



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* L.), 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* L.) or faba bean (*Vicia faba* L.) 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.

1. Introduction

Plant production is driven by the photosynthetic conversion of atmospheric CO₂ 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., 2020b,c, 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., 2017; 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, and they have a lower specific leaf area (SLA) and 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* L.) is frequently used in species mixtures, and maize often contributes substantially to intercropping yield advantages, more than other species do (Li et al., 2020c, 2023). It is therefore relevant to

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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 CO₂ 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 C₃ species, whereby the C₃ species is sown and harvested earlier than maize (Li et al., 2020c). 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., 2020c, 2023). In relay intercrops in which maize is sown later than its companion species, maize plants initially experience shading from the early-sown species (Gou et al., 2017; 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 C₃ species in a relay intercrop. A recent study in the Netherlands reported that in maize-wheat (*Triticum aestivum* L.) 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* L.) or alfalfa (*Medicago sativa* L.) compared to sole maize. The increases in leaf N may be due to N 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., 2020c). 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., 2020a, 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* L.) 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.

2. Materials and methods

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. 1). Each species strip had two border rows (one at each side of the strip) while the other rows are inner rows.

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. 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. 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 $\times$ 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 $\times$ 11 m in north-south directions for the sole maize crop, and 15 m in east-west $\times$ 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.

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 Supplementary Figs. S1-S3 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 (Supplementary Table S1). 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 (Supplementary Table S1).

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. 3). In both years, photosynthesis was furthermore measured at

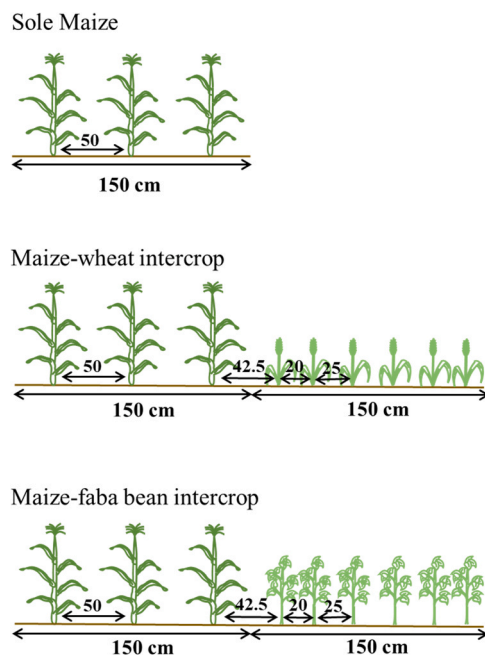


Fig. 1. Schematic illustration of row pattern in sole maize and maize intercropped with spring wheat or spring faba bean. Maize was sown at a 50 cm row distance in strips comprising three rows. The resulting 1.5 m-wide maize strips were alternated with 1.5 m-wide strips of wheat or faba bean. Wheat and 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.

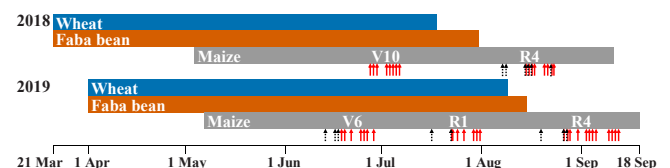


Fig. 2. Growing periods of wheat, faba bean, and maize in 2018 and 2019. Measurements on light distribution were made during maize grain-filling (R4) in 2018, and during six-leaf (V6), silking (R1), and R4 in 2019 (black arrows). Photosynthesis measurements were made at ten-leaf (V10) and R4 in 2018 and at V6, R1, and R4 in 2019 (red arrows).

