^{2} leaf g^{-1} leaf	mg N g^{-1} leaf	g N m^{-2} leaf
V10-Leaf10	Sole Maize	46.7 (1.6) a	0.31 (0.02) ab	60.1 (1.5) a	0.85 (0.00) a	213 (13) c	33.4 (0.9) ab	1.61 (0.06) a
	Maize-bean border	41.5 (1.7) b	0.25 (0.02) c	50.3 (1.3) c	0.83 (0.01) b	252 (13) a	31.1 (1.2) b	1.28 (0.06) c
	Maize-bean inner	46.7 (1.5) a	0.35 (0.01) a	54.7 (1.1) b	0.84 (0.00) ab	245 (7) ab	34.5 (1.0) a	1.48 (0.07) ab
	Maize-wheat border	47.6 (1.6) a	0.30 (0.02) b	53.0 (0.7) bc	0.83 (0.00) b	223 (9) bc	30.2 (1.6) b	1.40 (0.07) bc
	Maize-wheat inner	48.1 (1.1) a	0.31 (0.01) ab	55.0 (1.1) b	0.84 (0.01) ab	221 (5) c	33.1 (1.1) ab	1.56 (0.06) ab
R4-Leaf10	Sole Maize	30.6 (1.9) a	0.26 (0.03) a	62.0 (0.7) a	0.84 (0.01) a	210 (3) a	20.0 (0.8) a	0.92 (0.05) a
	Maize-bean border	35.2 (2.0) a	0.27 (0.02) a	50.8 (1) d	0.82 (0.01) b	204 (3) ab	21.7 (1.8) a	0.98 (0.08) a
	Maize-bean inner	33.8 (1.6) a	0.25 (0.03) a	54.5 (1) bc	0.84 (0.00) a	205 (6) ab	21.7 (2.0) a	0.98 (0.09) a
	Maize-wheat border	32.8 (2.5) a	0.26 (0.03) a	52.9 (1.8) cd	0.84 (0.01) ab	195 (5) b	19.2 (1.5) a	0.97 (0.09) a
	Maize-wheat inner	34.2 (1.6) a	0.26 (0.03) a	56.6 (1.3) b	0.84 (0.00) a	199 (6) ab	19.9 (1.6) a	0.98 (0.09) a

Table S4.4 Parameter values (standard errors in brackets) and the *P*-values of the regressions.

Year	Stage-Leaf	A_{1800} ~ SLA			A_{1800} ~ LNC		
		Intercept	Slope	*P*	Intercept	Slope	*P*
2019	V6-L6	60.2 (4.6)	-0.06 (0.02)	0.000	46.7 (13.6)	-0.14 (0.34)	0.675
	R1-L7	47.6 (5.1)	-0.07 (0.02)	0.000	23.0 (7.3)	0.18 (0.24)	0.473
	R1-L14	53.8 (4.7)	-0.06 (0.02)	0.015	29.0 (6.3)	0.41 (0.19)	0.041
	R4-L7	34.5 (8.3)	-0.04 (0.03)	0.166	-5.5 (3.2)	1.25 (0.14)	0.000
	R4-L14	29.6 (7.1)	0.04 (0.04)	0.354	14.8 (4.5)	0.87 (0.18)	0.000
2018	V10-L10	65.2 (5.8)	-0.08 (0.02)	0.003	37.5 (8.1)	0.27 (0.25)	0.294
	R4-L10	34.0 (14.8)	0.00 (0.07)	0.965	18.5 (3.9)	0.72 (0.19)	0.001

The acclimation of leaf photosynthetic traits of maize to interspecific competition in intercropping

Supplementary Information for Chapter 5:

Fig. S5.1 Daily air temperature and daily photosynthetically active radiation during the growing season

Fig. S5.2 Monthly precipitation, monthly irrigation, and monthly maize evapotranspiration during the growing season

Fig. S5.3 Measured A_n-C_i curves in different treatments at maize V10 and R4 stages

Fig. S5.4 Scatter plot matrices of leaf photosynthetic parameters at maize V10 and R4 stages

Fig. S5.5 Leaf traits of maize in different treatments at maize V10 and R4 stages

Fig. S5.6 Leaf N use efficiency for $A_{g,max}$, J_{atpmax}, and V_{cmax} in different treatments at maize V10 and R4 stages

Table S5.1 The application time and amount of fertilizer and pesticides

Table S5.2 Schedule of gas exchange and fluorescence measurements of maize leaves at maize V10 and R4 stages

Table S5.3 List of variables and their definitions, units, or values

Table S5.4 The AIC values of all models

Table S5.5 The fitted parameter values and standard errors (SE) in each treatment at each maize stage

Methods S5.1 Models used to estimate leaf photosynthetic parameters

Methods S5.2 Model parameterization

Methods S5.3 The process of model selection for the model with two parameters

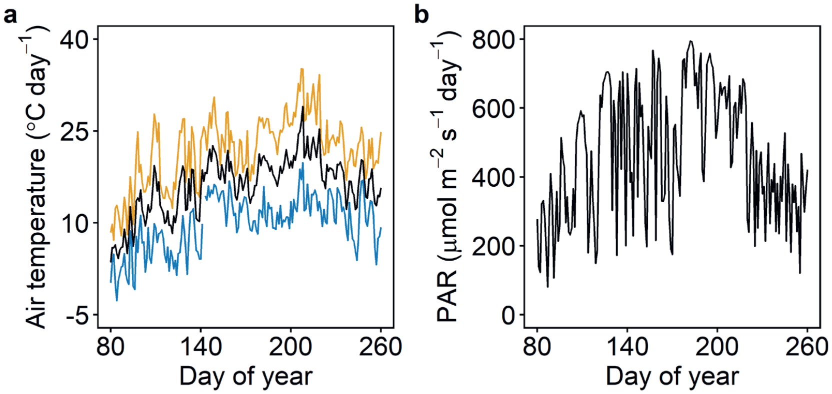


Fig. S5.1 Daily air temperature (°C) (a) and daily photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) (b) during the growing seasons of 2018. In panel a, black curve represents daily mean air temperature, yellow curve represents daily maximum air temperature, and blue curve represents daily minimum air temperature. Data was recorded at weather station De Veenkampen at 2.5 km distance from the experimental site. In panel b, data of daily global radiation (W m^{-2}) was recorded at weather station De Veenkampen. Daily PAR was calculated as: daily global radiation × 4.6 × 50%.

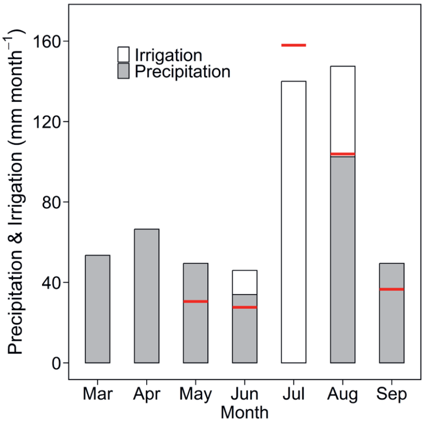


Fig. S5.2 Monthly precipitation (mm), irrigation (mm), and estimated maize evapotranspiration (mm) during the growing seasons of 2018. Monthly precipitation and irrigation were recorded at the experimental site. Red lines indicate monthly maize evapotranspiration. Monthly maize evapotranspiration was calculated by averaging daily maize evapotranspiration ET_c (mm day^{-1}) throughout the month. ET_c was calculated as: $ET_c = ET_0 \times K_c$. ET_0 (mm day^{-1}) is daily reference crop evapotranspiration, which was calculated using the FAO Penman-Monteith equation (Allen et al., 1998) with meteorological data from weather station De Veenkampen (2.5 km west of the experimental site). K_c is crop coefficient of maize, which is 0.3 during the maize early-season (May and June), 1.2 during the maize mid-season (July and August), and 0.6 during the maize late-season (September) (Allen et al., 1998).

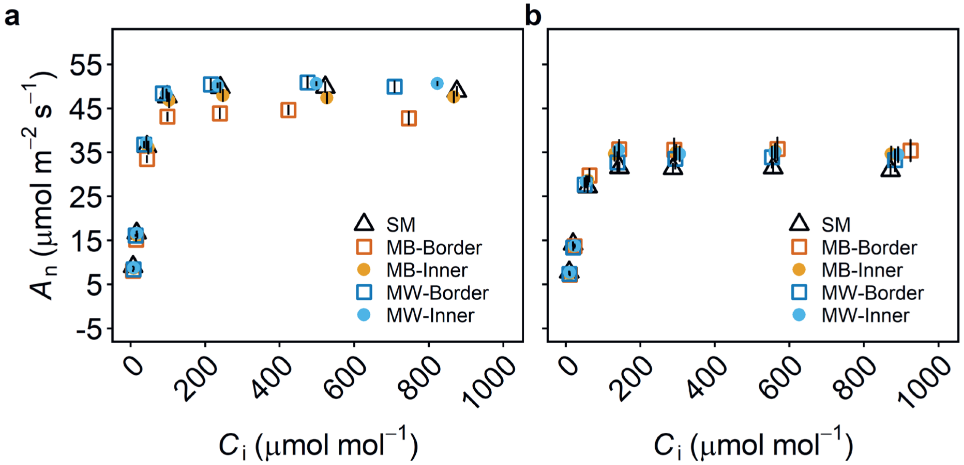


Fig. S5.3 Measured A_n-C_i in different treatments at maize V10 (a) and R4 (b) stages. Error bars represent standard errors of the means of measured A_n.

