Ecological, efficient and low-carbon cereal-legume intercropping systems

Edited by
Lingyang Feng, Muhammad Ali Raza and Feng Yang

Coordinated by
Ping Chen

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Topic editors
Lingyang Feng — Peking University, China
Muhammad Ali Raza — Islamia University of Bahawalpur, Pakistan
Feng Yang — Sichuan Agricultural University, China

Topic coordinator
Ping Chen — Sichuan Agricultural University, China

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EDITED AND REVIEWED BY
Leo Marcelis,
Wageningen University and Research,
Netherlands

*CORRESPONDENCE
Ping Chen
swust_pingchen@qq.com
Lingyang Feng
linyang.feng@pku-iaas.edu.cn
Feng Yang
f.yang@sicau.edu.cn
Muhammad Ali Raza
razaali0784@gmail.com

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Editorial: Ecological, efficient and low-carbon cereal-legume intercropping systems

Ping Chen[1]*, Lingyang Feng[2]*, Feng Yang[3]* and Muhammad Ali Raza[4,5]*

[1]School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, Sichuan, China, [2]Institute of Advanced Agricultural Sciences, Peking University, Weifang, Shandong, China, [3]College of Agronomy, Sichuan Agricultural University/Sichuan Engineering Research Center for Crop Strip Intercropping System/Key Laboratory of Crop Ecophysiology and Farming System in Southwest, Ministry of Agriculture and Rural Affair, Chengdu, Sichuan, China, [4]National Research Center of Intercropping, Islamia University of Bahawalpur, Bahawalpur, Pakistan, [5]Gansu Academy of Agricultural Sciences, Lanzhou, Gansu, China

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Editorial on the Research Topic
Ecological, efficient and low-carbon cereal-legume intercropping systems

The global population will reach 9.4–10.1 billion by 2050 (United Nations, 2019). Over the last number of decades, traditional agricultural production has met food demands by increasing resource input. However, excessive chemical fertilizer input results in severe environmental costs, e.g., soil acidification (Guo et al., 2010), global warming (Penuelas and Filella, 2001), water pollution (Yu et al., 2019), and finally cropland degradation, decreasing agricultural products and threatening human health (Han et al., 2016; Zhao et al., 2017). Moreover, increasing global food production by expanding cropland is unsustainable for the global ecosystem (Potapov et al., 2022). Expanding cropland also leads to the use of more chemical fertilizers and a high risk of global warming. Global warming increases yield losses to insect pests (Deutsch. et al., 2018), meaning more insecticide demands to guarantee crop production and a high risk of water pollution (Stehle and Schulz, 2015). Therefore, achieving global food security with environmentally friendly and sustainable development approaches is a great challenge in this century.

Intercropping is defined as simultaneously cultivating two or more crops on the same land (Willey, 1979). Intercropping is used worldwide to increase land productivity, to efficiently use resources (Li et al., 2020b; Li et al., 2021), to better control diseases and pests (Zhang et al., 2019; Chi et al., 2021), to suppress weeds (Gu et al., 2021), and to decrease environmental costs (Qin et al., 2013; Chen et al., 2019). Therefore, intercropping provides potential ways to achieve food security and sustainable agricultural development. In this Research Topic, we received recent studies revealing the mechanisms of yield advantages and the efficient use of resources in intercropping.

The complementary use of resources contributes to yield advantages in maize-legume intercropping (Li et al., 2020a). Raza et al. reported that optimizing the crop planting density maximizes the yield advantages of maize-soybean strip intercropping. Maize-soybean strip intercropping with a maize plant density of eight plants per square meter obtained a higher total leaf area index and total grain yield than other methods. The water equivalent ratios of intercropping are greater than one, suggesting that maize-soybean strip intercropping provides a potential way to achieve sustainable agricultural development. The optimized intercropping spares 20–50% of water and land. Maize-soybean intercropping with a N input of 250 kg N ha^{-1} obtained yield advantages (Nasar et al.). The underlying yield advantages include increased N use efficiency, e.g., N uptake efficiency and N agronomic efficiency since the N assimilatory enzymes of intercropped maize, e.g., nitrate reductase, nitrite reductase, and glutamate synthase, are more robust than the monoculture.

