Narrow-wide row planting pattern improves the light environment and seed yields of intercrop species in relay intercropping system

Lingyang Feng, Muhammad Ali Raza, Yuankai Chen, Muhammad Hayder Bin Khalid, Tehseen Ahmad Meraj, Faiza Ahsan, Yuanfang Fan, Junbo Du, Xiaoling Wu, Chun Song, Chuanyan Liu, George Bawa, Zhongwei Zhang, Shu Yuan, Feng Yang, Wenyu Yang

1 College of Agronomy, Sichuan Agricultural University, Chengdu, Sichuan, China, 2 Key Laboratory of Crop Eco-physiology and Farming System in Southwest, Ministry of Agriculture, Chengdu, Sichuan, P.R. China, 3 Maize Research Institute, Sichuan Agricultural University, Chengdu, Sichuan, PR China, 4 College of Resources, Sichuan Agricultural University, Chengdu, Sichuan, China

These authors contributed equally to this work.
* f.yang@sicau.edu.cn (FY); mssiyangwy@sicau.edu.cn (WY)

Abstract

Different planting patterns affect the light interception of intercrops under intercropping conditions. Here we revealed that narrow-wide-row relay-intercropping improves the light interception across maize leaves in wide rows (60cm) and narrow rows (40cm), accelerated the biomass production of intercrop-species and compensated the slight maize yield loss by considerably increasing the soybean yield. In a two-year experiment, maize was planted with soybean in different planting patterns (1M1S, 50:50cm and 2M2S, 40:60cm) of relay-intercropping, both planting patterns were compared with sole cropping of maize (M) and soybean (S). As compared to M and 1M1S, 2M2S increased the total light interception of maize leaves in wide rows (WR) by 27% and 23%, 20% and 10%, 16% and 9% which in turn significantly enhanced the photosynthetic rate of WR maize leaves by 7% and 5%, 12% and 9%, and 19% and 4%, at tasseling, grain-filling and maturity stage of maize, respectively. Similarly, the light transmittance at soybean canopy increased by 218%, 160% and 172% at V2, V5 and R1 stage in 2M2S compared with 1M1S. The improved light environment at soybean canopy in 2M2S considerably enhanced the mean biomass accumulation, and allocation to stem and leaves of soybean by 168%, and 131% and 207%, respectively, while it decreased the mean biomass accumulation, and distribution to stem, leaves and seed of maize by 4%, and 4%, 6% and 5%, respectively than 1M1S. Compared to 1M1S, 2M2S also increased the CR values of soybean (by 157%) but decreased the CR values of maize (by 61%). Overall, under 2M2S, relay-cropped maize and soybean produced 94% and 69% of the sole cropping yield, and the 2M2S achieved LER of 1.7 with net income of 1387.7 US $ ha\(^{-1}\) in 2016 and 1434.4 US $ ha\(^{-1}\) in 2017. Our findings implied that selection of optimum planting pattern (2M2S) may increase the light interception and influence the light distribution between maize and soybean rows under relay-intercropping conditions which will
significantly increase the intercrops productivity. Therefore, more attention should be paid to the light environment when considering the sustainability of maize-soybean relay-intercropping via appropriate planting pattern selection.

Introduction

In China, intensive farming has been practiced with high inputs of chemicals, fertilizers, seeds, and irrigation, due to the high food security pressure. This situation has raised serious environmental problems [1], including groundwater pollution by leaching of nitrogen from soil layers [2], acidification of soil [3] and emission of harmful gases to air [4]. The nitrogen loss during maize planting is an especial concern [5]. To guarantee both food production and environmental security, we have to adopt best agronomic practices such as appropriate planting systems which have the ability to use sunlight and land resources efficiently with minimum inputs, for instance, intercropping and relay intercropping systems [6,7]. Relay intercropping is one of the important agronomic practices to increase seed yield [8,9]. However, as compared to intercropping systems, the advantage of relay-intercropping system is higher because in intercropping systems both crops almost have the similar growth periods and they required high amount of inputs to produce higher intercrop yields, whereas under relay intercropping system both crop species have different growth periods and have complementary resource use in time [7,8,10,11]. In addition, for maize soybean relay-intercropping system, the land equivalent ratio (LER, described as the relative farmland that is needed for sole crops to produce similar crop yields as intercrops) often reaches 1.7–1.8 when both crops planted at their optimal planting density, which increases its popularity among farmers, especially among small farmers [10]. However, during the co-growth period per plant growth rates decrease and competition for sunlight, nutrients and land are exacerbated between maize and soybean under maize soybean relay intercropping system [11,12]. The reduced light intensity perceived by soybean plants promoted by maize canopy not only decreased the seed yield [13] but also lower the seed quality of soybean [14].

Planting pattern in intercropping systems changed the micro-climate, especially the light conditions of intercrops [8]. The adjacent growing of crops always cause mutual shading among individual intercropped plants [15]. Previously, it has been reported that upper canopy leaves shade middle strata leaves in maize and mutual shading of leaves reduces the photosynthetic capacity of maize plants which changes the crop morphology, electron transport chain in photosynthetic process and concentrations of enzymes related to carbohydrate assimilation [16,17]. Importantly, the major part of assimilates for seed filling process is obtained from the current carbohydrate production of maize leaves after tasseling, and subsequent translocation to the seeds [18,19]. Similarly, under this system, maize shading significantly affected the light environment of soybean canopy in terms of light quality and quantity [8]. The soybean is extremely sensitive to shading conditions [20] and soybean plants suffer from maize shading during their co-growth period under relay intercropping systems [21]. This shading environment inhibits the leaf growth and enlargement by controlling the cell proliferation of mesophyll cells in soybean [22]. Scientist have also confirmed that the stem diameter, root biomass, and plant biomass decrease under shading conditions that ultimately decrease the seed yield of soybean in relay intercropping system [8,11]. In addition, the shading conditions under relay intercropping systems considerably decrease the rate of sucrose transportation and stem breaking strength of soybean plants [23]. Therefore, by selecting the appropriate genotypes...
and planting pattern we can increase crop yield and quality under prevailing conditions [24,25,26].

In China, maize soybean relay-intercropping system follows the two main planting patterns: (i) modern narrow-wide row relay-intercropping; "40 cm: 60 cm: 40 cm: 60 cm" maize narrow-wide row planting, i.e., relay-intercropping combination of two crop strips with a total width of 200 cm, consisting of two rows of maize and two rows of soybean with 40-cm row width for maize and soybean, and 60-cm spacing between the adjacent rows of maize and soybean), 1M1S (100 cm; with width of one meter strip, i.e., one row of maize and one row of soybean with 50-cm spacing between the adjacent rows of maize and soybean), M (70 cm; with equal row configuration in one strip for maize row to maize row arrangement) and S (50 cm; with equal row configuration in one strip for soybean row to soybean row arrangement), respectively. 2M2S and 1M1S are intercropping system, M and S are sole cropping system of maize and soybean, respectively. The red film placed at the middle of maize and soybean leaves for one day from 6:00 am to 20:00 pm was used for the measurement PPFD as shown in the figure.

https://doi.org/10.1371/journal.pone.0212885.g001

Fig 1. Schematic representation of different maize-soybean planting patterns. A, B, C and D represent the general layout of 2M2S (40 cm + 160 cm; maize narrow-wide row planting, i.e., the relay intercropping combination of two crop strips with a total width of 200 cm, consisting of two rows of maize and two rows of soybean with 40-cm row width for maize and soybean, and 60-cm spacing between the adjacent rows of maize and soybean), 1M1S (100 cm; with width of one meter strip, i.e., one row of maize and one row of soybean with 50-cm spacing between the adjacent rows of maize and soybean), M (70 cm; with equal row configuration in one strip for maize row to maize row arrangement) and S (50 cm; with equal row configuration in one strip for soybean row to soybean row arrangement), respectively. 2M2S and 1M1S are intercropping system, M and S are sole cropping system of maize and soybean, respectively. The red film placed at the middle of maize and soybean leaves for one day from 6:00 am to 20:00 pm was used for the measurement PPFD as shown in the figure.

https://doi.org/10.1371/journal.pone.0212885.g001

and planting pattern we can increase crop yield and quality under prevailing conditions [24,25,26].