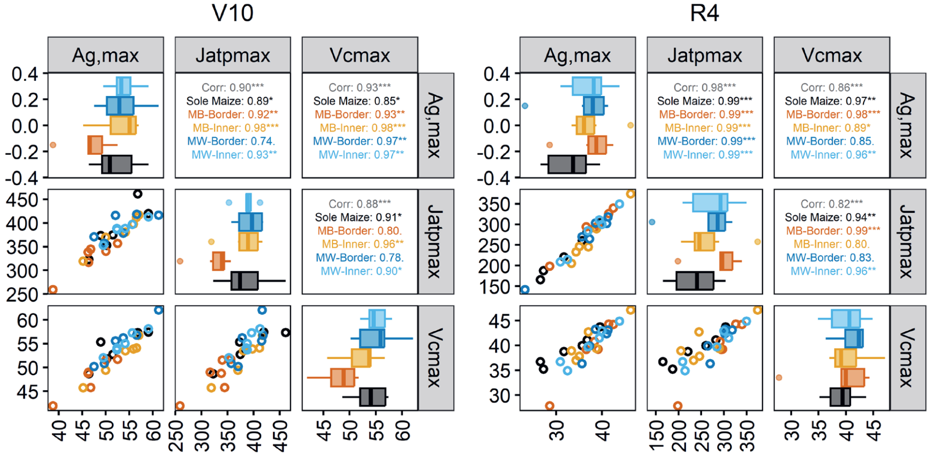


Fig. S5.4 Scatter plot matrices of leaf photosynthetic parameters at maize V10 (left) and R4 (right) stages. Leaf photosynthetic parameters were estimated by fitting the model to the data of each replicate for each treatment at each stage. The matrix was generated using the ggpairs function from the "GGally" package (Barret et al., 2021) in R (R Core Team, 2023). In the main diagonal, boxplots are shown. Scatter plots are shown in the lower triangular matrix. The upper triangular matrix shows the Pearson's correlation coefficients between leaf photosynthetic parameters. Single asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$.

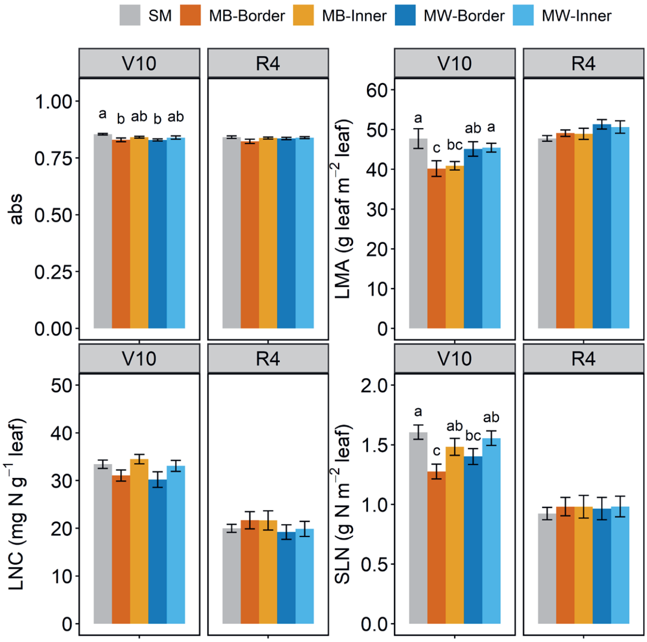


Fig. S5.5 Leaf traits of maize in different treatments at maize V10 and R4 stages. abs: light absorptance; LMA: leaf mass per unit area; LNC: leaf N concentration; SLN: leaf N concentration per unit area. Linear mixed effect models were used to compare means of five treatments at each maize stage. Treatment was a fixed effect and block a random effect. Models were fitted using the function lmer from the "lme4" package (Bates et al., 2015) in R (R Core Team, 2023). Significance of the fixed effect was determined with analysis of variance (ANOVA) (P = 0.05), using the Anova function from the "car" package (Fox and Weisberg, 2019). When the ANOVA showed significance of treatment ($P <$ 0.05), pairwise comparisons were conducted using Fisher's Least Significant Difference (LSD) in the "emmeans" package (Lenth, 2021). Different letters denote significant differences between treatments at each maize stage (Fisher's LSD test, $P < 0.05$).

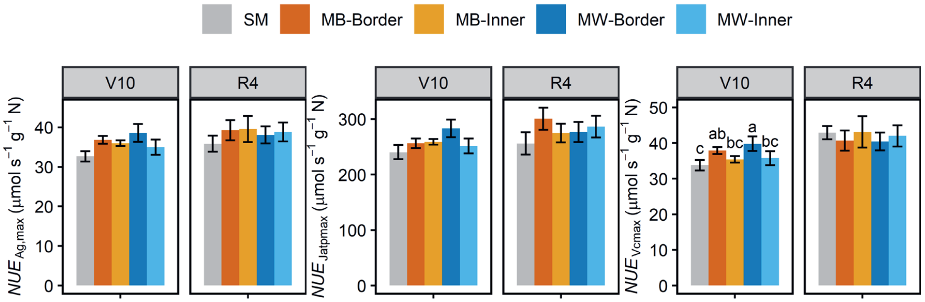


Fig. S5.6 Leaf N use efficiency for $A_{g,max}$ ($NUE_{Ag,max}$), J_{atpmax} ($NUE_{Jatpmax}$), and V_{cmax} (NUE_{Vcmax}) in different treatments at maize V10 and R4 stage. At each stage, $NUE_{Ag,max}$, $NUE_{Jatpmax}$, and NUE_{Vcmax} were calculated as leaf photosynthetic parameters divided by SLN, using the data of each replicate in each treatment. Linear mixed effect models were used to compare means of five treatments. Treatment was a fixed effect and block a random effect. Models were fitted using the function lmer from the "lme4" package (Bates et al., 2015) in R (R Core Team, 2023). Significance of the fixed effect was determined with analysis of variance (ANOVA) (P = 0.05), using the Anova function from the "car" package (Fox and Weisberg, 2019). When the ANOVA showed significance of treatment ($P <$ 0.05), pairwise comparisons were conducted using Fisher's Least Significant Difference (LSD) in the "emmeans" package (Lenth, 2021). Different letters denote significant differences between treatments at each maize stage (Fisher's LSD test, $P < 0.05$).

Table S5.1 The application time and amount of fertilizer and pesticides.

Year	Date	Fertilizer / pesticides	Amount
Mineral fertilizer application (kg ha^{-1}) (nutrients content)			
2018	28/Feb	K_2SO_4+$MgSO_4$, $Ca(H_2PO_4)_2\cdot H_2O$	350 (105 kg K_2O ha^{-1}), 150 (67.5 kg P_2O_5 ha^{-1})
	11/Apr	$NH_4NO_3\cdot CaMg(NO_3)_2$	296 (80 kg N ha^{-1} for wheat), 74 (20 kg N ha-1 for faba bean)
	4/May	$NH_4NO_3\cdot CaMg(NO_3)_3$	167 (45 kg N ha^{-1} for wheat), 296 (80 kg N ha^{-1} for maize)
	11/Jun	$NH_4NO_3\cdot CaMg(NO_3)_4$	333 (90 kg N ha^{-1} for maize)
Chemical pesticides, herbicides and fungicides utilization (g ha^{-1})			
2018	23/Mar	Pendimethalin	800
	13/Apr	Bentazon	480
	25/Apr	Bentazon	720
	7/May	Bentazon	720
	31/May	Tebuconazole, Prothioconazole, Pirimicarb	125, 125, 250

Table S5.2 Schedule of gas exchange and fluorescence measurements of leaves in sole maize (SM), border row and inner row maize in maize-faba bean intercrop (MB-Border; MB-Inner), and border row and inner row maize in maize-wheat intercrop (MW-Border; MW-Inner) at maize ten-leaf stage (V10) (DOY: 178-180, 183-187) and kernel dough stage (R4) (DOY: 226-229, 232-235). At both stages, measurements were conducted block by block. Measurements in blocks 1, 3, and 5 were made with LI-COR 6400XT. Measurements in blocks 2, 4, and 6 were made with LI-COR 6800. Treatments were randomly assigned to a morning (approx. 8:30 to 12:00) or an afternoon (approx. 12:00 to 15:00) measurement. For each leaf, the orders of three response curves, i.e., CO$_2$ response curve (A_n-C_a), light response curve (A_n-I_{inc}), and light response curve under limiting light levels and non-photorespiratory conditions (A_n-$I_{inc,LL}$), were also randomly assigned.