However, the underlying mechanisms of yield advantages of component crops in relay intercropping are different. Chen et al. revealed the mechanism for intercropped maize over-yielding in a low radiation area. The net yield of intercropped maize can be increased by 2.1 Mg ha^{-1} *via* the use of dense cultivation and high N input with plow tillage compared with normal farming practice. The over-yielding of intercropped maize mainly derives from an improved leaf area index (LAI) and net photosynthetic rate (Pn). Similarly, Zheng et al. showed that straw incorporation increases the aboveground N uptake and nitrogen recovery efficiency of intercropped soybean by 43.7% and 76.8%, respectively, compared with straw removal. In particular, straw incorporation at 30 kg N ha^{-1} achieved the greatest aboveground N uptake and nitrogen recovery efficiency compared with other N treatments. Although straw incorporation remarkably promotes CO_2 emission, the accumulated CO_2 emission of straw incorporation was lowest at 30 kg N ha^{-1}.

Legumes' performance in strip and relay intercropping differs (Zhang et al., 2023). In relay intercropping, the recovery growth of legumes benefits their yield advantage (Wu et al., 2021). In maize-peanut strip intercropping, the crop planted later, e.g., peanut, suffers from the shade of maize (Chen et al., 2020). Lu et al. pointed out that optimizing crop configurations increases light use and obtains yield advantages in maize-peanut strip intercropping. Although intercropped peanut suffers from the shade of maize, which decreases the leaf functional traits, intercropped peanut in eight rows allows higher light energy utilization than intercropped peanut in four or two rows. Previous studies reported that intercropped maize with legumes increases the usage efficiency of resources by optimizing crop root distribution and strengthening nutrient acquisition (Chen et al., 2017; Zheng et al., 2021; Zheng et al., 2022). Surigaoge et al. pointed out that cereal-legume intercropping improves soil nutrient cycling. Plant litter is decomposed more quickly in maize-peanut intercropping than in maize-soybean intercropping. Although N addition promotes plant litter decomposition, maize-peanut intercropping achieved a higher decomposition rate than maize-soybean intercropping. Moreover, a trade-off in yield advantage is observed in maize-wheat relay strip intercropping under rainfed conditions(Hussain et al.). N input contributes to a more robust yield advantage by strengthening the yield advantage of intercropped wheat in the border rows. Specifically, the yield advantage of intercropped wheat in the border rows is mainly attributed to a higher number of ears in the unit area. In contrast, yield disadvantage is obtained in intercropped maize due to the lower kernel number and thousand-grain weight of maize in the border rows compared with maize alone.

The practice of intercropping is not limited to staple crops; intercropping of vegetables or forage grass is also valuable (Stoltz and Nadeau, 2014).Pereira et al. pointed out that vegetable intercropping can mitigate greenhouse gas (GHG) emissions. Collard greens-spinach and collard greens-chicory intercropping decreased GHG emissions by 31% compared with the corresponding monoculture. Tahir et al. reported that a full mixture of legume-grass increases farmland productivity. The mixture is beneficial in improving the soil enzyme activity and in increasing the soil nutrient content. In return, the improved growth of forage leads to higher levels of crude protein than the monoculture, and the crude protein content of the mixture increases with increasing N input.

This Research Topic confirms the potential of intercropping to achieve food security using environmentally friendly approaches. Advisors and farmers can refer to this knowledge to optimize their decision-making in crop management and to improve food security and quality.