In China, maize soybean relay-intercropping system follows the two main planting patterns: (i) modern narrow-wide row relay-intercropping; “40 cm: 60 cm: 40 cm: 60 cm” maize narrow-wide row planting, i.e., relay-intercropping combination of 2 crop strips with a total width of 200 cm, consisting of 2 rows of soybean and 2 rows of maize with 40-cm row width (narrow) for soybean and maize, and 60-cm spacing (wide) between the rows of soybean and maize Fig 1A [7,11,27], and (ii) traditional row relay-intercropping; “50 cm + 50 cm” maize soybean equal row planting, i.e., 1 row of soybean and 1 row of maize with 50-cm spacing between the rows of soybean and maize Fig 1B [21]. Therefore, it is important to investigate the effects of different planting patterns on light interception and distribution in maize and soybean plants under relay intercropping system. In past studies, scientists have mainly focused on the morphological and photosynthetic characteristics of intercrop species under relay intercropping conditions [8,11]. However, no study has been carried out to investigate the light interception and distribution pattern in maize and soybean plants under relay intercropping system. In past studies, scientists have mainly focused on the morphological and photosynthetic characteristics of intercrop species under relay intercropping conditions [8,11]. However, no study has been carried out to investigate the light interception and distribution pattern in maize and soybean plants under relay intercropping system. Thus, a comprehensive study was required to understand the light environment of maize and soybean under the maize soybean relay intercropping system.

Therefore, a two-year field experiment was carried out to investigate the photosynthetically active radiation (PAR) distribution among maize and soybean plants under different planting patterns. Main objectives of the present experiment were (i) to quantify the total interception of PAR at maize plants under different planting patterns; (ii) to analyze how different planting patterns affect the PAR transmittance at soybean canopy; (iii) to investigate how the variations of PAR transmittance affect the biomass accumulation of maize and soybean at different growth stages and grain yields under different planting patterns.
Materials and methods

Ethics statement

No specific permissions were needed for these field experiments. All experiments were performed according to institutional guidelines of Sichuan Agricultural University, China.

Experimental location

The experiments were carried out in 2016 and 2017 at Renshou Research Farm of Sichuan Agricultural University, Sichuan Province, China (N30°16' 4", E104°12'53", altitude 482 m asl). The climate of the study area was humid and subtropical and it has the annual average temperature of 17.4°C, annual average rainfall 1009.4 mm, annual average sunshine 1196.6 h and a frost-free period of 312 days. Weather data during the growing seasons from 2016 to 2017 includes monthly rainfall, average temperature, humidity and wind speed (Table 1). Total rainfall in 2017 was less than 2016, irrigation was applied at the time of soybean sowing and fifth trifoliate stage of soybean. The soil has a purple clay texture with 6.8 pH, 13.6 g kg\(^{-1}\) organic matter, 0.43 g kg\(^{-1}\) total N, 0.36 g kg\(^{-1}\) total P, 7.16 g kg\(^{-1}\) total K, 52.9 mg kg\(^{-1}\) available N, 10.8 mg kg\(^{-1}\) available P, and 107.8 mg kg\(^{-1}\) available K in the 0–20 cm soil layer.

Experimental design and treatments

The Chuandan-418 (semi-compact maize) and Nandou-12 (shade-tolerant soybean) maize and soybean varieties respectively, were selected for the experiments. Different planting patterns described as follows in Fig 1: (2M2S) “40 cm: 60 cm: 40 cm: 60 cm” maize narrow-wide row planting (modern planting pattern generally used for maize and soybean production under relay intercropping), i.e., the relay intercropping combination of two crop strips with a total width of 200 cm, consisting of two rows of maize and two rows of soybean with 40-cm row width for maize and soybean, and 60-cm spacing between the adjacent rows of maize and soybean [8,11,27]; (1M1S) “50 cm: 50 cm” with width of one meter strip (traditional row relay-intercropping), i.e., one row of maize and one row of soybean with 50-cm spacing between the adjacent rows of maize and soybean [21]; (M) “70 cm” with equal row configuration in one strip for maize row to maize row arrangement; (S) “50 cm” with equal row configuration in one strip for soybean row to soybean row arrangement. 2M2S and 1M1S are the intercropping systems, M and S are sole cropping system of maize and soybean, respectively.

Table 1. Monthly rainfall, average temperature, humidity, and wind speed from March to October in the growing seasons of 2016 and 2017.

| Month   | Year   | 2016 | 2017 |
|---------|--------|------|------|
|         | Rainfall (mm) | Average T (˚C) | Humidity (%) | Wind Speed (ms\(^{-1}\)) | Rainfall (mm) | Average T (˚C) | Humidity (%) | Wind Speed (ms\(^{-1}\)) |
| March   | 41.9   | 15.41 | 58.32 | 0.36 | 29.5 | 13.17 | 55.33 | 0.35 |
| April   | 65.2   | 19.33 | 60.21 | 0.43 | 52   | 19.57 | 57.35 | 0.46 |
| May     | 93.3   | 22.71 | 62.35 | 0.51 | 40.7 | 23.57 | 56.32 | 0.52 |
| June    | 125.4  | 26.37 | 65.31 | 0.40 | 55.50| 25.01 | 56.41 | 0.42 |
| July    | 261.1  | 27.63 | 89.44 | 0.77 | 82.30| 29.11 | 62.34 | 0.36 |
| August  | 126.2  | 28.53 | 68.91 | 0.62 | 204.8| 27.77 | 80.13 | 1.21 |
| September | 172.8 | 22.47 | 73.25 | 0.82 | 48.10| 23.57 | 54.39 | 0.47 |
| October | 21.12  | 19.33 | 56.21 | 0.47 | 58.50| 17.61 | 57.82 | 0.42 |
| March-October | 907.02 | 22.72 | 66.75 | 0.55 | 571.40| 22.42 | 60.01 | 0.53 |

https://doi.org/10.1371/journal.pone.0212885.t001

Narrow-wide row planting pattern increases the seed yields of intercrop species in relay strip-intercropping.
The experiments were laid out using a randomized complete block design with three replicates. Every experimental block size was 36 m$^2$ (6 m x 6 m) in the intercropping system, including six rows of maize and six rows of soybean. In sole cropping system, the size of each experimental block was 42 m$^2$ (6 m x 7 m), consisting of 10 rows of maize and 14 rows of soybean in M and S, respectively. The maize crop was sown on 29th of March 2016 and 5th of April 2017 and harvested on 13th of August 2016 and 16th August 2017. Soybean was sown on 19th June 2016 and 18th June 2017 (when was at 12th leaf stage) and harvested on 22nd October 2016 and 23rd October 2017. All plots were treated with basal fertilizer. Basal nitrogen (N) at 45 kg ha$^{-1}$ as urea, phosphorus (P) at 40 kg ha$^{-1}$ as calcium superphosphate, and potassium (K) at 150 kg ha$^{-1}$ as potassium chloride were applied at the time of sowing in intercropped and sole-cropped maize. At the V6 stage of maize, the second dose of N was applied at 150 kg ha$^{-1}$ as urea in all plots. The P at 60 kg ha$^{-1}$ as calcium superphosphate, and K at 60 kg ha$^{-1}$ as potassium chloride sulfate were basally applied for soybean at the time of soybean sowing. Other measures were used according to the farmer’s practices.

**Sampling and measurements**

**Light interception.** For light interception measurement, photosynthetic photon flux density (PPFD) of all maize leaves in wide and narrow rows (from top to bottom) was measured at tasseling stage (TS), grain-filling stage (GFS) and maturation stage (MS). The PPFD of all odd (1, 3, 5, 7, 9, 11 and 13) and all even (2, 4, 6, 8, 10 and 12) number leaves were measured in wide rows (WR; right side) and narrow rows (NR; left side), respectively under 2M2S, 1M1S and M. In addition, PPFD at the top of soybean canopy was also determined at second trifoliolate stage ($V_2$), fifth trifoliolate stage ($V_5$) and flower initiation ($R_1$) stages corresponding to TS, GFS and MS stages of maize, respectively (Fig 2). The measurements were performed following a previously described method [28]. A color acetate film (O-1D, Taisei Chemical Industries, Tokyo, Japan) and Aquation Scientific Equipment (opto leaf, D-Meter RYO-470, Taisei Chemical Industries, Tokyo, Japan) were used to record and read the data. The film located at the middle of maize leaves for 1 day from 6:00 am to 20:00 pm for the collection of data. Six replicates were applied for the experiments and average was calculated. The PPFD was obtained as follows:

\[
PPFD_{total}(mol \ m^{-2}) = 540.6 - 270.3 \times \left[ \log_{10}\left(\frac{D}{D_0}\right) \right] \times 100
\]

Where $D_0$ is the initial light interception and $D$ is the light interception after exposure.