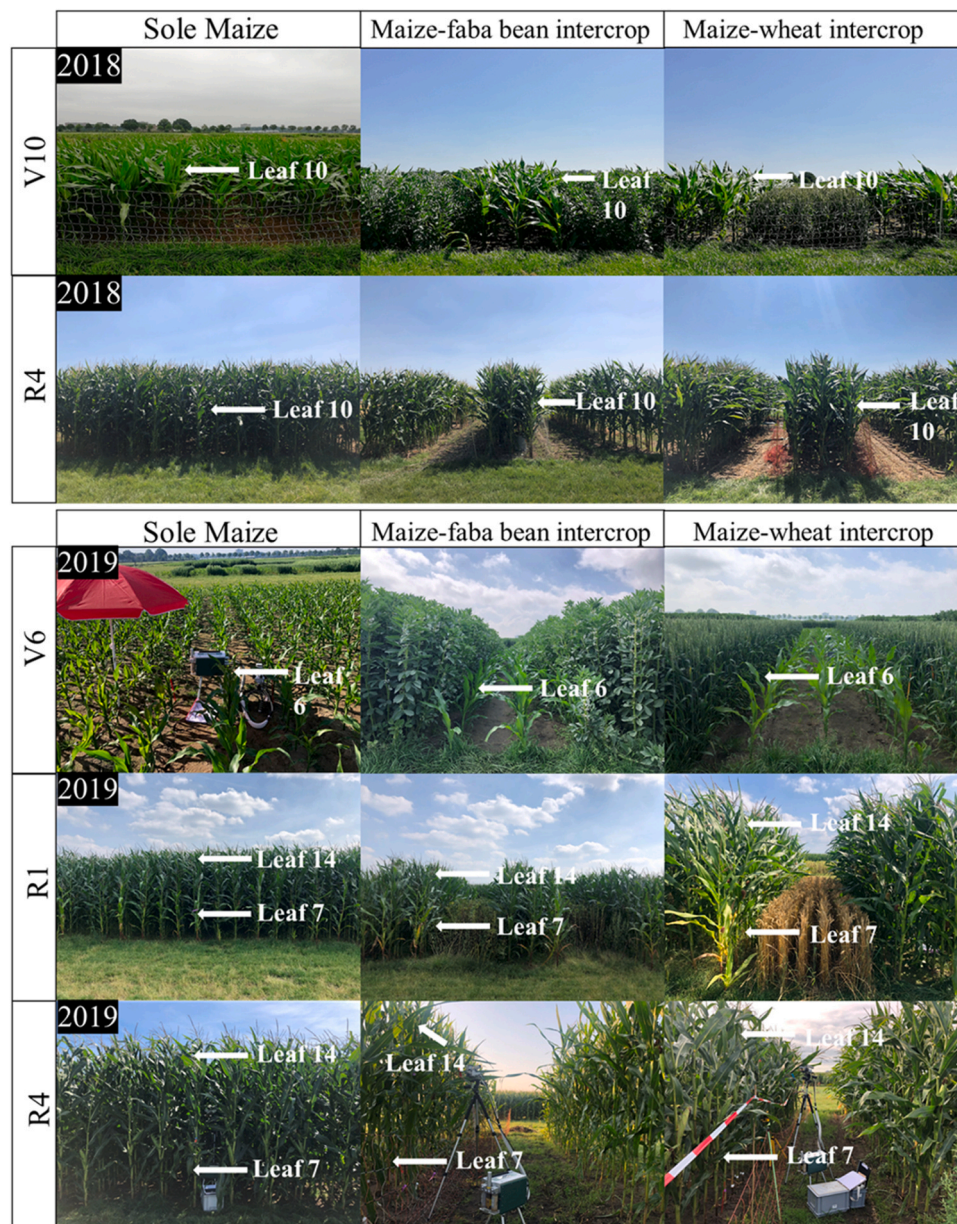


Fig. 3. Maize development stages and leaf positions for gas exchange measurements in sole maize, the maize-faba bean intercrop, and the maize-wheat intercrop in 2018 and 2019. White arrows represent the approximate positions of the measured leaves in the maize canopy.

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. 3). At R1, both a lower canopy leaf (leaf 7) and an 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.

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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a constant CO_2 level of 400 $\mu\text{mol mol}^{-1}$. The photosynthetic rate (A_{1800} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 a steady state. Both A_{1800} and stomatal conductance for water (g_{sw} ; $\text{mol m}^{-2} \text{s}^{-1}$) 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, absorbance (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; $\text{cm}^2 \text{ leaf g}^{-1}$ 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.