Author contributions

PC: Writing – original draft, Writing – review & editing. LF: Writing – original draft, Writing – review & editing. FY: Writing – original draft, Writing – review & editing. MR: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hans-Peter Kaul,
University of Natural Resources and Life Sciences Vienna, Austria

REVIEWED BY
Nasir Iqbal,
University of Adelaide, Australia
Sabin Saurav Pokharel,
Nanjing Agricultural University, China

*CORRESPONDENCE
Milan Skalicky
skalicky@af.czu.cz
Wenyu Yang
mssiyangwy@sicau.edu.cn

†These authors share first authorship

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Maize/soybean strip intercropping produces higher crop yields and saves water under semi-arid conditions

Muhammad Ali Raza[1,2,3†], Hassan Shehryar Yasin[1†], Hina Gul[4†], Ruijun Qin[5], Atta Mohi Ud Din[1,6], Muhammad Hayder Bin Khalid[1,2], Sajad Hussain[1,2], Harun Gitari[7], Amjed Saeed[1], Jun Wang[8], Esmaeil Rezaei-Chiyaneh[9], Ayman El Sabagh[10], Amir Manzoor[1], Akash Fatima[11], Shakeel Ahmad[12], Feng Yang[2], Milan Skalicky[13*] and Wenyu Yang[2*]

[1]National Research Center of Intercropping, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, [2]College of Agronomy, Sichuan Agricultural University, Chengdu, China, [3]Gansu Academy of Agricultural Sciences, Lanzhou, Gansu, China, [4]National Center for Industrial Biotechnology, Pir Mehar Ali Shah-Arid Agricultural University, Rawalpindi, Pakistan, [5]Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR, United States, [6]College of Agriculture, Nanjing Agricultural University, Nanjing, China, [7]Department of Agricultural Science and Technology, School of Agriculture and Enterprise Development, Kenyatta University, Nairobi, Kenya, [8]Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xian, China, [9]Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University, Urmia, Iran, [10]Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt, Turkey, [11]Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Sharif-University of Agriculture, Multan, Pakistan, [12]Department of Agronomy, Bahauddin Zakariya University, Multan, Multan, Pakistan, [13]Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czechia

Sustainable increases in crop production require efficient use of resources, and intercropping can improve water use efficiency and land productivity at reduced inputs. Thus, in a three-year field experiment, the performance of maize/soybean strip intercropping system differing with maize plant density (6 maize plants m-2, low, D1; 8 maize plants m-2, medium, D2; and 10 maize plants m-2, high, D3) was evaluated in comparison with sole maize or soybean cropping system. Results revealed that among all intercropping treatments, D2 had a significantly higher total leaf area index (maize LAI + soybean LAI; 8.2), total dry matter production (maize dry matter + soybean dry matter; 361.5 g plant-1), and total grain yield (maize grain yield + soybean grain yield; 10122.5 kg ha-1) than D1 and D3, and also higher than sole maize (4.8, 338.7 g plant-1, and 9553.7 kg ha-1) and sole soybean (4.6, 64.8 g plant-1, and 1559.5 kg ha-1). The intercropped maize was more efficient in utilizing the radiation and water, with a radiation use efficiency of 3.5, 5.2, and 4.3 g MJ-1 and water use efficiency of 14.3, 16.2, and 13.3 kg ha-1 mm-1, while that of intercropped soybean was 2.5, 2.1, and 1.8 g MJ-1 and 2.1, 1.9, and 1.5 kg ha-1 mm-1 in D1, D2, and D3, respectively. In intercropping, the land and water equivalent ratios ranged from 1.22 to 1.55, demonstrating that it is a sustainable strategy to improve land and water use efficiencies; this maximization is likely associated with the species complementarities for radiation, water, and land in time and space, which resulted in part from competition avoidance responses that maximize the economic profit (e. g., 1300 US $ ha-1 in D2) over sole maize (798 US $ ha-1) or sole soybean (703 US $ ha-1). Overall, these results indicate that optimizing strip intercropping systems can save 20–50% of water and land, especially under the present scenario of limited resources and climate change. However, further research is required to fully understand the resource capture mechanisms of intercrops in intercropping.