**Chlorophyll content.** Six ear leaves (three from narrow and three from wide rows) of maize plants at TS, GFS and MS, and five fully expanded trifoliolate soybean plants at $V_2$, $V_5$, and $R_1$, were collected from each treatment (Fig 2). The chlorophyll contents including Chl a, Chl b, and the ratio of Chl a/Chl b were extracted from all the leaf samples, and two leaf discs (1.130 cm$^2$) were cut from the middle part of each middle lobules by a puncher (1.2 diameters), and dipped the samples in 10 ml of 80 percent aqueous acetone solution in the dark for 24 h at room temperature [29]. The extraction mixture was then measured at wavelengths of 663, 645 and 470 nm by using a spectrophotometer DU-730 (Beck Man Coulter Inc., USA).

**Photosynthetic parameters.** As described previously, the photosynthetic parameters of maize and soybean, including photosynthetic rate ($Pn$), transpiration rate ($Tr$), stomatal conductance ($Gs$), and intercellular CO$_2$ concentration ($Ci$) were measured using Li-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) under a CO$_2$ concentration of 400 (μmol mol$^{-1}$) [25]. In all treatments, six fully expanded ear leaves (three from narrow and three from wide rows) of maize leaves at TS, GFS, and MS, and five fully expanded soybean...
leaves at V2, V5, and R1 were selected (Fig 2), and the photosynthetic parameters were determined. The data collection of photosynthetic parameters was carried out from 10:00 to 12:00 h.

**Morphological characteristics and leaf area.** Ten maize and soybean plants were selected from each treatment to measure the leaf area at TS, GFS and MS, and V2, V5, and R1, respectively. Importantly, leaf area of all odd (1, 3, 5, 7, 9, 11 and 13) and all even (2, 4, 6, 8, 10 and 12) maize leaves were measured separately in wide rows (WR; right side) and narrow rows (NR; left side), respectively. While plant height and stem diameter of maize and soybean were measured once at MS and R1, respectively and averaged was calculated. The plant height was measured from base to top and vernier caliper was used to measure stem diameter. In addition, the following equation was used to calculate the leaf area of maize and soybean [30]:

\[ A_i \text{ (cm}^2) = k(L \times W) \]

Where, \( A_i \) (cm\(^2\)) represents leaf area of maize and soybean, L (cm) and W (cm) represent the maximum length and width values of maize and soybean leaves, for maize and soybean k was 0.7356 (\( R^2 = 0.9553, p = 0.002 \)) and 0.6903 (\( R^2 = 0.9765, p = 0.001 \)) in 2016 and 0.7298 (\( R^2 = 0.9609, p = 0.001 \)) and 0.6982 (\( R^2 = 0.9732, p = 0.001 \)) in 2017, respectively.

**Biomass accumulation and distribution.** Ten maize (five from narrow and five from wide rows) and soybean plants from each treatment were sampled destructively with at least one meter away from the last sampling at TS, GFS and MS, and V2, V5 and R1, of maize and soybean (Fig 2), respectively for biomass accumulation and distribution among different plant
parts. Then all the sampled plants were divided into different plant parts of maize (leaves, stem and seed) and soybean (stem and leaves), and placed in oven for one hour at 65˚C to kill the fresh-tissues and then dried at 80˚C to obtain constant weight before weighing of each plant part of maize and soybean for total biomass accumulation (g plant\(^{-1}\)) and distribution analysis.

**Grain yield, land equivalent ratio and competition ratio.** Furthermore, thirty-six ears (18 ears from narrow rows and 18 ears from wide rows) and forty soybean plants were sampled from the middle rows of each treatment at maturity. These samples were used to analyze the grain yield of maize and soybean. All the harvested sampled ears and soybean plants were sun-dried for six days, dried ears and pods were threshed by hand and weighed to measure the grain yield of every treatment and then converted into kg ha\(^{-1}\). Land equivalent ratio (LER) was also calculated by using the following equations \[31,32\].

\[
LER_m = \frac{LER_m}{LER_s}
\]

\[
LER = LER_m + LER_s
\]

\[Y_{sm} \text{ and } Y_{im}\] are maize yields (kg ha\(^{-1}\)) of sole cropping and intercropping system, respectively. \[Y_{ss}\] and \[Y_{is}\] are soybean yields (kg ha\(^{-1}\)) of sole cropping and intercropping system, respectively. LER\(_m\) and LER\(_s\) are the partial land equivalent ratio of maize and soybean, respectively. LER more than 1 means that production in intercropping system is higher as compared to sole cropping system of its component species \[31\]. In addition, competition ratio (CR) is another parameter to investigate the competition between two crop species. The CR is determined by using the following formula:

\[
CR_m = \frac{LER_m}{LER_s} \times \frac{Z_{sr}}{Z_{mr}}
\]

\[
CR_s = \frac{LER_s}{LER_m} \times \frac{Z_{mr}}{Z_{sr}}
\]

Where LER\(_m\) and LER\(_s\) are the land equivalent ratio of maize and soybean respectively. \[Z_{sr}\] and \[Z_{mr}\] are the ratios of the area occupied by soybean and maize under the relay intercropping system relative to that of the corresponding monoculture, respectively (in this study, ratios of the area occupied by soybean and maize were the same) \[32\]. When the value of CR\(_m\) and CR\(_s\) is higher than ‘one’ suggested the competitive ability of maize and soybean greater than soybean and maize, respectively.

**Economic analysis.** To evaluate the economics of different planting patterns, an economic-analysis was conducted. Total expenditure for intercrops (maize and soybean) production was included farm-land rent, preparation of seedbed, seed and fertilizer cost of both intercrops (N, P and K), hand-weeding and thinning, harvesting and threshing of maize and soybean crops. Total income was calculated according to the local market price for maize and soybean at Chengdu in P. R. China in 2016 and 2017. Additionally, net income (NI) was measured by subtracting the total expenditure from total income and benefit to cost ratio (BCR) was assessed as the ratio of total income to total expenditure \[25\].

**Data analysis.** All parameters of planting models on the light environment, photosynthesis, chlorophyll content, biomass, and grain yield were analyzed using SPSS v17.0. Origin Pro 9.1 and Microsoft Excel program was employed to the graphical presentation of data. The least
significance difference (LSD) test was used to compare the means at one percent or five percent probability level.

**Results**

**Light interception.**  Fig 3A and 3C show the photosynthetic photon flux density (PPFD) at maize leaves at TS, GFS and MS under 2M2S, 1M1S and M. The different planting patterns treatments significantly ($P < 0.05$) affected the PPFD of maize leaves in wide rows (WR) and narrow rows (NR) at TS, GFS, and MS in both years. The mean highest PPFD of maize leaves was recorded in WR (right side; all odd leaves), and lowest PPFD of maize leaves was observed in NR (left side; all even leaves) at TS, GFS and MS, respectively for both years. Overall, the mean PPFD of maize leaves in WR under 2M2S was increased by 23, 10 and 9% in 1M1S and 27, 20 and 16% in M. In addition, we also calculated the total PPFD of whole maize plant (PPFD of odd leaves + PPFD of even leaves) and planting pattern 2M2S significantly increased the PPFD of whole maize plant at TS and MS by 5 and 7%, and 10 and 14% (Table 2), compared to 1M1S and M, suggesting that extra PPFD at odd leaves of maize in wide rows under 2M2S compensated the reduced PPFD effect at even leaves in narrow rows.

The different planting treatments considerably ($P < 0.05$) changed the PPFD at soybean canopy in both two years (Fig 3B and 3D). However, the PPFD at soybean canopy in sole cropping system was always found higher than those under 2M2S and 1M1S at all sampling stages ($V_2$, $V_5$, and $R_1$). In relay intercropping patterns, the average maximum and minimum PPFD at the top of soybean canopy were 153.0 and 48.2 mol m$^{-2}$ at $V_2$, 113.3 and 43 mol m$^{-2}$ at $V_5$, and 229.2 and 84.3 mol m$^{-2}$ at $R_1$ under treatments 2M2S and 1M1S, respectively (Table 2).