V10	DOY 178 DOY 226		DOY 179 DOY 227		DOY 180 DOY 228		DOY 183 DOY 229		DOY 184 DOY 232		DOY 185 DOY 233		DOY 186 DOY 234		DOY 187 DOY 235	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
	Block 1 (LI-COR 6400 XT)						Block 3 (LI-COR 6400 XT)				Block 5 (LI-COR 6400 XT)					
SM					A_n-I_{inc} A_n-C_a A_n-$I_{inc,LL}$	A_n-$I_{inc,LL}$ A_n-I_{inc} A_n-C_a										A_n-C_a A_n-$I_{inc,LL}$ A_n-I_{inc}
MB-Border			A_n-$I_{inc,LL}$ A_n-I_{inc} A_n-C_a							A_n-$I_{inc,LL}$ A_n-C_a A_n-I_{inc}	A_n-I_{inc} A_n-$I_{inc,LL}$ A_n-C_a					
MB-Inner				A_n-$I_{inc,LL}$ A_n-C_a A_n-I_{inc}					A_n-C_a A_n-I_{inc} A_n-$I_{inc,LL}$			A_n-I_{inc} A_n-$I_{inc,LL}$ A_n-C_a				
MW-Border	A_n-C_a A_n-$I_{inc,LL}$ A_n-I_{inc}						A_n-$I_{inc,LL}$ A_n-C_a A_n-I_{inc}							A_n-I_{inc} A_n-C_a A_n-$I_{inc,LL}$		
MW-Inner		A_n-I_{inc} A_n-C_a A_n-$I_{inc,LL}$						A_n-I_{inc} A_n-$I_{inc,LL}$ A_n-C_a					A_n-C_a A_n-I_{inc} A_n-$I_{inc,LL}$			

R4	DOY 178 DOY 226		DOY 179 DOY 227		DOY 180 DOY 228		DOY 183 DOY 229		DOY 184 DOY 232		DOY 185 DOY 233		DOY 186 DOY 234		DOY 187 DOY 235	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
	Block 2 (LI-COR 6800)					Block 4 (LI-COR 6800)					Block 6 (LI-COR 6800)					
SM					A_n-I_inc A_n-$I_\mathrm{inc,LL}$ A_n-C_a	A_n-$I_\mathrm{inc,LL}$ A_n-C_a A_n-I_inc										A_n-C_a A_n-I_inc A_n-$I_\mathrm{inc,LL}$
MB-Border		A_n-C_a A_n-$I_\mathrm{inc,LL}$ A_n-I_inc						A_n-C_a A_n-I_inc A_n-$I_\mathrm{inc,LL}$						A_n-I_inc A_n-C_a A_n-$I_\mathrm{inc,LL}$		
MB-Inner	A_n-C_a A_n-$I_\mathrm{inc,LL}$ A_n-I_inc						A_n-I_inc A_n-C_a A_n-$I_\mathrm{inc,LL}$						A_n-$I_\mathrm{inc,LL}$ A_n-I_inc A_n-C_a			
MW-Border				A_n-$I_\mathrm{inc,LL}$ A_n-I_inc A_n-C_a						A_n-C_a A_n-I_inc A_n-$I_\mathrm{inc,LL}$	A_n-I_inc A_n-$I_\mathrm{inc,LL}$ A_n-C_a					
MW-Inner			A_n-$I_\mathrm{inc,LL}$ A_n-C_a A_n-I_inc						A_n-$I_\mathrm{inc,LL}$ A_n-I_inc A_n-C_a			A_n-C_a A_n-$I_\mathrm{inc,LL}$ A_n-I_inc				

Table S5.3 List of variables and their definitions, units, or values.

Variables	Definition	Unit or value
abs	Leaf absorptance	-
A_n	Net rate of CO_2 assimilation	μmol CO_2 m^{-2} s^{-1}
A_EE	A_n when both C_4 and C_3 cycle are limited by enzyme activity	μmol CO_2 m^{-2} s^{-1}
A_ET	A_n when the C_4 cycle is limited by enzyme activity and the C_3 cycle is limited by chloroplastic ATP production rate	μmol CO_2 m^{-2} s^{-1}
A_TE	A_n when the C_4 cycle is limited by chloroplastic ATP production rate and the C_3 cycle is limited by enzyme activity	μmol CO_2 m^{-2} s^{-1}
A_TT	A_n when both C_4 and C_3 cycle are limited by chloroplastic ATP production rate	μmol CO_2 m^{-2} s^{-1}
$A_\mathrm{g,max}$	Light-saturated gross rate of CO_2 assimilation	μmol CO_2 m^{-2} s^{-1}
C_c	CO_2 concentration at the carboxylation sites of Rubisco in the bundle sheath cell	μmol mol^{-1}
C_i	CO_2 concentration in intercellular air spaces	μmol mol^{-1}
C_m	CO_2 concentration in the mesophyll cell	μmol mol^{-1}
$F_\mathrm{m}{}'$	Maximum fluorescence yield	-
F_s	Steady-state fluorescence yield	-
g_bs	Bundle sheath conductance to CO_2 leakage	mol m^{-2} s^{-1}
g_m	Mesophyll diffusion conductance	100 mol m^{-2} s^{-1} [a]
I_abs	Absorbed light	μmol photon m^{-2} s^{-1}
J_atp	Rate of ATP production in the chloroplast	μmol ATP m^{-2} s^{-1}
J_atpmax	Maximum rate of ATP production in the chloroplast under saturated light	μmol ATP m^{-2} s^{-1}
K_p	Michaelis-Menten constant of PEPc for CO_2	80 μbar [b]
K_mc	Michaelis-Menten constants of Rubisco for CO_2	650 μbar [b]
K_mo	Michaelis-Menten constants of Rubisco for O_2	450 mbar [b]
L	Rate of CO_2 leakage from the bundle sheath to the mesophyll cell	μmol CO_2 m^{-2} s^{-1}
O_c	O_2 concentration at the carboxylation sites of Rubisco in the bundle sheath cell	mmol mol^{-1}
O_i	O_2 concentration in intercellular air spaces	210 mmol mol^{-1}
$S_\mathrm{c/o}$	Relative CO_2/O_2 specificity factor of Rubisco	2590 [b]

s'	A lumped parameter used to calculate J_{atp} from chlorophyll fluorescence-based data	µmol ATP µmol^{-1} electron
V_{c}	Rate of RuBP carboxylation	µmol CO_2 m^{-2} s^{-1}
V_{cmax}	Maximum rate of Rubisco carboxylation	µmol CO_2 m^{-2} s^{-1}
V_{p}	Rate of PEP carboxylation	µmol CO_2 m^{-2} s^{-1}
V_{pmax}	Maximum rate of PEPc carboxylation	µmol CO_2 m^{-2} s^{-1}
R_{d}	Day respiration	µmol CO_2 m^{-2} s^{-1}
R_{m}	Day respiration in the mesophyll cell	µmol CO_2 m^{-2} s^{-1}
Φ_2	Photon use efficiency of Photosystem II e$^-$ transport	µmol electron µmol^{-1} photon
$\Phi_{2\mathrm{LL}}$	Φ_2 under limiting light levels	µmol electron µmol^{-1} photon
ε	Photon use efficiency of CO_2-assimilation based on absorbed irradiance under limiting light levels	µmol CO_2 µmol^{-1} photon
γ^*	Half the inverse of Rubisco specificity	$0.5/S_{\mathrm{c/o}}$ [b]
α	Fraction of O_2 evolved in the bundle sheath cell	0.1 [c]
x	Fraction of ATP partitioned to the C_4 cycle	0.4 [b]
θ_{A}	Curvature factor for the response of A_{n} to I_{abs}	-
θ_{J}	Convexity factor for the response of J_{atp} to I_{abs}	0.7 [b]

[a] g_{m} is hard to estimate reliably from our data as it is not the main limiting factor for C_4 photosynthesis (Yin et al., 2021). In order to better fit the measured data, g_{m} was assumed to be non-limiting.

[b] von Caemmerer and Furbank 1999

[c] Chapman et al., 1980

Table S5.4 The AIC values of all models. The AIC value of each model version was calculated based on negative log-likelihood and a penalty term for the number of parameters: $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The **bold** AIC value represents the selected model. ΔAIC is the difference compared to the selected model.