KEYWORDS

land productivity, water use efficiency, competition, sustainability, economic profit

Introduction

Food security is a prerequisite for ensuring national security and human survival. The global human population is projected to cross nine billion in 2050 (Thornton et al., 2014). Thus, to fulfill the enhanced demands of an increasing population for food and feed, it is estimated that the current crop yield needs to be increased by 50% in 2030 and 100% in 2050 (Li et al., 2020). The continuous decline in cultivable lands due to urbanization and industrialization has limited the further expansion in cultivation area of cereals (e. g., maize; *Zea mays* L.) and legumes (e. g., soybean; *Glycine max* L.). This situation is more serious in the developing countries (e. g., China, Pakistan, and India) that have more population and less cultivable land (Du et al., 2017; Iqbal et al., 2018). Furthermore, researchers have reported that the expansion in the cultivation area for food crops is the leading cause of deforestation in many regions that adversely affect the environment (Barona et al., 2010). Therefore, in the present scenario of limited resources (i. e., land and water) and climate change, it is important to develop new cropping systems (i. e., intercropping or agroforestry), which can increase crop yields by effectively using the limited resources without affecting the environment.

Intercropping, the cultivation of two or more crop species on the same land, provides opportunities for sustainable crop production and agricultural intensification (Feng et al., 2019). Intercropping results in higher crop yield at the system level (grain yield of species one + grain yield of species two) and less yield variation than mono-cropping systems (Martin-Guay et al., 2018). This higher and stable yield, particularly with reduced inputs, are mainly ascribed to resources (i. e., water, sunlight, and nutrients) complementarity (Liu et al., 2017; Gitari et al., 2018; Raza et al., 2019), in which intercrop species utilize available resources more adequately due to different spatial (Raza et al., 2021a), temporal (Yang et al., 2017), and phenological characteristics (Li et al., 2013). The intra- and interspecific competition (Yang et al., 2015), availability of environmental resources (Liu et al., 2017), and planting density of the intercrop species influenced the degree of resource complementarity (Ren et al., 2016) and the yield of intercropping (Hauggaard-Nielsen and Jensen, 2005). For instance, maize and soybean produced larger relative grain yields in strip intercropping than in mono-cropping (Chen et al., 2017; Du et al., 2017); and intercropping of maize with soybean achieved high land productivity (estimated as a land equivalent ratio; LER) with high maize planting density compared to low maize planting density under strip intercropping (Muoneke et al., 2007). These findings conclude that strip intercropping produces higher yields at the system level than mono-cropping due to complementarity and facilitation interactions.

Determining the optimum planting density of intercrop species is a paramount for higher crop yields in intercropping. Compared with mono-cropping, crops in intercropping use planting space more efficiently and effectively (Raza et al., 2020). The optimum planting density in intercropping outweighs the optimum planting density in mono-cropping (Willey and Osiru, 1972). Nevertheless, the optimum planting density of one intercrop species at one location, i. e., maize in maize/soybean intercropping at Sichuan under high-rainfall conditions (Feng et al., 2020), maize in maize/wheat intercropping at Wageningen under medium-rainfall conditions (Gou et al., 2016), maize in maize/pea intercropping at Gansu under low-rainfall conditions (Mao et al., 2012), and maize in maize/pigeon pea intercropping at Trinidad under irrigated conditions (Dalal, 1974), may not be applicable to other sites because of the regional variations in soil properties (water holding capacity, total available nitrogen, phosphorus, and potassium, and organic matter) and weather (precipitation, temperature, and solar radiation). However, lack of appropriate study and relevant literatures on determining the optimum planting density of maize in cereal/legume intercropping systems under irrigated conditions, especially in semi-arid areas (high-temperature regions, where farmers are using extra water for the production of cereals and legumes).