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. 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 (Supplementary Fig. S5), 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). Measurements were conducted with either clear sky or steady overcast sky, within two hours from solar noon (around 11:45–15:45) (for details see in Supplementary Figs. S6 to S9).

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, 2022), using the “ggplot2”

package (Wickham, 2016).

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., 2011; Wu et al., 2022; Zhao et al., 2023):

$$OY_m(\%) = \frac{Y_{im} - 0.5Y_{sm}}{0.5Y_{sm}} \times 100$$

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); $0.5 \times Y_{sm}$ 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.

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, 2022). 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, 2022) was used.

3. Results

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

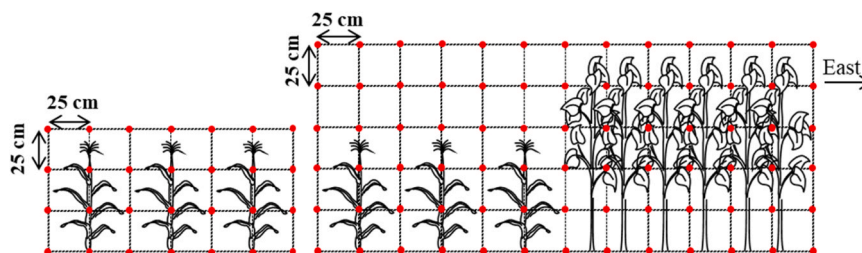


Fig. 4. Schematic illustration of the positions of the SunScan probe in the canopy in a maize strip in sole maize and in an intercrop strip in maize-faba bean intercrop at maize V6 stage in 2019. Every red dot was a measuring point with the probe oriented parallel to the rows. The measurements were made 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 (Supplementary Fig. S4).

therefore 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. 5 A). 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. 5 E, G; for substantial correlation between SLN and SLA see [Supplementary Fig. S10](#)). 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. 5 F).

At R1, leaf 7 was a lower canopy leaf, with seven additional leaves above it (Fig. 3). Leaf 7 of inner row maize in maize-wheat had higher A_{1800} than the same leaf in sole maize (Fig. 5 A). In intercrops, maize leaf 14 was fully above the canopy of faba bean or wheat. Leaf 14 had higher A_{1800} than leaf 7 in all treatments. No differences in A_{1800} 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. 5 E). However, leaf 14 in border row maize in maize-wheat had lower LNC than the same leaf in sole maize (Fig. 5 F). Both LNC and SLN of leaf 14 were reduced in border row maize in maize-faba compared to sole maize (Fig. 5 F 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 A_{1800} of leaf 14 was not increased in any intercrop