Maize Stage	Model	Groups of data used to fit model	Parameter differing among groups	Parameter number (k) [a]	-2log(L)	AIC	ΔAIC
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	ε [b]	7	292	**306**	0
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	ε	5	300	310	4
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	ε	4	301	309	4
	M4	One group: all data combined	None	3	308	314	8
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	ε	7	226	240	4
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	ε	5	226	**236**	0
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	ε	4	232	240	4
	M4	One group: all data combined	None	3	234	240	4
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	s' and R_d	11	500	522	9
	M2		R_d	7	502	516	2
	M3		s'	7	501	515	2
	M4	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	s' and R_d	7	503	517	3
	M5		R_d	5	504	514	0
	M6		s'	5	503	513	0
	M7	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	s' and R_d	5	504	514	1
	M8		R_d	4	505	513	0
	M9		s'	4	504	512	-1
	M10	One group: all data combined	None	3	508	**514**	0
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	s' and R_d	11	399	421	14
	M2		R_d	7	400	414	6
	M3		s'	7	400	414	6
	M4	Three groups: SM, (MB-Border, MB-Inner),	s' and R_d	7	401	415	7
	M5		R_d	5	401	411	3

	M6	(MW-Border, MW-Inner)	s'	5	401	411	3
	M7	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	s' and R_d	5	401	411	3
	M8		R_d	4	401	409	1
	M9		s'	4	401	409	1
	M10	One group: all data combined	None	3	402	**408**	0
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	$A_{\mathrm{g,max}}$	6	1054	**1066**	0
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	$A_{\mathrm{g,max}}$	4	1076	1084	18
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	$A_{\mathrm{g,max}}$	3	1090	1096	29
	M4	One group: all data combined	None	2	1090	1094	28
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	$A_{\mathrm{g,max}}$	6	1093	1105	4
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	$A_{\mathrm{g,max}}$	4	1094	1102	1
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	$A_{\mathrm{g,max}}$	3	1095	**1101**	0
	M4	One group: all data combined	None	2	1116	1120	19
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	V_{pmax}	6	619	631	5
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	V_{pmax}	4	619	627	2
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	V_{pmax}	3	622	628	2
	M4	One group: all data combined	None	2	622	**626**	0
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	V_{pmax}	6	521	533	7
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	V_{pmax}	4	522	530	3
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	V_{pmax}	3	523	529	2

	M4	One group: all data combined	None	2	523	**527**	0
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	g_{bs} and V_{cmax}	11	3782	3804	561
	M2		V_{cmax}	7	3229	**3243**	0
	M3		g_{bs}	7	3251	3265	22
	M4	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	g_{bs} and V_{cmax}	7	3817	3831	587
	M5		V_{cmax}	5	3236	3246	3
	M6		g_{bs}	5	3254	3264	20
	M7	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	g_{bs} and V_{cmax}	5	3443	3453	210
	M8		V_{cmax}	4	3253	3261	17
	M9		g_{bs}	4	3253	3261	18
	M10	One group: all data combined	None	3	3253	3259	16
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	g_{bs} and V_{cmax}	11	3327	3349	673
	M2		V_{cmax}	7	2684	2698	23
	M3		g_{bs}	7	2661	**2675**	0
	M4	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	g_{bs} and V_{cmax}	7	3268	3282	607
	M5		V_{cmax}	5	2684	2694	19
	M6		g_{bs}	5	2670	2680	4
	M7	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	g_{bs} and V_{cmax}	5	2912	2922	247
	M8		V_{cmax}	4	2684	2692	17
	M9		g_{bs}	4	2672	2680	5
	M10	One group: all data combined	None	3	2684	2690	15
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	Φ_{2LL} [c]	7	-530	-516	-1
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	Φ_{2LL}	5	-525	-515	1
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	Φ_{2LL}	4	-522	-514	2
	M4	One group: all data combined	None	3	-522	**-516**	0
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	Φ_{2LL}	7	-119	-105	291
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	Φ_{2LL}	5	-407	-397	-1

	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	Φ_{2LL}	4	-403	**-395**	0
	M4	One group: all data combined	None	3	331	337	733
V10	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	J_{atpmax}	6	1677	**1689**	0
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	J_{atpmax}	4	1731	1739	50
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	J_{atpmax}	3	1767	1773	84
	M4	One group: all data combined	None	2	1769	1773	84
R4	M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, MW-Inner	J_{atpmax}	6	2011	2023	0
	M2	Three groups: SM, (MB-Border, MB-Inner), (MW-Border, MW-Inner)	J_{atpmax}	4	2017	2025	1
	M3	Two groups: SM, (MB-Border, MB-Inner, MW-Border, MW-Inner)	J_{atpmax}	3	2017	**2023**	0
	M4	One group: all data combined	None	2	2032	2036	13

[a] In each model version, first, maximum likelihood estimate of the parameters was obtained through the mle2's formula interface. In the mle2's formula interface, equations of the model were used as the deterministic model, and a normal distribution was used as the stochastic model (including standard deviation to be estimated). The standard deviation was assumed to be a common value in each model version, which was also counted in the parameter number.

[b] ε was estimated as the slope of the linear regression of A_n against I_{abs}, using the data measured under limiting light levels ($I_{inc} \leq 200$ µmol m^{-2} s^{-1}) and 400 µmol mol^{-1} C_a. In the linear model, there was two parameters to be estimated: one is ε (slope) and another one is the intercept. The intercept was assumed to be a common value in each model version, which was also counted in the parameter number. The result of the intercept was not shown in this study.

[c] Φ_{2LL} was estimated as the linear intercept of Φ_2 against I_{abs}, using data of $I_{inc} \leq 200$ µmol m^{-2} s^{-1} under 400 µmol mol^{-1} C_a. In the linear model, there was two parameters to be estimated: one is the slope and another one is Φ_{2LL} (intercept). The slope was assumed to be a common value in each model version, which was also counted in the parameter number. The result of the slope was not shown in this study.

Table S5.5 The fitted parameter values and standard errors (SE) in each treatment at each maize stage. At each maize stage, maximum likelihood estimate of the parameter of each treatment was obtained, fitting to the data of each treatment.

Parameter	Stage	SM		MB-Border		MB-Inner		MW-Border		MW-Inner	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ε (μmol CO_2 μmol^{-1} photon)	V10	0.062	0.003	0.061	0.005	0.068	0.003	0.070	0.003	0.064	0.004
	R4	0.065	0.003	0.073	0.002	0.069	0.003	0.069	0.003	0.071	0.002
R_d (μmol CO_2 m^{-2} s^{-1})	V10	3.09	0.70	3.33	0.83	3.53	0.84	2.89	0.73	3.25	0.65
	R4	2.29	0.54	2.20	0.36	2.38	0.71	2.34	0.33	2.24	0.43
$A_{g,max}$ (μmol CO_2 m^{-2} s^{-1}) [a]	V10	50.9	0.9	47.2	1.1	53.2	0.9	51.8	0.7	52.6	0.7
	R4	33.4	0.9	37.6	0.7	38.1	0.8	36.3	1.0	37.1	0.7
Φ_{2LL} (μmol electron μmol^{-1} photon)	V10	0.68	0.01	0.66	0.02	0.68	0.02	0.68	0.01	0.68	0.01
	R4	0.61	0.04	0.67	0.01	0.64	0.02	0.64	0.02	0.65	0.02
s' (μmol ATP μmol^{-1} electron)	V10	0.33	0.03	0.34	0.04	0.36	0.03	0.35	0.03	0.35	0.03
	R4	0.37	0.02	0.36	0.02	0.37	0.03	0.37	0.01	0.36	0.02
J_{atpmax} (μmol ATP m^{-2} s^{-1})	V10	362	7	322	5	388	5	391	4	384	3
	R4	243	8	288	7	272	8	276	10	278	8
V_{pmax} (μmol CO_2 m^{-2} s^{-1})	V10	104.7	6.4	96.0	6.9	96.6	7.0	112.4	9.5	103.6	8.5
	R4	68.0	4.7	66.1	4.8	69.6	1.9	73.2	4.3	68.8	3.9
V_{cmax} (μmol CO2 m^{-2} s^{-1})	V10	56.2	1.5	48.7	1.3	52.4	2.1	55.0	2.6	54.3	2.3
	R4	38.2	1.6	40.7	1.3	44.0	1.5	42.0	1.5	43.0	1.5
g_{bs} (mmol m^{-2} s^{-1})	V10	0.23	0.16	0.01	0.15	0.10	0.32	0.22	0.39	0.01	0.29
	R4	0.87	0.36	0.11	0.21	1.39	0.32	1.15	0.41	0.96	0.33

[a] At each maize stage, $A_{g,max}$ was estimated with a common θ_A (0.95 at the V10; 0.91 at the R4) as input. The common θ_A for each stage was estimated using the combined data of all treatments.

Methods S5.1 Models used to estimate leaf photosynthetic parameters
Variables used in models and constant values of variables are listed in Table S5.3.