**Chlorophyll content.** In this study, different planting treatments considerably changed the contents of chlorophyll a and b in maize and soybean (Fig 4A and 4B). The Chl a content

---

**Fig 3.** The photosynthetic photon flux density (PPFD) of relay-intercropped maize (a, c) and soybean (b, d) at tasseling, grain filling and maturity stage of maize corresponding to second trifoliate ($V_2$), fifth trifoliate ($V_5$) and flower initiation ($R_1$) stage of soybean, respectively as affected by different planting pattern from 2016 to 2017. The 2M2S (40 cm + 160 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. The WR and NR wide rows (WR; right side) and narrow rows (NR; left side), respectively. Means are averaged over three replicates. Bars show ± standard errors, ($n = 3$). Within a bar, different lowercase and same letters show a significant and non-significant difference ($P < 0.05$) between treatments.

https://doi.org/10.1371/journal.pone.0212885.g003
was significantly ($P < 0.05$) higher in maize under 2M2S at TS and MS as compared to 1M1S and M, while at GFS it was found maximum in 1M1S than 2M2S and M (Fig 4A). In addition, the Chl a and Chl b in soybean leaves under treatment S were increased significantly compared to those under 2M2S and 1M1S at V$_2$, V$_5$, and R$_1$ stages of soybean (Fig 4B). Compared with 1M1S, the Chl a and Chl b contents were increased considerably by 81 and 106%, 75 and 27%, and 44 and 57% at V$_2$, V$_5$, and R$_1$ under 2M2S, respectively (Fig 4B).

**Photosynthetic rate.** The photosynthetic characteristics of maize plants at TS, GFS, and MS under 2M2S, 1M1S and M are presented in Table 3. In our field experiment, different planting treatments significantly affected the photosynthetic rate of maize and soybean plants ($P < 0.05$). The mean maximum $P_n$ of maize ear leaves was recorded in WR (right side) under 2M2S and in NR (left side) was measured in 1M1S at TS, GFS, and MS, respectively. The mean minimum $P_n$ in WR, and in NR was noticed in M and 2M2S, respectively. Importantly,
treatment 2M2S increased the Pn of maize ear leaves by 4 and 19% in WR and 21 and 40% in NR at MS than 1M1S and M (Table 3). The consistent pattern was observed for Pn of maize plants for 2016 and 2017 in WR and NR under different planting treatments. In addition, the values of Tr and Gs were found higher in wide rows than narrow rows under 2M2S as compared to 1M1S and M treatments for both years, while the Ci values were represented the opposite trend.

The values of Pn, Gs, and Tr of soybean plants increased with the increase in PPFD at soybean canopy, and average values of Pn, Gs and Tr in treatment S at V2, V5 and R1 were found significantly higher than those under 2M2S and 1M1S for both years. However, planting pattern 2M2S significantly increased the mean Pn (by 64, 126 and 30%), Gs (by 100, 48 and 119%) and Tr (by 23, 15 and 12%) at V2, V5 and R1, respectively than 1M1S. Whereas, the maximum Ci was found in 1M1S and S at V2, and V5 and R1, respectively (Table 3).

**Morphological parameters and leaf area.** In this study, different planting treatments significantly (P < 0.05) affected the morphological parameters and leaf area of maize (WR and

---

**Table 3. Photosynthetic parameters of relay-intercropped maize and soybean as affected by different planting pattern from 2016 to 2017.**

| Years | Stages | Treatments | Photosynthetic Rate (μmol CO2 m⁻² s⁻¹) | Stomatal Conductance (mol H₂O m⁻² s⁻¹) | Transpiration Rate (mmol H₂O m⁻² s⁻¹) | Intercellular CO₂ Concentration (μmol CO₂ m⁻² s⁻¹) |
|-------|--------|------------|------------------------------------------|------------------------------------------|------------------------------------------|---------------------------------------------|
|       |        |            | WR | NR | WR | NR | WR | NR | WR | NR | WR | NR | WR | NR |
| 2016  | TS—V1  | 2M2S       | 28.8a | 28.1b | 9.7b | 0.32a | 0.29b | 0.14a | 2.93a | 2.89a | 3.3b | 211.9b | 216.5b | 252.2b |
|       |        | 1M1S       | 28.5a | 28.7a | 5.5c | 0.3a | 0.31a | 0.08b | 2.91a | 2.91a | 2.5c | 222.3b | 229.2a | 271.4a |
|       |        | M          | 27.9b | 28.2b | -    | 0.26b | 0.27b | -    | 2.87b | 2.83b | -    | 248.5a | 236.4a | -    |
|       |        | S          | -    | -    | 13.4a | -    | 0.16a | -    | -    | 5.3a | -    | -    | 205.2c | -    |
|       | GFS—V5 | 2M2S       | 26.8a | 21.3c | 11.6b | 0.21a | 0.11b | 0.86b | 3.51a | 1.98c | 3.9b | 110.2b | 176.3a | 293.6c |
|       |        | 1M1S       | 24.2b | 24.5a | 5.6c | 0.17b | 0.15a | 0.59c | 3.26a | 3.19a | 3.5c | 145.6a | 142.8b | 312.5a |
|       |        | M          | 23.6c | 23.5b | -    | 0.14b | 0.14a | -    | 2.69b | 2.64b | -    | 153.2a | 156.8a | -    |
|       |        | S          | -    | -    | 14.8a | -    | 0.76a | -    | -    | 3.9a | -    | -    | 305.6b | -    |
|       | MS—R1  | 2M2S       | 19.3a | 22.8a | 12.9b | 0.19a | 0.20a | 0.63b | 2.88a | 2.92a | 3.8a | 121.6c | 119.8b | 306.8a |
|       |        | 1M1S       | 18.6a | 18.5b | 8.8c | 0.17a | 0.15b | 0.43c | 2.76b | 2.68b | 3.4b | 133.5b | 135.2b | 290.1b |
|       |        | M          | 16.8b | 17.1c | -    | 0.13b | 0.13b | -    | 2.63c | 2.58b | -    | 221.4a | 226.5a | -    |
|       |        | S          | -    | -    | 14.5a | -    | -    | -    | -    | 3.9a | -    | -    | 309.7a | -    |
| 2017  | TS—V1  | 2M2S       | 26.6a | 22.3c | 9.3b | 0.21a | 0.19b | 0.24b | 2.69b | 2.66b | 4.1b | 278.6a | 289.4a | 313.6b |
|       |        | 1M1S       | 24.1b | 24.4a | 6.1c | 0.21a | 0.22b | 0.11c | 2.72a | 2.73a | 3.5c | 266.4b | 251.3b | 328.1a |
|       |        | M          | 23.8c | 23.8b | -    | 0.23a | 0.24a | -    | 2.69b | 2.68b | -    | 276.4a | 273.5a | -    |
|       |        | S          | -    | -    | 13.1a | -    | -    | -    | -    | 3.8a | -    | -    | 296.9c | -    |
|       | GFS—V5 | 2M2S       | 24.3a | 20.8b | 12.1b | 0.18a | 0.09b | 0.93a | 3.22a | 1.67b | 4.5b | 148.5b | 183.4a | 318.9b |
|       |        | 1M1S       | 22.6b | 22.4a | 4.9c | 0.13b | 0.13a | 0.62b | 2.75b | 2.73a | 3.8c | 162.8a | 163.7b | 333.2a |
|       |        | M          | 22.1b | 22.1a | -    | 0.13b | 0.12a | -    | 2.69b | 2.68a | -    | 163.1a | 168.9b | -    |
|       |        | S          | -    | -    | 16.3a | -    | 1.03a | -    | -    | 5.2a | -    | -    | 320.5b | -    |
|       | MS—R1  | 2M2S       | 17.8a | 21.3a | 12.3b | 0.15a | 0.16a | 0.86a | 2.84a | 2.84a | 3.5b | 158.2b | 143.8b | 286.9a |
|       |        | 1M1S       | 17.1a | 17.8b | 10.6c | 0.14a | 0.13b | 0.25b | 2.65b | 2.66b | 3.2c | 162.8b | 163.7b | 273.3b |
|       |        | M          | 14.5b | 14.4c | -    | 0.11b | 0.11b | -    | 2.45c | 2.38c | -    | 256.8a | 249.7a | -    |
|       |        | S          | -    | -    | 15.7a | -    | 0.93a | -    | -    | 3.9a | -    | -    | 292.5a | -    |

TS, GFS and MS refers to tasseling, grain filling and maturity stage of maize corresponding to second trifoliate (V2), fifth trifoliate (V5) and flower initiation (R1) stage of soybean, respectively. The 2M2S (40 cm: 60 cm: 40 cm: 60 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. The WR and NR wide rows (WR; right side) and narrow rows (NR; left side), respectively. Means are averaged over three replicates. Different lowercase letters in the same line are significantly different at 0.05 probability level.