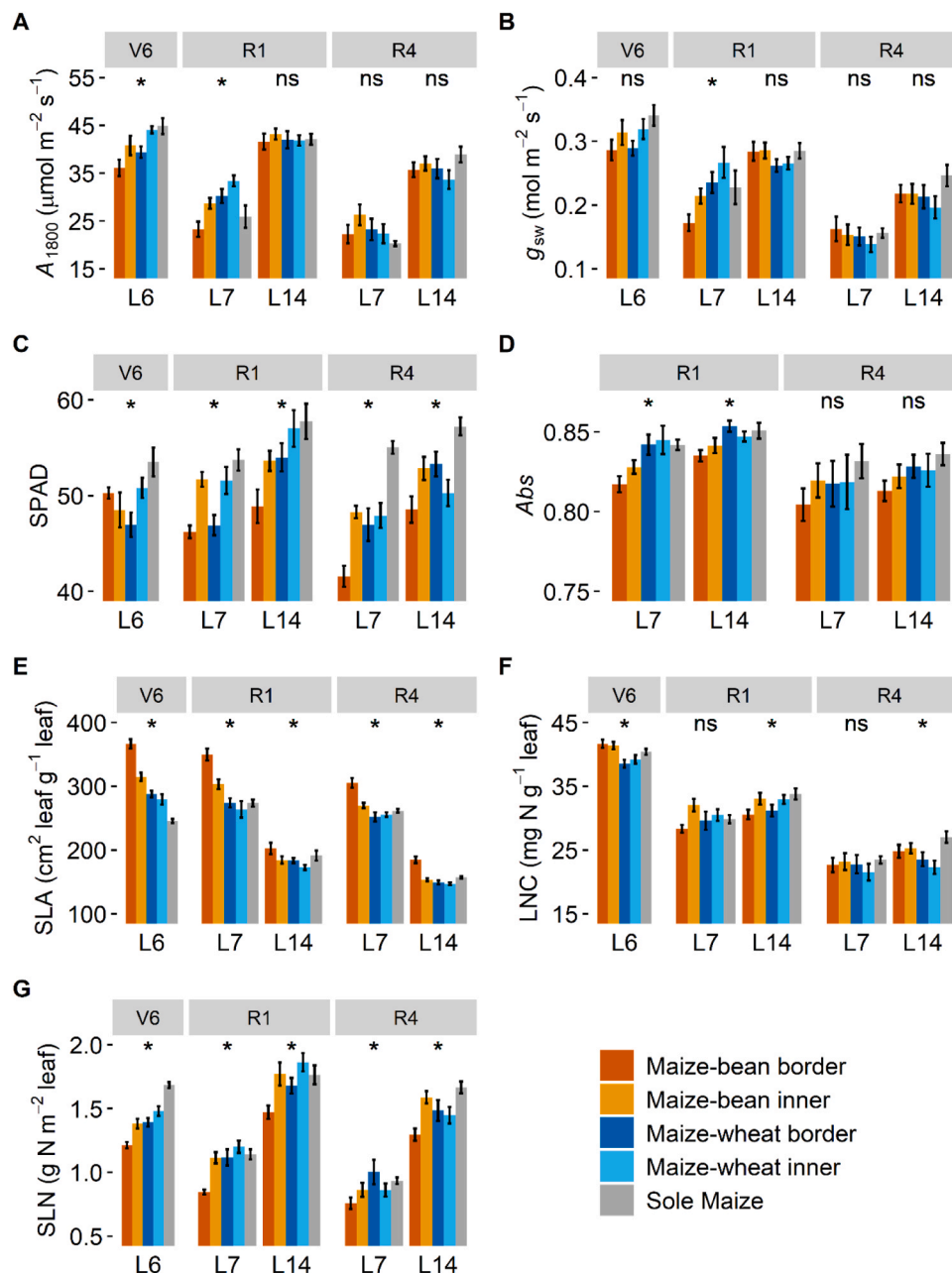


Fig. 5. Leaf traits of maize in different treatments at different growth stages and leaf positions in 2019. L6: leaf 6; L7: leaf 7; L14: leaf 14; A_{1800} : leaf photosynthetic capacity; g_{sw} : stomatal conductance for water; SPAD: a proxy for chlorophyll content; Abs: light absorbance; SLA: specific 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 [Supplementary Table S2](#).

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 A_{1800} of maize leaves were found among treatments (Fig. 5 A), 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. 5 F and G; for substantial correlation between SLN and LNC see Supplementary Fig. S14). Thus, both leaf N and A_{1800} of maize leaves were not increased by intercropping with faba bean or wheat.

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. 6 A), larger SLA (Fig. 6 E), and lower SLN (Fig. 6 G) 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.

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. 7 A, 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. 7 F, 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.

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. 8 A 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. 8 D to F). At R4 in both years, intercropped maize showed a deeper penetration of radiation into the canopy compared to sole maize (Fig. 8 G to K).

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 1).

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. 5 A, 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. 6 A, 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. 5 E). 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. 5 F 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