Light-saturated gross photosynthetic rate ($A_{g,max}$)

$A_{\mathrm{g,max}}$ was estimated using a nonrectangular hyperbola equation:

$$A_\mathrm{n} = \frac{\varepsilon I_\mathrm{abs} + A_\mathrm{g,max} - \sqrt{(\varepsilon I_\mathrm{abs} + A_\mathrm{g,max})^2 - 4\theta_\mathrm{A}\varepsilon I_\mathrm{abs} A_\mathrm{g,max}}}{2\theta_\mathrm{A}} - R_\mathrm{d} \qquad \text{Eq. S5.1}$$

where I_{abs} ($= I_\mathrm{inc} \times abs$) is the absorbed light, ε is the photon use efficiency of CO_2-assimilation based on absorbed light under limiting light levels, R_d is the day respiration, θ_A is the curvature factor for the response of A_n to I_abs.

Maximum PEPc carboxylation rate (V_{pmax}), maximum Rubisco carboxylation rate (V_{cmax}), and bundle sheath conductance (g_{bs})

A biochemical C_4 photosynthesis model was adopted to estimate V_pmax, V_cmax, and g_bs (Yin et al., 2011), which is based on the model of von Caemmerer and Furbank (1999). In C_4 photosynthesis, CO_2 first diffuses into the mesophyll cell and is fixed by PEP carboxylation at the rate V_p into C_4 acids. The C_4 acids are transported to the bundle sheath cell and then decarboxylated to CO_2. In the bundle sheath cell, CO_2 can either be fixed by RuBP carboxylation at the rate V_c, or leaks back to the mesophyll cell. The C_3 cycle in the bundle sheath cell relies on decarboxylation of C_4 acids as source of CO_2.

A_n can be written in terms of C_4 cycle as:

$$A_\mathrm{n} = V_\mathrm{p} - L - R_\mathrm{m} \qquad \text{Eq. S5.2}$$

where L is the rate of CO_2 leakage from the bundle sheath to the mesophyll cell, and R_m ($= 0.5 \times R_\mathrm{d}$) is the day respiration in the mesophyll cell. The rate of PEP carboxylation, V_p, can be limited either by the activity of enzyme PEPc or by the regeneration rate of substrate PEP. For the enzyme-limited case, V_p can be described as:

$$V_\mathrm{p} = \frac{C_\mathrm{m} V_\mathrm{pmax}}{C_\mathrm{m} + K_\mathrm{p}} \qquad \text{Eq. S5.3}$$

where C_m is the CO_2 concentration in the mesophyll cell, V_pmax is the maximum carboxylation rate of PEPc, and K_p is the Michaelis-Menten constant of PEPc for CO_2 (Table S5.3). The regeneration of PEP requires ATP driven by the electron transport chain. Because ATP is shared between the mesophyll and the bundle sheath cell, a simple approach is taken where ATP as a whole is allocated in a fixed fraction to the C_4 cycle. Two mol ATP is required for the regeneration of one mol PEP in maize-type C_4 photosynthesis.

For the PEP regeneration rate-limited case, V_p can be described as:

$$V_\mathrm{p} = xJ_\mathrm{atp}/2 \qquad \text{Eq. S5.4}$$

where J_atp is the ATP production rate in the chloroplast, and x is the fraction of ATP partitioned to the C_4 cycle (Table S5.3).

L in Eq. S5.2 is described as:

$$L = g_\mathrm{bs}(C_\mathrm{c} - C_\mathrm{m}) \qquad \text{Eq. S5.5}$$

where g_bs is the bundle sheath conductance to CO_2 leakage, and C_c is the CO_2 concentration at the carboxylation sites of Rubisco in the bundle sheath cell.

C_m in Eqs S5.3 and S5.5 is given as:

$$C_\mathrm{m} = C_\mathrm{i} - A_\mathrm{n}/g_\mathrm{m} \qquad \text{Eq. S5.6}$$

where C_i is the CO_2 concentration in intercellular air spaces which can be obtained from gas exchange measurements, and g_m is the mesophyll diffusion conductance (Table S5.3).

A_n in terms of C_3 cycle can be given as:

$$A_\mathrm{n} = \left(1 - \gamma^* \frac{O_\mathrm{c}}{C_\mathrm{c}}\right) V_\mathrm{c} - R_\mathrm{d} \qquad \text{Eq. S5.7}$$

where γ^* is half the inverse of Rubisco specificity ($S_\mathrm{c/o}$, Table S5.3), and O_c is the O_2 concentration at the carboxylation sites of Rubisco in the bundle sheath cell. The rate of RuBP carboxylation, V_c, can be limited either by the activity of enzyme Rubisco or by the regeneration rate of substrate RuBP.

For the enzyme-limited case, V_c can be given as:

$$V_\mathrm{c} = \frac{C_\mathrm{c} V_\mathrm{cmax}}{C_\mathrm{c} + K_\mathrm{mc}(1 + \frac{O_\mathrm{c}}{K_\mathrm{mo}})} \qquad \text{Eq. S5.8}$$

where V_cmax is the maximum rate of Rubisco carboxylation, and K_mc and K_mo are the Michaelis-Menten constants of Rubisco for CO_2 and O_2 respectively (Table S5.3).

V_c limited by RuBP regeneration rate can be described as the function of J_atp:

$$V_\mathrm{c} = \frac{(1-x)J_\mathrm{atp}}{3 + 7\gamma^* O_\mathrm{c}/C_\mathrm{c}} \qquad \text{Eq. S5.9}$$

where $(1-x)$ is the fraction of ATP partitioned to the C_3 cycle.

O_c in Eqs S5.7 to S5.9 can be described as:

$$O_\mathrm{c} = \frac{\alpha A_\mathrm{n}}{0.047 g_\mathrm{bs}} + O_\mathrm{i} \qquad \text{Eq. S5.10}$$

where α is the fraction of O_2 evolution occurring in Photosystem II in the bundle sheath cell (Table S5.3), 0.047 is the ratio of diffusivities and solubilities for O_2 to CO_2 in water at 25 °C (von Caemmerer and Furbank, 1999), $0.047 g_\mathrm{bs}$ is the conductance to leakage of O_2 across the bundle sheath, and O_i is the O_2 concentration in intercellular air spaces which is assumed to be equal to the ambient 21% O_2 (Table S5.3). Following Yin et al. (2011), we assume that the O_2 level in mesophyll cells is identical with that in intercellular air spaces.

In the model, J_atp is described as:

$$J_\mathrm{atp} = \frac{I_\mathrm{abs} \Phi_2 s'}{1-x} \qquad \text{Eq. S5.11}$$

where Φ_2 is the photon use efficiency of Photosystem II electron transport (obtained from chlorophyll fluorescence measurements), and s' is the calibration factor which lumps a number of hard-to-measure parameters including the fraction of absorbed irradiance partitioned to Photosystem II, fractions of basal cyclic electron transport, stoichiometric constants with regard to the Q-cycle activity and proton requirement for ATP synthesis, and the fraction of ATP partitioned to the C_3 cycle $(1-x)$.

The biochemical C_4 photosynthesis model (Yin et al., 2011) predicts A_n as the minimum of four rates:

$$A_\mathrm{n} = min\ (A_\mathrm{EE}, A_\mathrm{ET}, A_\mathrm{TT}, A_\mathrm{TE}) \qquad \text{Eq. S5.12}$$

where A_EE is A_n when both C_4 and C_3 cycles are limited by enzyme activity, A_ET is A_n when the C_4 cycle is limited by enzyme activity and the C_3 cycle is limited by ATP production rate, A_TT is A_n when both C_4 and C_3 cycles are limited by ATP production rate, and A_TE is A_n when the C_4 cycle is limited by ATP production rate and the C_3 cycle is limited by enzyme activity.

Mathematical solutions to A_{EE}, A_{ET}, A_{TT}, and A_{TE} are according to Appendix in Yin et al. (2011) and are given below.