https://doi.org/10.1371/journal.pone.0212885.t003
NR of leaves) and soybean under 2M2S, 1M1S and M. During both study years, the plant height of maize plants in M were significantly higher than 2M2S and 1M1S (Table 4). However, the mean maximum stem diameter (26.1 cm) of maize plants was noticed under 1M1S as compared to 2M1S and M (Table 4). In addition, at MS, the mean maximum leaf area in WR and NR was recorded under 2M2S, whereas mean minimum leaf area was measured in M (Fig 5A–5C). Importantly, maize plants under 2M2S displayed the longer duration of green leaf area than M. For example, narrow-wide row planting pattern (2M2S) led to an increase in leaf

Table 4. Plant height, stem diameter and total biomass of relay-intercropped maize and soybean as affected by different planting pattern from 2016 to 2017.

| Years | Treatments | Maize | Soybean |
|-------|------------|-------|---------|
|       |            | PH (cm) | SD (cm) | Total Biomass (g plant⁻¹) | PH (cm) | SD (cm) | Total Biomass (g plant⁻¹) |
|       |            | TS | GFS | MS | TS | GFS | MS |
| 2016  | 2M2S       | 262.8b | 24.2b | 127.8b | 142.3c | 89.6b | 8.6b | 0.26b | 3.57b | 17.90b |
|       | 1M1S       | 260.9bc | 25.9a | 131.5a | 145.9a | 110.3a | 5.2c | 0.20c | 0.93c | 7.68c |
|       | M          | 273.1a | 24.8b | 130.0a | 143.8b | - | - | - | - | - |
|       | S          | 272.8a | 25.6b | 127.5a | 135.1a | 250.2a | - | - | - | - |
| 2017  | 2M2S       | 260.9b | 23.8c | 124.5b | 130.9b | 240.6b | 91.2b | 8.3b | 0.27b | 0.86b | 17.52b |
|       | 1M1S       | 259.6b | 26.2a | 127.3a | 135.7a | 251.4a | 106.8a | 5.4c | 0.21c | 0.86c | 5.52c |
|       | M          | 272.8a | 25.6b | 127.5a | 135.1a | 250.2a | - | - | - | - |
|       | S          | 272.8a | 25.6b | 127.5a | 135.1a | 250.2a | - | - | - | - |

TS, GFS and MS refers to tasseling, grain filling and maturity stage of maize corresponding to second trifoliate (V₂), fifth trifoliate (V₅) and flower initiation (R₁) stage of soybean, respectively. The 2M2S (40 cm: 60 cm: 40 cm: 60 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. Means are averaged over three replicates. Different lowercase letters in the same line are significantly different at 0.05 probability level.

https://doi.org/10.1371/journal.pone.0212885.t004

Fig 5. The leaf area of relay-intercropped maize (a, c) and soybean (b, d) at tasseling, grain filling and maturity stage of maize corresponding to second trifoliate (V₂), fifth trifoliate (V₅) and flower initiation (R₁) stage of soybean, respectively as affected by different planting pattern from 2016 to 2017. The 2M2S (40 cm + 160 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. The WR and NR refer wide rows (WR; right side) and narrow rows (NR; left side), respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase and same letters show a significant and non-significant difference (P < 0.05) between treatments.

https://doi.org/10.1371/journal.pone.0212885.g005
area at MS by 4% in WR and 4% in NR, indicating that leaf senescence in 2M2S was delayed (Fig 2).

All the planting treatments significantly (P < 0.05) affected the plant height (Table 4), stem diameter (Table 4) and leaf area (Fig 5B–5D) of soybean in both years. The mean maximum stem diameter and leaf area were observed in sole cropping of soybean. Whereas, the average highest plant height at all measured stages was recorded under 1M1S than 2M2S and S. However, treatment 2M2S increased the leaf area by 44, 72 and 68% at V2, V5, and R1, respectively (Fig 5B–5D), and stem diameter by 59% (Table 4) compared to 1M1S at R1.

**Biomass accumulation and distribution.** The different planting treatments significantly (P < 0.05) affected the total biomass accumulation (g plant⁻¹) in maize and soybean at all measured stages (Table 4). For maize plant, the mean highest biomass was found in 1M1S which was statically at par with M, while mean lowest biomass was recorded in 2M2S at all stages (TS, GFS and MS). For soybean, at R1, the mean maximum biomass 33.8 g plant⁻¹ was noted under treatment S, while mean minimum biomass 6.6 g plant⁻¹ was recorded under planting pattern treatment 1M1S, respectively. Furthermore, different planting treatments significantly changed the pattern of biomass distribution among different plant organs of maize and soybean (Fig 6). For maize, at TS and GFS, the maximum biomass allocation was observed in stem but after that (at MS) the highest biomass allocation was measured in seed followed by stem and leaves under 1M1S and M. On average, planting treatment 1M1S increased the seed biomass at MS by 8 and 5% in 2016 and 2017, respectively compared to the 2M2S (Fig 6A–6C). In addition, during all the measured stages, under S and 2M2S, highest distribution of

---

**Fig 6.** The biomass distribution of relay-intercropped maize (a, c) and soybean (b, d) at tasseling, grain filling and maturity stage of maize corresponding to second trifoliate (V₂), fifth trifoliate (V₅) and flower initiation (R₁) stage of soybean, respectively as affected by different planting pattern from 2016 to 2017. The 2M2S (40 cm + 160 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. Means are averaged over three replicates. Bars show ± standard errors, (n = 3). Within a bar, different lowercase and same letters show a significant and non-significant difference (P < 0.05) between treatments.

[https://doi.org/10.1371/journal.pone.0212885.g006](https://doi.org/10.1371/journal.pone.0212885.g006)
biomass was recorded in leaves than stem, while in 1M1S maximum biomass distribution was noted in stem than leaves for both experimental years. Importantly, between 1M1S and 2M2S planting pattern treatments, the mean maximum leaf (9.93 g plant⁻¹) and stem biomass (7.77 g plant⁻¹) of soybean were found in 2M2S, while mean minimum (3.24 g plant⁻¹) and stem biomass (3.36 g plant⁻¹) of soybean were noticed at R₁ under 1M1S (Fig 6B–6D).

Grain yield, land equivalent ratio and competition ratio. Table 5 showed the seed yields, land equivalent ratio (LER) and competition ratio (CR) of relay-intercropped maize and soybean as affected by different planting pattern from 2016 to 2017.

Table 5. Seed yield (kg ha⁻¹), land equivalent ratio (LER) and competition ratio (CR) of relay-intercropped maize and soybean as affected by different planting pattern from 2016 to 2017.

| Years | Treatments | Maize | Soybean |
|-------|------------|-------|---------|
|       | Seed Yield | LERₘ | CRₘ | Seed Yield | LERₛ | CRₛ |
| 2016  | 2M2S       | 8472.3b | 0.92b | 1.25b | 1642.1b | 0.74a | 0.80a | 1.67a |
|       | 1M1S       | 9114.6a | 0.99a | 3.21a | 686.6c  | 0.31b | 0.31b | 1.30b |
|       | M          | 9168.7a | -    | -    | -       | -    | -    | -    |
|       | S          | -      | -    | -    | 2214.5a | -    | -    | -    |
| 2017  | 2M2S       | 8550.2b | 0.95b | 1.22b | 1702.2b | 0.78a | 0.80a | 1.73a |
|       | 1M1S       | 8952.5a | 1.00a | 3.07a | 707.1c  | 0.32b | 0.31b | 1.32b |
|       | M          | 8990.5a | -    | -    | -       | -    | -    | -    |
|       | S          | -      | -    | -    | 2181.8a | -    | -    | -    |

The LERₘ and CRₘ, and LERₛ and CRₛ represent the land equivalent ratio and competition ratio of maize and soybean, respectively. The 2M2S (40 cm: 60 cm: 40 cm: 60 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively. Means are averaged over three replicates. Different lowercase letters in the same line are significantly different at 0.05 probability level.

Grain yield, land equivalent ratio and competition ratio. Table 5 showed the seed yields, land equivalent ratio (LER) and competition ratio (CR) of maize and soybean under different planting treatments. The planting treatments significantly (P < 0.05) affected the maize and soybean seed yields, and LER under 2M2S, 1M1S. Higher seed yield of soybean (1642.1 in 2016 and 1702.2 kg ha⁻¹ in 2017) was measured in 2M2S as compared to 1M1S (686.6 in 2016 and 707.1 kg ha⁻¹ in 2017). On average, LER of 2M2S was increased by 27% and 31% in 2016 and 2017, respectively in comparison with 1M1S. Furthermore, the average maximum CRₘ and CRₛ values of maize and soybean were found in 1M1S and 2M2S, respectively, while minimum CRₘ and CRₛ values of maize and soybean were calculated under 2M2S and 1M1S, respectively in both study years.