Researchers have previously reported that a higher planting density of intercropped maize resulted in greater intercropping advantages (Willey and Osiru, 1972; Muoneke et al., 2007). Whereas it significantly affects the competitive interactions between intercrops; for instance, the dominance of maize over soybean was enhanced with increased maize density, which ultimately decreased the grain yield of soybean in maize/soybean intercropping (Muoneke et al., 2007). In addition, the planting density of intercrop species, especially of tall crops, adversely affects the root growth and distribution (Hauggaard-Nielsen et al., 2001), sunlight transmittance (Li et al., 2001), leaf area development (Prasad and Brook, 2005), dry matter production (Ren et al., 2016), and resource capturing (Gao et al., 2009) of understory crops in cereal/legume intercropping systems. However, most past studies on the plant density response of intercrops have mainly been conducted by changing the row ratio or sowing proportions (Ofori and Stern, 1987; Ijoyah and Fanen, 2012; Mao et al., 2012). Thus, the response of intercrops to equal row-ratio and sowing proportion under strip intercropping systems remains unclear. The interaction (below and above ground) of intercrops species has been reported to enhance the water and light utilization efficiency. Furthermore, it has been rarely investigated how changing maize planting density affects the interspecific interactions, competition for the acquisition of available resources (i. e., water and radiation), and land productivity of maize/soybean strip intercropping (maize/soybean intercropping) under irrigated conditions. Therefore, the main aims of this study were to determine the effects of changing maize planting density on (i) growth and crop yields of maize and soybean in maize/soybean intercropping, (ii) resource (water or sunlight) utilization dynamics of intercrops under maize/soybean intercropping, and (iii) land productivity and economic viability of maize/soybean intercropping compared to sole cropping of maize and soybean using data from a three-year field experiment.

Materials and methods

Field experiments

The field study was conducted in 2018, 2019, and 2020 at Khairpur Tamewali (29.57°N, 72.25°E; altitude 130 m), Bahawalpur, Punjab Province, Pakistan, a research site of Sichuan Agricultural University, P. R. China. The research site has a continental monsoon climate, with a mean annual precipitation of 143 mm and a temperature of 25.7°C. The soil was a sandy clay loam, with 7.7 pH, 7.3 g kg^{-1} organic matter, 0.5 g kg^{-1} total nitrogen (N), 5.0 mg kg^{-1} available phosphorus (P), 341.5 mg kg^{-1} available potassium (K), and 1.47 Mg m^{-3} bulk density. Daily incident solar radiation, air temperature, and rainfall of 2018, 2019, and 2020 are shown in Figure 1. During the planting period (from sowing to harvest), total rainfall was 77, 105, and 280 mm in 2018, 2019, and 2020, respectively.

The experiment was laid out in a randomized complete block design with three replications. The study consisted of three maize/soybean intercropping treatments differing with maize plant density (6 maize plants m^{-2}, low, D_1; 8 maize plants m^{-2}, medium, D_2; and 10 maize plants m^{-2}, high, D_3) and two sole cropping treatments of maize (M) and soybean (S). The intercropping treatments comprised of two rows of maize with two rows of soybean in each intercropping strip (Figure 2); six intercropping strips were arranged in each intercropping plot. The size of each plot was 144 m^2 (12 m in width and 12 m in length). The plant configuration (i. e., row spacings, plant distances, and planting densities) in D_1, D_2, D_3, M, and S are presented in Table 1. According to the local recommended planting densities, both sole crops were planted: 80000 plants ha^{-1} for maize and 140000 plants ha^{-1} for soybean. In addition, all agronomic practices, i. e., sowing, weeding, and harvesting, were done manually.

The soybean (determinate) variety 'NARC-16' and maize (semi-compact) variety 'DK-6317' were used in the study. Both crops were planted and harvested on the same date, on February 03rd in 2018, February 05th in 2019, and February 7th in 2020; and harvested on June 30th in 2018, July 7th in 2019, and July 5th in 2020. Before sowing, for maize, basal N at 120 kg ha^{-1} as urea, P at 205 kg ha^{-1} as diammonium phosphate (DAP), and K at 150 kg ha^{-1} as potassium sulfate (SOP) were applied between maize rows in D_1, D_2, D_3, and M. For soybean, basal N at 75 kg ha^{-1} as urea, P at 150 kg ha^{-1} as DAP, and K at 100 kg ha^{-1} as SOP were used between soybean rows in D_1, D_2, D_3, and S. At the V_6 and tasseling stages of maize, the second and third doses of N were applied at 60 and 100 kg ha^{-1}, respectively, as urea between maize rows under D_1, D_2, D_3, and M. Besides, all treatments were irrigated with the same amount of water across the whole experiment, and the detailed information is shown in Table 2. According to the local water application advisory for maize and soybean production, irrigation water was applied, which is equal to 550 ± 100 mm water for both crops depending on the crop or weather conditions. Groundwater was pumped out using a tube well and applied *via* the furrow irrigation method.