(i) The quadratic expression for A_{TE} and A_{TT} and its solution

A_{TE} is the combination of Eqs S5.2, S5.4 to S5.8, S5.10 and S5.11. A_{TT} is the combination of Eqs S5.2, S5.4 to S5.7, and S5.9 to S5.11. A_{TE} or A_{TT} can be expressed in a standard quadratic expression as:

$aA_{\mathrm{n}}{}^2 + bA_{\mathrm{n}} + c = 0$

where $A_{\mathrm{n}} = (-b + \sqrt{b^2 - 4ac})/(2a)$

$a = x_2 g_{\mathrm{m}} \alpha/0.047 - g_{\mathrm{m}} - g_{\mathrm{bs}}$

$b = g_{\mathrm{m}}(C_{\mathrm{i}} g_{\mathrm{bs}} + V_{\mathrm{p}} - R_{\mathrm{m}}) + (x_3 + x_2 O_{\mathrm{i}}) g_{\mathrm{m}} g_{\mathrm{bs}} + (x_1 \gamma^* + x_2 R_{\mathrm{d}}) g_{\mathrm{m}} \alpha/0.047 + (g_{\mathrm{m}} + g_{\mathrm{bs}})(x_1 - R_{\mathrm{d}})$

$c = -g_{\mathrm{m}}(C_{\mathrm{i}} g_{\mathrm{bs}} + V_{\mathrm{p}} - R_{\mathrm{m}})(x_1 - R_{\mathrm{d}}) + g_{\mathrm{m}} g_{\mathrm{bs}}[x_1 \gamma^* O_{\mathrm{i}} + R_{\mathrm{d}}(x_3 + x_2 O_{\mathrm{i}})]$

For A_{TE}, $x_1 = V_{\mathrm{cmax}}$, $x_2 = K_{\mathrm{mc}}/K_{\mathrm{mo}}$, and $x_3 = K_{\mathrm{mc}}$. For A_{TT}, $x_1 = (1\text{-}x)J_{\mathrm{atp}}/3$, $x_2 = 7\gamma^*/3$, and $x_3 = 0$.

(ii) The cubic expression for A_{EE} and A_{ET} and its solution

A_{EE} is the combinations of Eqs S5.2, S5.3, S5.5 to S5.8, and S5.10. A_{ET} is the combinations of Eqs S5.2, S5.3, S5.5 to S5.7, and S5.9 to S5.11. A_{EE} or A_{ET} can be expressed in a standard cubic expression as:

$A_{\mathrm{n}}^3 + pA_{\mathrm{n}}^2 + qA_{\mathrm{n}} + r = 0$

where $A_{\mathrm{n}} = -2\sqrt{Q}\, cos(\psi/3) - p/3$

$Q = (p^2 - 3q)/9$

$\psi = cos^{-1}(U/\sqrt{Q^3})$

$U = (2p^3 - 9pq + 27r)/54$

where p, q, and r can be expressed as:

$p = m/(g_{\mathrm{m}} + g_{\mathrm{bs}} - x_2 g_{\mathrm{m}} \alpha/0.047)$

$q = n/(g_{\mathrm{m}} + g_{\mathrm{bs}} - x_2 g_{\mathrm{m}} \alpha/0.047)$

$r = o/(g_{\mathrm{m}} + g_{\mathrm{bs}} - x_2 g_{\mathrm{m}} \alpha/0.047)$

where m, n, and o can be expressed as:

$m = d - (x_3 + x_2 O_{\mathrm{i}}) g_{\mathrm{m}} g_{\mathrm{bs}} + (R_{\mathrm{d}} - x_1)(g_{\mathrm{m}} + g_{\mathrm{bs}}) - (x_1 \gamma^* g_{\mathrm{m}} + x_2 R_{\mathrm{d}} g_{\mathrm{m}} - x_2 k/g_{\mathrm{bs}})\alpha/0.047$

$n = f + (x_3 + x_2 O_{\mathrm{i}})k + d(R_{\mathrm{d}} - x_1) - g_{\mathrm{m}} g_{\mathrm{bs}}[x_1 \gamma^* O_{\mathrm{i}} + R_{\mathrm{d}}(x_3 + x_2 O_{\mathrm{i}})] + (x_1 \gamma^* + x_2 R_{\mathrm{d}})k\alpha/(0.047 g_{\mathrm{bs}})$

$o = R_{\mathrm{d}}[f + (x_3 + x_2 O_{\mathrm{i}})k] - x_1(f - k\gamma^* O_{\mathrm{i}})$

where d, f, k can be expressed as:

$d = g_{\mathrm{m}}\left[R_{\mathrm{m}} - V_{\mathrm{pmax}} - C_{\mathrm{i}}(g_{\mathrm{m}} + 2g_{\mathrm{bs}}) - K_{\mathrm{p}}(g_{\mathrm{m}} + g_{\mathrm{bs}})\right]$

$f = g_{\mathrm{m}}^2\left[C_{\mathrm{i}} V_{\mathrm{pmax}} + (C_{\mathrm{i}} + K_{\mathrm{p}})(g_{\mathrm{bs}} C_{\mathrm{i}} - R_{\mathrm{m}})\right]$

$k = g_{\mathrm{m}}^2 g_{\mathrm{bs}}(C_{\mathrm{i}} + K_{\mathrm{p}})$

For A_{EE}, $x_1 = V_{\mathrm{cmax}}$, $x_2 = K_{\mathrm{mc}}/K_{\mathrm{mo}}$, and $x_3 = K_{\mathrm{mc}}$. For A_{ET}, $x_1 = (1\text{-}x)J_{\mathrm{atp}}/3$, $x_2 = 7\gamma^*/3$, and $x_3 = 0$.

Maximum ATP production rate (J_{atpmax})

J_{atpmax} is estimated using a nonrectangular hyperbola equation:

$$J_{\mathrm{atp}} = \frac{I_{\mathrm{abs}}\Phi_{\mathrm{2LL}}s'/(1-x) + J_{\mathrm{atpmax}} - \sqrt{\left(I_{\mathrm{abs}}\Phi_{\mathrm{2LL}}s'/(1-x) + J_{\mathrm{atpmax}}\right)^2 - 4\theta_{\mathrm{J}} J_{\mathrm{atpmax}} I_{\mathrm{abs}}\Phi_{\mathrm{2LL}}s'/(1-x)}}{2\theta_{\mathrm{J}}} \qquad \text{Eq. S5.13}$$

where $\Phi_{2\mathrm{LL}}$ is Φ_2 under limiting light levels, $(\Phi_{2\mathrm{LL}}s'/(1-x))$ is the efficiency of converting I_{abs} into ATP under limiting light levels, θ_{J} is the convexity factor for the response of J_{atp} to I_{abs} (Table S5.3).

Methods S5.2 Model parameterization

$A_{\mathrm{g,max}}$ was estimated stepwise. ε was first estimated as the slope of the linear regression of A_{n} against I_{abs}, using the data measured under limiting light levels ($I_{\mathrm{inc}} \leq 200$ μmol m^{-2} s^{-1}) and 400 μmol mol^{-1} C_{a}. Estimated $A_{\mathrm{g,max}}$ depends on the curvature factor θ_{A}. To avoid this confounding effect of variable θ_{A} on comparing $A_{\mathrm{g,max}}$ among the treatments, the common θ_{A} at maize V10 stage (= 0.95) and R4 stage (= 0.91) was first estimated using the combined data of all treatments at each stage. $A_{\mathrm{g,max}}$ was then estimated using Eq. S5.1 to fit A_{n}-I_{abs} curves, using the data measured under 400 μmol mol^{-1} C_{a}, with the already estimated common θ_{A} of each maize stage, already estimated ε and R_{d} (see below) as inputs.

R_{d} and s' were estimated as the intercept and slope, respectively, of the linear regression of A_{n} against $(I_{\mathrm{abs}}\Phi_2)/3$, using the data measured under limiting light levels ($I_{\mathrm{inc}} \leq 200$ μmol m^{-2} s^{-1}) and high C_{a} (2000 μmol mol^{-1}) (Yin et al., 2011). The s' was used to calculate values of J_{atp} at all C_{a} and I_{inc} levels using Eq. S5.11.

V_{pmax}, V_{cmax}, and g_{bs} can be estimated using the C_4 photosynthesis model (Yin et al., 2011) (Eqs S5.2 to S5.12 combined with solutions (i) and (ii)), fitting to all data of combined gas exchange and chlorophyll fluorescence measurements on light response curves at the two C_{a} levels (400 μmol mol^{-1} and 2000 μmol mol^{-1}) and CO_2 response curves. There was an overfitting when fitted with the three parameters simultaneously. In order to have a better fit of measured data, V_{pmax} was estimated first. At low CO_2 levels, the leakage of CO_2 into the mesophyll cell is low. Eq. S5.2 can be approximated to (von Caemmerer and Furbank, 1999):

$$A_{\mathrm{n}} = \frac{C_{\mathrm{i}}V_{\mathrm{pmax}}}{(C_{\mathrm{i}}+K_{\mathrm{p}})} - R_{\mathrm{m}} \qquad \text{Eq. S5.14}$$

V_{pmax} was estimated using data under low C_{a} ($C_{\mathrm{a}} \leq 250$ μmol mol^{-1}) of A_{n}-C_{i} curves, with the already estimated R_{m} ($R_{\mathrm{m}} = 0.5 \times R_{\mathrm{d}}$) and constant K_{p} (Table S5.3) as inputs. V_{cmax} and g_{bs} were then estimated simultaneously by fitting the C_4 photosynthesis model to all A_{n}-I_{abs} (two C_{a} levels) and A_{n}-C_{i} curves with the already estimated V_{pmax} and R_{d}, calculated J_{atp}, and constant parameters (Table S5.3) as inputs.