Economic analysis. Results of the economic analysis are shown in Table 6. In this study, among different planting pattern treatments, 2M2S gave the highest net income (NI) (1387.7 US $ ha⁻¹ for 2016 and 1434.4 US $ ha⁻¹ for 2017), while average lowest NI (217.8 US $ ha⁻¹ for 2016 and 188.7 US $ ha⁻¹ for 2017) was obtained in S treatment. However, the mean maximum and minimum benefit to cost ratio (BCR) was measured with treatment M and 1M1S, respectively.

Table 6. Economic analysis (US $ ha⁻¹) for the effect of different planting patterns on maize and soybean performance pattern from 2016 to 2017.

| Treatments | Total Expenses | Gross Income | Net Income | Benefit-Cost Ratio |
|------------|----------------|--------------|------------|--------------------|
|            | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| 2M2S       | 3339.9 | 3141.5 | 4482.4 | 4575.8 | 1341.0 | 1434.4 | 1.4 | 1.5 |
| 1M1S       | 3339.9 | 3141.5 | 3570.1 | 3544.5 | 428.6 | 403.1 | 1.1 | 1.1 |
| M          | 1843.1 | 1733.6 | 2790.5 | 2736.2 | 1056.9 | 1002.6 | 1.6 | 1.6 |
| S          | 1815.7 | 1707.8 | 1925.7 | 1896.5 | 217.8 | 188.7 | 1.1 | 1.1 |

The 2M2S (40 cm: 60 cm: 40 cm: 60 cm) and 1M1S (50 cm: 50 cm). The SM and SS refer to sole cropping system of maize and soybean, respectively.
respectively during both years. Overall, relay intercropping of maize and soybean with narrow wide row planting arrangement (2M2S) had 233% higher NI as compared to 1M1S.

Discussion

Effect of different planting treatments on light interception

The crop competition for sunlight is investigated in several studies about intercropping, for maize [33] and other annual crop species, such as soybean [8] and wheat [34,35]. In intercropping system, the planting system, row arrangement and spacing, and crop architecture can reduce the negative effects of taller crop shade on the middle strata leaves within the rows and between the rows. In our experiment, different planting systems considerably changed the light interception at maize leaves and soybean canopy, maximum light interception at odd and even leaves were observed in 2M2S and 1M1S, respectively (Fig 3). We noticed in another study of maize and soybean relay intercropping system that light interception and utilization was increased in 2M2S than 1M1S and S [12]. Furthermore, we observed, as Liu et al., (2017) did, the planting system 2M2S is favorable for higher maize and soybean seed yields because the increasing distance between maize strip was more advantageous to improve light interception at odd leaves of maize and soybean canopy, and past studies reported similar results [12,36,37]. Overall, wide rows in 2M2S increased the light interception at soybean canopy and compensate the decreased light intensity effect at even leaves of maize in narrow rows.

Effect of different planting treatments on morphological parameters

Variations in light quantity can initiate crop morphological responses [38]. Generally, shading conditions under 2M2S and 1M1S significantly increased the plant height at the expense of leaves but it reduced the crop productivity [39,40]. Similarly, stem diameter of crops also reduced under low light conditions [41,42]. In our current experiment, minimum plant height, and maximum stem diameter and leaf area of soybean was observed in S (Table 4). However, between 2M2S and 1M1S, the higher leaf area of soybean plants was noticed under 2M2S as compared to 1M1S but the opposite results were found for maize leaf area (Fig 5). Furthermore, the leaf area of soybean is inversely proportional to shading [40], a decrease in light intensity reduces the leaf area of soybean by controlling the leaf proliferation under maize soybean relay intercropping system [22]. But narrow-wide row planting system significantly increased the leaf area of maize and delayed the leaf senescence process by increasing the leaf area at maturity (by 4% in WR and 4% in NR) in 2M2S. Therefore, our findings indicate that relay intercropping of maize and soybean in severe shading conditions under 1M1S probably promoted the stem elongation to obtain high amounts of light at the expense of leaf growth, which eventually reduced the crop growth and development of intercrop species. However, by using the narrow-wide row planting pattern (2M2S) we can grow soybean plants with higher stem diameter and leaf area, and it will be more beneficial to the initial growth of soybean plants under intercropping systems. Because increasing distance between maize and soybean rows under 2M2S reduced the maize shade, increased the light transmittance on soybean canopy, and decreased competition for land and water resources which eventually improved the maize and soybean growth.

Effect of different planting treatments on photosynthetic characteristics

Under shading conditions, the investigation of chlorophyll content helps as an index for light absorption [21]. Several studies have documented that Chl a and Chl b contents decrease with the increase in shade [40,43]. On the other hand, other studies have argued that chlorophyll
contents increase as shading density increases, especially Chl b content [16]. Our results showed that with the increase in the shade (1M1S) the Chl a and b contents were increased in maize leaves as compared to 2M2S and M in both years. In addition, the narrow-wide row planting treatments (2M2S) significantly increased the chlorophyll content of soybean leaves than 1M1S. This increase in chlorophyll content might be linked with the improved light environment and growing conditions for soybean plants under 2M2S (Fig 4B).

Plant leaves are responsive to the light conditions, and shading reduces photosynthetic capacity of crops [40,44]. This environment was consistent with our findings, which demonstrated that increasing maize narrow row distance (decreasing mutual-shading of leaves) enhanced ear-leaf photosynthesis at TS and GFS in 1M1S (Table 3) as compared to 2M2S and consequently significantly increased maize yield (Table 5). However, the net photosynthetic rate in wide rows under 2M2S was higher than those in 1M1S and M, which was the supplement (photosynthetic rate) for maize narrow row leaves. This increase in the photosynthetic rate of ear leaves in wide rows due to the improved light interception, leaf area and growing space for maize in 2M2S. Additionally, maize is a C₄ and cereal crop that possess high photosynthetic and carbon gain activities [45]. By contrast, shading by relay-intercropped maize decreased the photosynthetic rate of soybean by reducing leaf area (Fig 5). The shading of relay-intercropped soybean became serious when the maize narrow row distance increased (1M1S, 50 cm: 50 cm). Whereas, the planting system 2M2S significantly improves the transmitted light at soybean canopy than 1M1S (Table 2). Therefore, these results indicated that the narrow wide planting pattern (2M2S) exhibited a higher photosynthetic rate of soybean than equal row planting system (1M1S) which increased the dry matter production and final seed yield of soybean plants by maintaining optimum maize yield.

Effect of different planting treatments on intercrop yields, LER and CR

The remarkable increase in intercrop maize and soybean has been attributed mainly to the high use of inputs, which makes plant to use and intercept sunlight more efficiently [33]. By managing the planting density, row arrangement, and spacing, we can increase crop yield in relay intercropping system [11]. In this experiment, significant differences were noted in the biomass accumulation (Fig 6) and seed yield (Table 5) of maize and soybean in 2M2S, 1M1S, M, and S for both years in field conditions. These variations in biomass accumulation and yield are likely due to the differences in light interception and planting arrangements. Moreover, mutual shading of intercrop crops considerably changed the light interception [46] and any change in light interception directly affect the photosynthetic capacity (leaf area) of crops [40]. The narrow-wide row planting arrangement of maize and soybean under relay intercropping condition substantially increased the soybean yield as compared to equal row planting arrangement which was might be due to the higher light transmission at soybean canopy, improved leaf area and enhanced photosynthetic rate of soybean canopy in wide rows especially during the co-growth period because initial growth and development of crops is very important to obtain higher seed yield [25]. Furthermore, the decreased seed yield of individual intercrop can be counterbalanced by an increase in total grain seed yield on an annual basis [47]. For example, reducing 5.78% seed yield of relay-intercropped maize from 1M1S to 2M2S treatment increased the relay-intercropped soybean seed yield by 140% (Table 4).

Total LER values were always higher than one in both relay intercropping systems (Table 5), which exhibits the yield benefit of the relay-intercropping system over sole cropping systems (M and S) due to the better utilization of land and environmental resources for crops growth and development [27]. Particularly, the mean values of LER under 2M2S was 1.7, which means that 70% extra farmland will be needed by the sole cropping of maize and
soybean to equal the seed yields of relay-intercropping systems, showing intercrops advantage of using resources as compared with sole crops [11]. Similarly, Liu et al., (2017) reported higher LER values 1.3–1.4 in narrow wide row intercropping system of maize and soybean [13], which suggesting that increasing the distance between maize and soybean rows (40 cm: 60 cm: 40 cm: 60 cm) under relay intercropping system improved the growing conditions (light environment) and decreased the competition especially for nutrients [48]. In addition, less distance (52 cm) between maize and soybean rows negatively affected the light interception at soybean canopy [8,49,50], therefore it is an effective method to ameliorate the negative effects of maize shade on soybean in maize soybean relay intercropping system, which resulted in higher intercrop seed yields and LER under relay-intercropping system.