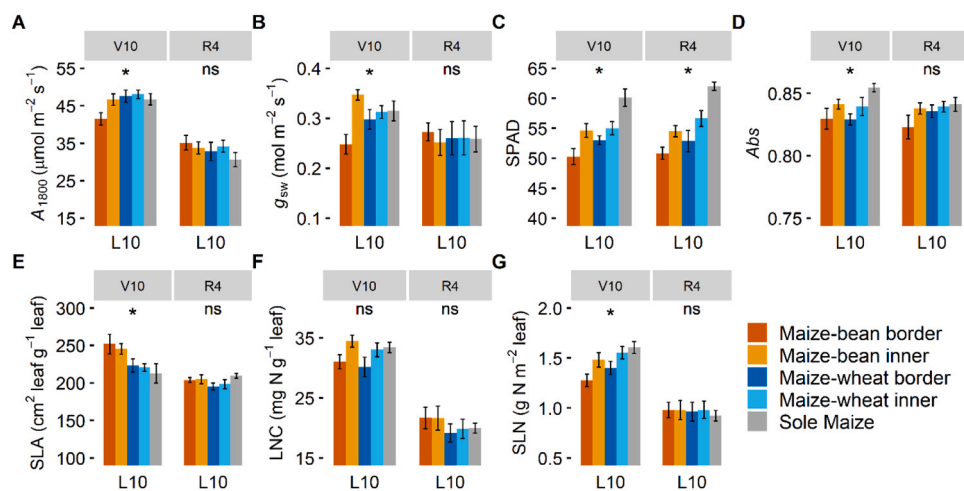


Fig. 6. Leaf traits of maize leaf 10 (L10) in different treatments at maize V10 and R4 stages in 2018. In each stage and leaf position, significance of treatment was determined using ANOVA with treatment as the fixed effect (* = $P < 0.05$; ns = $P > 0.05$). Details showing the pairwise comparison are presented in Supplementary Table S3.

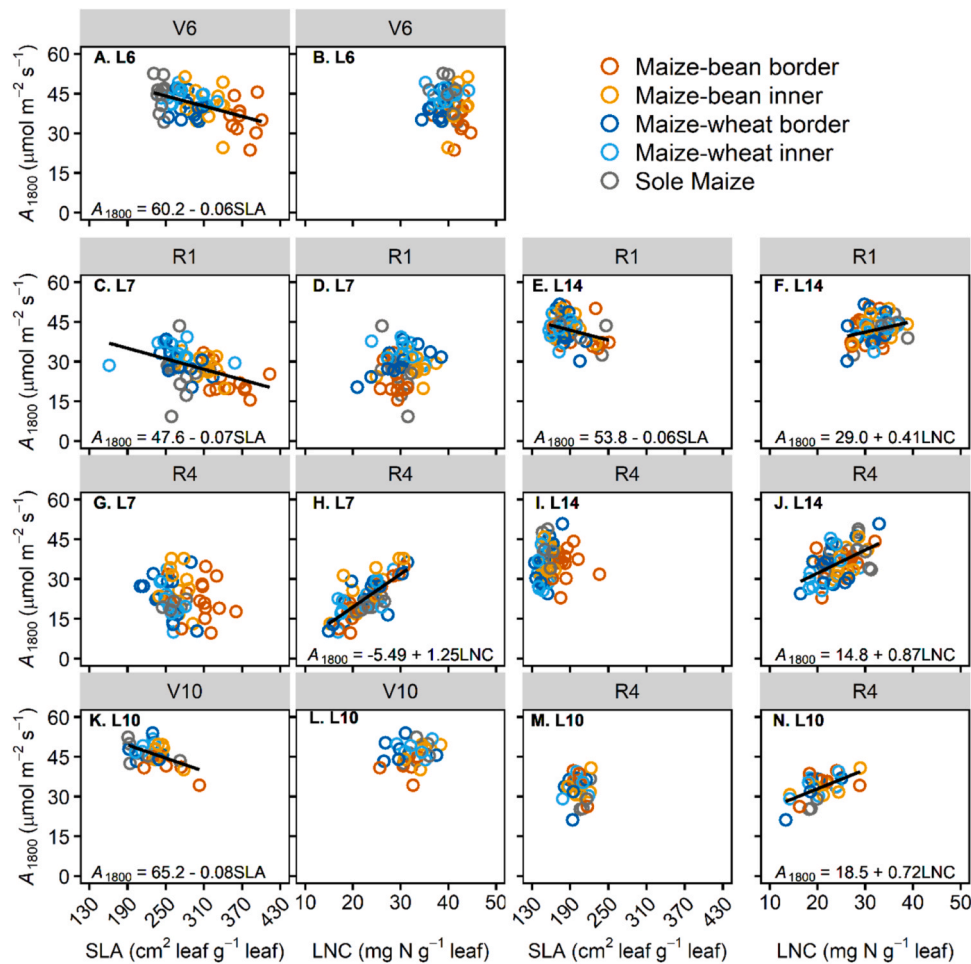


Fig. 7. Relationships between leaf photosynthetic capacity (A_{1800}) and specific leaf area (SLA), and A_{1800} and leaf nitrogen concentration (LNC) in 2019 (A to J) and 2020 (K to N). In each stage and leaf position per year, a linear regression was fit through the combined data from the five treatments. Only the lines for regressions with $P < 0.05$ are presented. Details on coefficients ($\pm$ SE) and P -values of the regressions are presented in [Supplementary Table S4](#).