J_{atpmax} was estimated stepwise using the data of A_{n}-I_{abs} curves. $\Phi_{2\mathrm{LL}}$ was first estimated as the linear intercept of Φ_2 against I_{abs}, using data of $I_{\mathrm{inc}} \leq 200$ μmol m^{-2} s^{-1} under 400 μmol mol^{-1} C_{a}. J_{atpmax} was then estimated by fitting Eq. S5.13 to J_{atp}-I_{abs} curves, with the already estimated $\Phi_{2\mathrm{LL}}$, s', calculated J_{atp}, and constant x and θ_{J} (Table S5.3) as inputs.

Methods S5.3 The process of model selection for the model with two parameters

There were four main model versions (as mentioned in the main text): (i) separate parameterization for the data of SM, MB-Border, MB-Inner, MW-Border, and MW-Inner; (ii) the model was fitted to three groups of data- data of SM, combined data of MB-Border and MB-Inner, and combined data of MW-Border and MW-Inner; (iii) the model was fitted to two groups of data- data of SM, and combined data of MB-Border, MB-Inner, MW-Border, and MW-Inner; (iv) the model was fitted to the combined data of all five treatments, where one common value for each of the two parameters were estimated.

Under each of model version i, ii, and iii, we also tested the combination of the two parameters differing between groups of data. There were three cases for each model version. For model i: first, both parameter 1 and 2 were different among five treatments, and ten values were estimated. Second, parameter 1 was different among five treatments while parameter 2 was the common value, and six values were estimated. Third, parameter 2 was different while parameter 1 was common, and six values were estimated. For model ii: as before, first, the two parameters were fitted to three groups of data and six values were estimated. Then, one of the two parameters was different among three groups while the other one was the common value, and four values were estimated. For model iii: as before, first, the two parameters were fitted to two groups of data, and four values were estimated. Then, one of the two parameters was fitted to two groups of data while the other one was the common value, and three values were estimated. An example is given below:

Model	Groups of data used to fit model	Parameter differing among groups	Parameter number[a]	$-2\log(L)$[b]	AIC[b]	ΔAIC[b]
M1	Five groups: SM, MB-Border, MB-Inner, MW-Border, and MW-Inner.	g_{bs} and V_{cmax}	11	3782	3804	561
M2		V_{cmax}	7	3229	**3243**	0
M3		g_{bs}	7	3251	3265	22
M4	Three groups: SM, combined data of MB-Border and MB-Inner, and combined data of MW-Border and MW-Inner.	g_{bs} and V_{cmax}	7	3817	3831	587
M5		V_{cmax}	5	3236	3246	3
M6		g_{bs}	5	3254	3264	20
M7	Two groups: SM, and combined data of MB-Border, MB-Inner, MW-Border, and MW-Inner.	g_{bs} and V_{cmax}	5	3443	3453	210
M8		V_{cmax}	4	3253	3261	17
M9		g_{bs}	4	3253	3261	18
M10	Combined data of all treatments.	None	3	3253	3259	16

[a] g_{bs} and V_{cmax} were estimated simultaneously using the biochemical C_4 photosynthesis model (Methods S5.1 and S5.2). First, maximum likelihood estimate of the parameters of each model version were obtained through the mle2's formula interface. In the mle2's formula interface, equations of the C_4 photosynthesis model were used as deterministic model (including g_{bs} and V_{cmax} to be estimated), and a normal distribution was used as stochastic model (including standard deviation to be estimated). The standard deviation was assumed to be a common value in each model version, which was also counted in the parameter number.

[b] The AIC value of each model version was calculated according to $\mathrm{AIC} = -2\log(L) + 2k$, where $\log(L)$ is the log-likelihood of the fit, and k is the number of parameters. The model with the smallest AIC fitted the data best. In this case, the smallest AIC was 3243 (M2), indicating that V_{cmax} was different among five treatments while g_{bs} was the same in all treatments. ΔAIC is the difference compared to the selected model, which is M2 in this case.

Summary

Intercropping is the mixed cultivation of multiple crop species in a field for the whole or part of their growing periods and is considered an option for sustainable intensification of crop production. Compared to sole crops, intercropping has advantages in land productivity, absolute yield gain, and it is characterized by improved use efficiency of resources (light, water, and nutrients). These advantages arise from complementary resource capture between species in intercrops. Temporal complementarity exists when intercropped species use resources during different periods of the season, either due to differences in sowing and harvesting dates, as in relay intercropping, or due to differences in growth dynamics when sowing and harvesting are simultaneous. Combining cereals and legumes in intercrops decreases competition for soil nitrogen (N) as the legume can fix N from the atmosphere. To date, intercropping has been well studied in conventional high-input agriculture in China, using strip designs wherein component species are cultivated in alternating strips consisting of several rows. However, limited information is available on the potential of strip intercropping in conventional agriculture in Europe, which tends to have lower nutrient inputs than in China. There is information only on wheat-maize relay strip intercropping, but no information is available yet on intercropping cereals and legumes or simultaneous strip intercropping under European growing conditions. Further analysis is required to investigate performance of more crop combinations to obtain results consistent with European standards for “Good Agricultural Practices”, i.e., moderate fertilization.

In this thesis, I studied eco-physiological responses of crop species in strip intercropping at the field, plant, and leaf levels in conventional agriculture in the Netherlands. At the field level, the study aimed to investigate whether N uptake of crop species is increased in intercrops compared to sole crops. I focused on four commonly grown species (maize, wheat, faba bean, and pea) in all their bi-specific intercrops and corresponding sole crops. Combining maize and one of the C_3 species in relay intercrops, with maize as the late-sown and late-harvest species, enables temporal complementarity due to heterogeneity in sowing and harvest dates between the two species. The combinations of two of the three C_3 species were all simultaneous intercrops, where the component species had the same sowing date and similar harvesting date.

The responses of crop species at both the plant and leaf levels were studied in relay strip intercropping maize with wheat or faba bean. I focused on wheat and faba bean as two contrasting companion species for relay intercropping with maize, in which wheat competes for soil N with maize, while such competition is expected to be diminished when maize is intercropped with faba bean due to the ability for N_2 fixation. At the plant level, I investigated shoot plasticity of component species in relay intercrops. At the leaf level, I aimed to fill a gap in elucidating the physiological responses of maize to relay intercropping, obtaining results from maize leaf photosynthetic capacity, leaf traits, and dynamic parameters related to the processes of leaf photosynthesis.

Chapter 2 reports on intercropping effects on N uptake. Compared to the sole crop, C_3 species had higher N uptake in the relay intercrop with maize, with the exception of pea, which showed different N uptake between years due to lodging in one of the years. Maize had increased N uptake in the intercrop compared to its sole crop only when intercropped with wheat or pea and only in a year with a larger temporal niche differentiation. In simultaneous intercrops involving faba bean, N uptake of the companion wheat or pea decreased compared to their sole crops. When evaluating at the whole intercrop level, relay intercrops, rather than simultaneous intercrops, had advantages in saving land for N uptake, requiring less land to give the same N uptake as the combined sole crops. The findings indicate that complementarity in N uptake in the strip intercrops studied was associated with temporal complementarity rather than complementarity in N uptake between cereals and legumes. Relay strip intercropping maize with a firm-stemmed C_3 species (wheat and faba bean) is a promising system for western-European conditions. The lodging of pea in the strip indicates that this weak-stemmed species is not very fit to the strip setting.

In Chapter 3, I zoomed in, studying shoot plasticity of wheat, faba bean, and maize in wheat-maize and faba bean-maize relay intercropping. I linked this plasticity to changes in red to far-red ratio (R:FR) and transmitted photosynthetically active radiation (PAR) in intercrops compared to sole crops. Wheat had increased tiller number per plant and faba bean had increased branch number per plant and shorter internodes in intercrops compared to sole crops. Plasticity in earlier sown species coincided with higher PAR and R:FR in intercrops as compared to sole crops. Maize showed shade avoidance responses (longer sheaths) in both intercrops compared to sole maize. The degree of this response was larger in the intercrop with faba bean than with wheat, which was related with lower PAR and R:FR perceived by maize plants in the faba bean-maize as compared to the wheat-maize intercrop. Thus, interspecific competition for light was stronger when relay intercropping maize with faba bean than with wheat. The observed trait values help to understand the yield performance of crop species in relay strip intercropping of maize with species of different statures under western-European growing conditions.