The partial values of CR clearly showed maize as the dominant crop specie the under relay-intercropping system. Similarly, in previous investigations, it has been proved that the CR values of maize were always higher than soybean [10,27]. Moreover, higher competitive ability of maize crop to exploit and use available resources i.e. light, land, and water in association with soybean or groundnut or chickpea has been confirmed by other scientists [32,51,52]. Whereas, in pea-rye intercropping the partial values of CRp of legume (pea) were greater than cereal (rye), which was the different trend which we observed as cereal (maize) was more aggressive and competitive (higher CRm than CRs values) than legume (soybean) [32]. In this study, fertilizer and water were not the limiting factors in all planting patterns, but the row spacing and arrangement were the vital factors which may dominate and become more important for increasing the maize and soybean seed yields under maize sowing relay intercropping system.

Local farming communities only approve that new planting pattern or innovation which produces more profit with fewer expenses [25]. In this experiment, the economic analysis revealed that net income (higher profit) were obtained by using the narrow wide row planting pattern (2M2S, 40 cm: 60 cm: 40 cm: 60 cm) for maize and soybean production under relay-intercropping systems in both years than 1M1S (Table 6). Importantly, our results of the present study revealed that optimum light transmission and distribution at soybean canopy and in maize plants, respectively have significantly increased the total biomass accumulation and distribution towards the reproductive parts in maize and soybean plants under relay intercropping system and ultimately it can be a source of maximum profit to the farmer.

Conclusion

In the present study, light interception and distribution patterns were evaluated by using different planting patterns. As compared to traditional planting pattern (1M1S, 50 cm: 50 cm), modern narrow-wide-row planting pattern (2M2S, 40 cm: 60 cm: 40 cm: 60 cm) greatly improved the light environment of maize and soybean plants. Our results indicate that greater contributions for relay-intercrop advantages can be attributed to better light interception and transmission between relay-intercropped species in maize-soybean relay intercropping system. Additionally, the high LER (1.7) and net income (1411.1 US $ ha⁻¹) of 2M2S (40 cm: 60 cm: 40 cm: 60 cm) was a result of high maize and soybean seed yields (Tables 5 and 6). In relay intercropping with a enough distance between maize and soybean strips for growing soybeans, the improved light interception at maize narrow row and wide row leaves close to the ear increased their photosynthetic rate, and potentially maintained the maize biomass production and seed yield; the increased light at top of soybean plants significantly enhanced the light transmittance and photosynthetic rate of soybean, which then considerably improved its biomass production, competitive ability and seed yield. Therefore, advantage of relay intercropping can be improved by decreasing the competitive ability of maize.
Acknowledgments

The authors are grateful to Professor Yang Wenyu for his expert advices throughout this difficult research project. Muhammad Ali Raza thanks Muhammad Khan Khichi (Managing Director, PASSCO) for encouragement and support during the PhD study and research.

Author Contributions

Conceptualization: Lingyang Feng, Yuanfang Fan.

Data curation: Muhammad Hayder Bin Khalid, Junbo Du, Chuanyan Liu.

Formal analysis: Lingyang Feng, Yuankai Chen, Muhammad Hayder Bin Khalid, Junbo Du, Zhongwei Zhang, Shu Yuan.

Funding acquisition: Yuankai Chen, Wenyu Yang.

Investigation: Lingyang Feng, Yuankai Chen, Yuanfang Fan, Junbo Du, Xiaoling Wu, Chun Song, George Bawa, Zhongwei Zhang, Shu Yuan, Feng Yang, Wenyu Yang.

Methodology: Lingyang Feng, Muhammad Ali Raza, Yuankai Chen, Yuanfang Fan, Junbo Du, Xiaoling Wu, Chun Song, Zhongwei Zhang, Shu Yuan, Feng Yang, Wenyu Yang.

Software: Faiza Ahsan.

Supervision: Feng Yang, Wenyu Yang.

Validation: Xiaoling Wu.

Visualization: Xiaoling Wu.

Writing – original draft: Lingyang Feng, Muhammad Ali Raza.

Writing – review & editing: Lingyang Feng, Muhammad Ali Raza, Tehseen Ahmad Meraj.

References

1. Zhang F, Li L. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant and Soil. 2003; 248(1–2):305–12.

2. Ju XT, Kou CL, Zhang F, Christie P. Nitrogen balance and groundwater nitrate contamination: comparison among three intensive cropping systems on the North China Plain. Environmental Pollution. 2006; 143(1):117–25. https://doi.org/10.1016/j.envpol.2005.11.005 PMID: 16364521

3. Blumenberg M, Bemümmeyer C, Moros M, Muschalla M, Schmale O, Thiel V. Bacteriohopanepolyols record stratification, nitrogen fixation and other biogeochemical perturbations in Holocene sediments of the central Baltic Sea. Biogeosciences. 2013; 10(4):2725–35.

4. Zhang Y, Liu J, Mu Y, Xu Z, Pei S, Lun X, et al. Nitrous oxide emissions from a maize field during two consecutive growing seasons in the North China Plain. Journal of Environmental Sciences. 2012; 24(1):160–8.

5. Ju X-T, Xing G-X, Chen X-P, Zhang S-L, Zhang L-J, Liu X-J, et al. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proceedings of the National Academy of Sciences. 2009;pnas. 0813417106.

6. Zhang Y, Liu J, Zhang J, Liu H, Liu S, Zhai L, et al. Row ratios of intercropping maize and soybean can affect agronomic efficiency of the system and subsequent wheat. PloS one. 2015; 10(6):e0129245. https://doi.org/10.1371/journal.pone.0129245 PMID: 26061566

7. Yang F, Lou Y, Liao D, Gao R, Yong T, Wang X, et al. Effects of row spacing on crop biomass, root morphology and yield in maize-soybean relay strip intercropping system. Acta Agron Sin. 2015; 41:642–50.

8. Yang F, Huang S, Gao R, Liu W, Yong T, Wang X, et al. Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red: far-red ratio. Field Crops Research. 2014; 155:245–53. https://doi.org/10.1016/j.fcr.2013.08.011

9. Iqbal N, Hussain S, Ahmed Z, Yang F, Wang X, Liu W, et al. Comparative analysis of maize-soybean strip intercropping systems. A review. Plant Production Science. 2018;(just-accepted).
10. Chen P, Du Q, Liu X, Zhou L, Hussain S, Lei L, et al. Effects of reduced nitrogen inputs on crop yield and nitrogen use efficiency in a long-term maize-soybean relay strip intercropping system. PloS one. 2017; 12(9):e0184503. https://doi.org/10.1371/journal.pone.0184503 PMID: 28910355

11. Yang F, Liao D, Wu X, Gao R, Fan Y, Raza MA, et al. Effect of aboveground and belowground interactions on the intercrop yields in maize-soybean relay cropping systems. Field Crops Research. 2017; 203:16–23. https://doi.org/10.1016/j.fcr.2016.12.007

12. Liu X, Rahman T, Song C, Su B, Yang F, Yong T, et al. Changes in light environment, morphology, growth and yield of soybean in maize-soybean intercropping systems. Field Crops Research. 2017; 200:38–46.

13. Liu X, Rahman T, Yang F, Song C, Yong T, Liu J, et al. PAR Interception and Utilization in Different Maize and Soybean Intercropping Patterns. PloS one. 2017; 12(1):e0169218. https://doi.org/10.1371/journal.pone.0169218 PMID: 28056056

14. Liu J, Yang C-q, Zhang Q, Lou Y, Wu H-j, Deng J-c, et al. Partial improvements in the flavor quality of soybean seeds using intercropping systems with appropriate shading. Food Chemistry. 2016; 207:107–14. https://doi.org/10.1016/j.foodchem.2016.03.059 PMID: 27080886

15. Li T, Liu L-N, Jiang C-D, Liu Y-J, Shi L. Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. Journal of Photochemistry and Photobiology B: Biology. 2014; 137:31–8. https://doi.org/10.1016/j.jphotobiol.2014.04.022 PMID: 24935099

16. Li R, Wen T, Tang Y, SUN X, XIA C. Effect of shading on photosynthetic and chlorophyll fluorescence characteristics of soybean. Acta Prataculturae Sinica. 2014; 23(3):198–206.