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. 8), confirming earlier studies in the Netherlands (Gou et al., 2017; Zhu et al., 2014). The maize leaves accordingly showed shade traits during their early growth, such as a large SLA (Figs. 5 and 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. 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 (Supplementary Fig. S17) and the shade cast by faba bean (Fig. 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 absorbance values between treatments was found (Fig. 5 D; Fig. 6 D), 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 $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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,

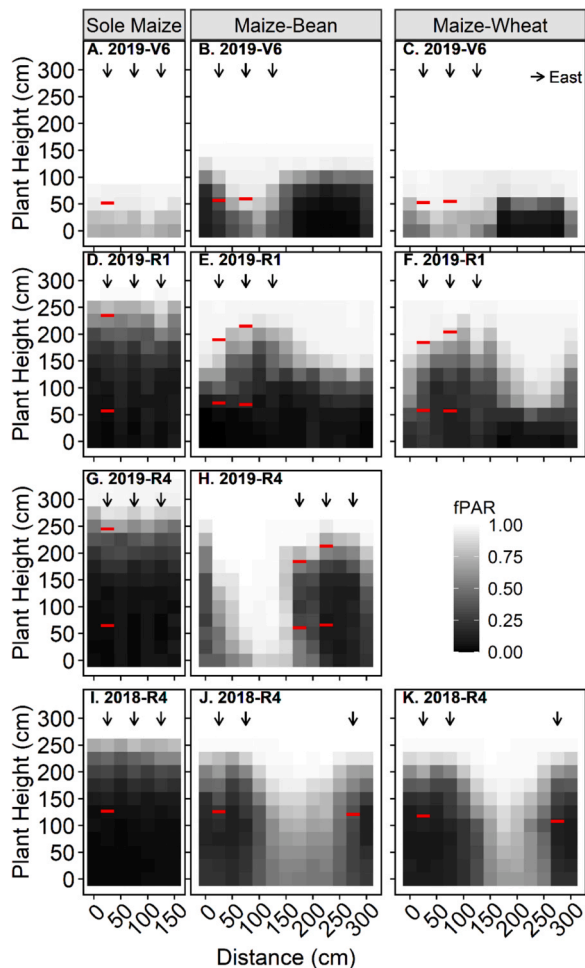


Fig. 8. Light distribution in sole maize, maize-faba bean intercropping, and maize-wheat intercropping at maize V6, R1, and R4 stages in 2019 (A to H), and at maize R4 stage in 2018 (I to K). The arrows indicate the position of maize rows. The red lines indicate the position of photosynthesis measurements. 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 [Supplementary Figs. S6 to S9](#).

Table 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

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 ([Supplementary Fig. S17](#)). 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 thus be reduced, which would then constrain leaf photosynthetic capacity ([Fig. 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., 2011](#)), 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 still 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 variety. 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 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 ([Supplementary Fig. S18](#)). 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., 2017; 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., 2017; Li et al., 2021; Zhu et al., 2015).

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 with 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.

CRediT authorship contribution statement

Bei Dong: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zishen Wang:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Jochem B. Evers:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Tjeerd Jan Stomph:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Peter E.L. van der Putten:** Resources, Methodology, Investigation. **Xinyou Yin:** Writing – review & editing, Conceptualization. **Jin L. Wang:** Writing – review & editing, Investigation. **Timo Sprangers:** Writing – review & editing, Investigation. **Xuebing Hang:** Investigation. **Wopke van der Werf:**

Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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>.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eja.2024.127119](https://doi.org/10.1016/j.eja.2024.127119).

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