In Chapters 4 and 5, I zoom in further, studying the responses of maize leaf photosynthetic traits to wheat-maize and faba bean-maize relay strip intercropping. In Chapter 4, I focused on maize leaf photosynthetic capacity (A_{1800}) and the related leaf traits, i.e., specific leaf area (SLA), leaf N concentration (LNC), and specific leaf N (SLN). The distribution of PAR within canopies was measured to characterize the light conditions to which maize leaves were exposed. During its vegetative growth, maize in both relay intercrops responded to shading from the earlier sown species, having thinner leaves (larger SLA) with lower SLN and lower A_{1800} than maize in sole crops. These negative effects were stronger when maize was intercropped with faba bean than with wheat. During maize reproductive growth, maize leaves in both intercrops were exposed to better light conditions than leaves in sole crops. However, both LNC and A_{1800} were not increased in intercropped maize compared to sole maize. Thus, light competition during the vegetative stage and negative effects of intercropping on maize leaf N during its reproductive stage negatively affected maize leaf photosynthetic capacity in relay strip intercropping.

In Chapter 5, I focused on dynamic parameters associated with the process of maize leaf photosynthesis, i.e., light-saturated gross photosynthetic rate ($A_{\mathrm{g,max}}$), the maximum rate of ATP production (J_{atpmax}), and the maximum carboxylation rate of Rubisco (V_{cmax}). During the vegetative stage, values of maize leaf photosynthetic parameters ($A_{\mathrm{g,max}}$, J_{atpmax}, and V_{cmax}) increased with leaf mass area (LMA), indicating shade response as a mechanism for acclimation of maize leaf photosynthesis in intercrops. The parameters were lower in thinner leaves (lower LMA) in maize grown next to faba bean than in sole maize or in maize intercropped with wheat. During the reproductive stage, values of maize leaf photosynthetic parameters correlated with LNC rather than leaf thickness (LMA), indicating access to soil N as a possible mechanism for acclimation of maize leaf photosynthesis in intercrops. Intercropped maize had higher values of J_{atpmax} and $A_{\mathrm{g,max}}$ than sole maize; however, maize

LNC was not increased in intercrops compared to sole crops. Therefore, interspecific competition for light decreased Rubisco carboxylation capacity, ATP production capacity, and thereby $A_{\mathrm{g,max}}$ in maize leaves during vegetative stage. During the reproductive stage, maize leaf photosynthesis was likely restricted by decreased access to soil N in relay intercrops.

In conclusion, complementarity in N uptake in the strip intercrops studied was associated with temporal complementarity, rather than complementarity in N uptake mechanisms resulting from the legume's N fixation ability. Relay strip intercropping maize with a firm-stemmed species (wheat and faba bean) is likely to provide productivity gains under western-European growing conditions. The earlier sown wheat and faba bean in relay intercrops benefited from an improved light environment compared to sole crops. A higher N uptake in intercropped maize than sole maize was found only in the wheat-maize and pea-maize relay intercrop and only in one of the two years. Maize showed shade avoidance traits in response to stronger signals for light competition in relay intercrops than in sole crops, and interspecific competition for light was stronger when intercropping with faba bean than with wheat. The responses of maize leaf photosynthetic capacity to relay intercropping did not contribute to the higher maize yield in the wheat-maize intercrop than sole maize. The findings indicate that in relay strip intercropping, lower temporal niche differentiation between species, interspecific competition for light during the maize vegetative stage, and N deficiency during its reproductive stage negatively affected maize performance. In the general discussion of this thesis, I elaborated on options for relaxing competition for maize during the vegetative stage and preventing N shortage in the soil during the reproductive stage, aiming to mitigate or overcome the observed negative intercropping effects on maize leaf traits.

While the effects of intercropping on maize leaf traits were generally either neutral (when intercropped with wheat) or negative (when intercropped with faba bean), the performance of maize in intercrops was still satisfactory to good, due to the temporal complementarity with the other species. Thus, increased resource capture rather than improved photosynthesis underlies the good performance of relay-intercropped maize and maize-based relay intercrops under European growing conditions.

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April 4, 2024

Curriculum vitae

Bei Dong was born on April 4, 1993, in Hebei, China. From 2011, she majored in Resource Use in Agriculture during her Bachelor study in Hebei Agricultural University, China. After obtaining her Bachelor degree in 2015, she started her Master study of Agricultural Meteorology in China Agricultural University. She worked in an intercropping project and did field work in Inner Mongolia, China. She received her Master degree in 2017 and directly started as a PhD candidate at the Centre for Crop Systems Analysis (CSA) at Wageningen University & Research (WUR), the Netherlands. The PhD project was Redesigning European cropping systems based on species MIXtures (ReMIX). She was under the supervision of Dr Wopke van der Werf, Prof. Dr Jochem B. Evers, and Dr Tjeerd Jan Stomph. In this project, she focused on eco-physiological responses of plants to intercropping in different species combinations under conventional management in Western Europe. The results of her PhD research are described in this thesis. She will defend her thesis on May 14, 2024. At this moment, she is looking for a job that could allow her to stay in academia.

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List of publications

Dong, B., Wang, Z., Evers, J.B., Stomph, T.J, van der Putten, P.E.L., Yin, X., Wang, J.L., Sprangers, T., Hang, X., van der Werf, W., 2024. Competition for light and nitrogen with an earlier-sown species negatively affects leaf traits and leaf photosynthetic capacity of maize in relay intercropping. European Journal of Agronomy 155, 127119. https://doi.org/10.1016/j.eja.2024.127119

Wang, Z., **Dong, B**., Stomph, T.J., Evers, J.B., van der Putten, P.E.L, Ma, H., Missale, R., van der Werf, W., 2023. Temporal complementarity drives species combinability in strip intercropping in the Netherlands. Field Crops Res 291, 108757. https://doi.org/10.1016/j.fcr.2022.108757

Stomph, T.J., Dordas, C., Baranger, A., de Rijk, J., **Dong, B**., Evers, J.B., Gu, C., Li, L., Simon, J., Jensen, E.S., Wang, Q., Wang, Y., Wang, Z., Xu, H., Zhang, C., Zhang, L., Zhang, W.P., Bedoussac, L., van der Werf, W., 2020. Designing intercrops for high yield, yield stability and efficient use of resources: Are there principles?, in: Advances in Agronomy. Elsevier Inc., pp. 1–50. https://doi.org/10.1016/bs.agron.2019.10.002

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 (10.5 ECTS)

- Resource capture and resource use efficiency in intercropping
- Maize photosynthesis in intercropping: connecting leaf traits to crop performance

Post-graduate courses (6.2 ECTS)

- Introduction to R for statistical analysis PE&RC and SENSE (2017)
- Tidy data transformation and visualization with R; PE&RC (2020)
- Linear models PE&RC and WIMEK (2021)
- Advances in intercropping: principles and implementation; PE&RC (2021)
- Crop physiology and climate change: understanding fundamental processes to counter the challenge; PE&RC and University of Florida (2022)

Invited review of journal manuscripts (1 ECTS)

- European journal of agronomy: canopy light interception, photosynthesis, and yield in intercropping (2022)

Deficiency, refresh, brush-up courses (24 ECTS)

- Advanced statistics; Biometrics, WUR (2017)
- Systems analysis, simulation and systems management; PPS, WUR (2017)
- Functional diversity for sustainable crop production; CSA, WUR (2018)
- Ecological modelling and data analysis in R; CSA, WUR (2018)

Competence, skills and career-oriented activities (4.5 ECTS)

- Scientific writing; Wageningen in'to Languages (2019)
- Presenting with impact; Wageningen in'to Languages (2021)
- Ethics in plant and environmental sciences; WGS (2022)
- Career orientation; WGS (2023)
- Scientific integrity; WGS (2023)

PE&RC Annual meetings, seminars and PE&RC weekend/retreat (1.2 ECTS)

- PE&RC Weekend for first years (2017)
- PE&RC Day (2021)

Discussion groups/local seminars or scientific meetings (9 ECTS)

- ReMix thesis ring (2017, 2018)
- Functional-Structural Plant Modelling (2018-2020)
- Statistical Rethinking (2019)
- Automating agroecology: how to make an ecofeminist robot (2019)
- Final congress ReMIX (2021)
- Sharing knowledge in photosynthesis research (2019, 2022, 2023)

International symposia, workshops and conferences (5.2 ECTS)

- Crop mixture workshop with Bonn University; oral presentation; online (2020)
- AAB conference: intercropping for sustainability; poster presentation; online (2021)
- Netherlands annual ecology meeting; poster presentation; online (2021)
- Agricultural green development workshop with China Agricultural University; oral presentation; Wageningen (2023)

BSc/MSc thesis supervision (12.5 ECTS)

- The effects of intercropping with faba bean or wheat on photosynthetic rate and specific leaf nitrogen of maize
- Light distribution, leaf photosynthetic capacity, and yield of maize in intercropping
- Yield and light capture of wheat in intercropping
- Light distribution in faba-bean/maize and faba bean/wheat intercropping
- Yield and yield component of faba bean in intercropping
- Yield and plant architecture of pea in intercropping
- Yield and yield component in both relay intercropping and simultaneous intercropping
- Maize leaf photosynthesis in intercropping

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