17. Marchiori PE, Machado EC, Ribeiro RV. Photosynthetic limitations imposed by self-shading in field-grown sugarcane varieties. Field Crops Research. 2014; 155:30–7.

18. Wardlaw IF. Tansley Review No. 27 The control of carbon partitioning in plants. New phytologist. 1990; 116(3):341–81.

19. Borrellás, Slafer GA, Otegui ME. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Research. 2004; 86(2–3):131–46.

20. Wolff XY, Coltman RR. Productivity under shade in Hawaii of five crops grown as vegetables in the tropics. Journal of the American Society for Horticultural Science. 1990; 115(1):175–81.

21. Fan Y, Chen J, Cheng Y, Raza MA, Wu X, Wang Z, et al. Effect of shading and light recovery on the growth, leaf structure, and photosynthetic performance of soybean in a maize-soybean relay-strip intercropping system. PloS one. 2018; 13(5):e0198159. https://doi.org/10.1371/journal.pone.0198159 PMID: 29851989

22. Wu Y, Gong W, Yang W. Shade inhibits leaf size by controlling cell proliferation and enlargement in soybean. Scientific Reports. 2017; 7(1):9259. https://doi.org/10.1038/s41598-017-10026-5 PMID: 28835715

23. Liu W, Deng Y, Hussain S, Zou J, Yuan J, Luo L, et al. Relationship between cellulose accumulation and lodging resistance in the stem of relay intercropped soybean [Glycine max (L.) Merr.]. Field Crops Research. 2016; 196:261–7. https://doi.org/10.1016/j.fcr.2016.07.008

24. Raza MA, Feng LY, Manaf A, Wasaya A, Ansar M, Hussain A, et al. Sulphur application increases seed yield and oil content in sesame seeds under rainfed conditions. Field Crops Research. 2018; 218:51–8. https://doi.org/10.1016/j.fcr.2017.12.024

25. Raza M, Feng L, Isqal N, Manaf A, Khalid M, Wasaya A, et al. Effect of Sulphur Application on Photosynthesis and Biomass Accumulation of Sesame Varieties under Rainfed Conditions. Agronomy. 2018; 8(8):149.

26. Liu X, Rahman T, Song C, Yang F, Su B, Cui L, et al. Relationships among light distribution, radiation use efficiency and land equivalent ratio in maize-soybean strip intercropping. Field Crops Research. 2018; 224:91–101. https://doi.org/10.1016/j.fcr.2018.05.010

27. Yang F, Wang X, Liao D, Lu F, Gao R, Liu W, et al. Yield response to different planting geometries in maize–soybean relay strip intercropping systems. Agronomy Journal. 2015; 107(1):296–304. https://doi.org/10.2134/agronj14.01263

28. Kawamura K, Cho M, Takeda H. The applicability of a color acetate film for estimating photosynthetic photon flux density in a forest understory. Journal of forest research. 2005; 10(3):247–9.

29. Porcel R, Redondo-Gómez S, Mateos-Naranjo E, Aroca R, Garcia R, Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. Journal of plant physiology. 2015; 185:75–83. https://doi.org/10.1016/j.jplph.2015.07.006 PMID: 26291919

30. Francis C, Rutger J, Palmer A. A Rapid Method for Plant Leaf Area Estimation in Maize (Zea mays L.) 1. Crop Science. 1969; 9(5):537–9.
31. Li L, Yang S, Li X, Zhang F, Christie P. Interspecific complementary and competitive interactions between intercropped maize and faba bean. Plant and Soil. 1999; 212(2):105–14.
32. Lithourgidis A, Vlachostergios D, Dordas C, Damalas C. Dry matter yield, nitrogen content, and competition in pea–cereal intercropping systems. European Journal of agronomy. 2011; 34(4):287–94.
33. Xue J, Gou L, Shi Z-g, Zhao Y, Zhang W. Effect of leaf removal on photosynthetically active radiation distribution in maize canopy and stalk strength. Journal of Integrative Agriculture. 2017; 16(1):85–96.
34. Li F, Meng P, Fu D, Wang B. Light distribution, photosynthetic rate and yield in a Paulownia-wheat intercropping system in China. Agroforestry Systems. 2008; 74(2):163–72.
35. Chirko CP, Gold MA, Nguyen PV, Jiang J. Influence of direction and distance from trees on wheat yield and photosynthetic photon flux density (Qp) in a Paulownia and wheat intercropping system. Forest ecology and management. 1996; 83(3):171–80.
36. Keating B, Carberry P. Resource capture and use in intercropping: solar radiation. Field Crops Research. 1993; 34(3–4):273–301.
37. Wang Z, Zhao X, Wu P, He J, Chen X, Gao Y, et al. Radiation interception and utilization by wheat/maize strip intercropping systems. Agricultural and forest meteorology. 2015; 204:58–66.
38. Kurepin LV, Emery RN, Pharis RP, Reid DM. The interaction of light quality and irradiance with gibberellins, cytokinins and auxin in regulating growth of Helianthus annuus hypocotyls. Plant, cell & environment. 2007; 30(2):147–55. PMID: 12234731
39. Ruberti I, Sessa G, Ciolfi A, Possenti M, Carabelli M, Morelli G. Plant adaptation to dynamically changing environment: the shade avoidance response. Biotechnology advances. 2012; 30(5):1047–58. PMID: 21889962
40. Feng LY, Raza MA, Li ZC, Chen Y, Khalid MHB, Du J, et al. The Influence of Light Intensity and Leaf Movement on Photosynthesis Characteristics and Carbon Balance of Soybean. Frontiers in plant science. 2018; 9:1952. https://doi.org/10.3389/fpls.2018.01952 PMID: 30673355
41. Morelli G, Ruberti I. Light and shade in the photocontrol of Arabidopsis growth. Trends in plant science. 2002; 7(9):399–404. PMID: 12234731
42. Nagasuga K, Kubota F. Effects of shading on hydraulic resistance and morphological traits of internode and node of napiergrass (Pennisetum purpureum Schumach.). Plant Production Science. 2008; 11(3):352–4.
43. Wittmann C, Aschan G, Pfanz H. Leaf and twig photosynthesis of young beech (Fagus sylvatica) and aspen (Populus tremula) trees grown under different light regime. Basic and Applied Ecology. 2001; 2(2):145–54. https://doi.org/10.1078/1439-1791-00047
44. Jiang C-D, Wang X, Gao H-Y, Shi L, Chow FW. Systemic regulation of leaf anatomical structure, photosynthetic performance and high-light tolerance in sorghum. Plant physiology. 2011; pp. 11.172213.
45. Omoto E, Taniguchi M, Miyake H. Adaptation responses in C4 photosynthesis of maize under salinity. Journal of plant physiology. 2012; 169(5):469–77. https://doi.org/10.1016/j.jplph.2011.11.009 PMID: 22209164
46. Midmore D, Berrios D, Roca J. Potato (Solanum spp.) in the hot tropics V. Intercropping with maize and the influence of shade on tuber yields. Field Crops Research. 1988; 18(2–3):159–76.
47. Monzon JP, Mercau JL, Andrade J, Caviglia OP, Cerrudo A, Cirilo AG, et al. Maize–soybean intensification alternatives for the Pampas. Field Crops Research. 2014; 162:48–59.
48. Gou F, van Ittersum MK, Wang G, van der Putten PE, van der Werf W. Yield and yield components of wheat and maize in wheat–maize intercropping in the Netherlands. European Journal of agronomy. 2016; 76:17–27.
49. Oseni TO. Evaluation of sorghum-cowpea intercrop productivity in savanna agro-ecology using competition indices. Journal of Agricultural Science. 2010; 2(3):229.
50. Liu W, Zou J, Zhang J, Yang F, Wan Y, Yang W. Evaluation of soybean (Glycine max) stem vining in maize-soybean relay strip intercropping system. Plant Production Science. 2015; 18(1):69–75. https://doi.org/10.1626/pps.18.69
51. Banik P, Sasmal T, Ghosal P, Bagchi D. Evaluation of Mustard (Brassica compestris Var. Toria) and Legume Intercropping under 1: 1 and 2: 1 Row-Replacement Series Systems. Journal of Agronomy and Crop Science. 2000; 185(1):9–14.
52. Ghosh P. Growth, yield, competition and economics of groundnut/cereal fodder intercropping systems in the semi-arid tropics of India. Field Crops Research. 2004; 88(2–3):227–37.