Measurements

Leaf area of maize and soybean was measured five times at 45, 65, 85, 105, and 125 days after sowing (DAS) in all years of this study. For this purpose, three maize and five soybean plants

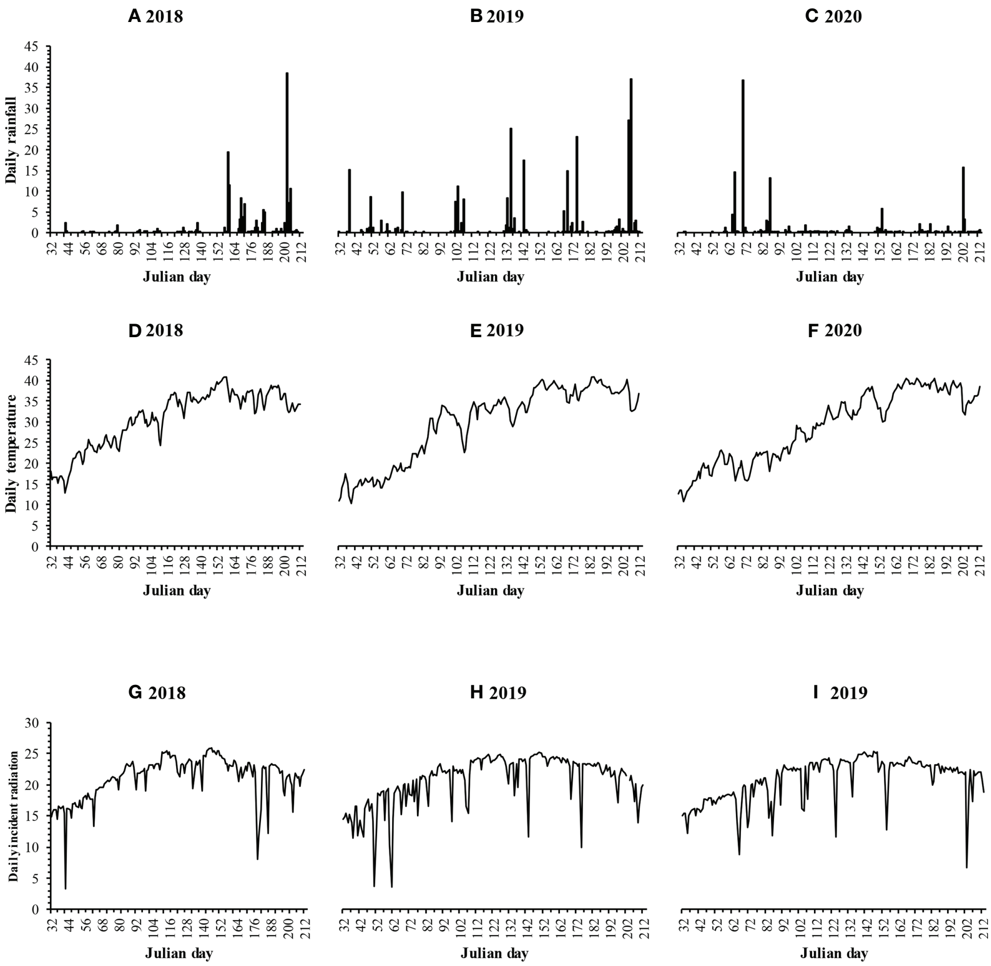

FIGURE 1
Daily rainfall (mm), temperature (°C), and incident radiation (MJ m^{-2} day^{-1}) during the summer season of maize and soybean in 2018, 2019, and 2020.

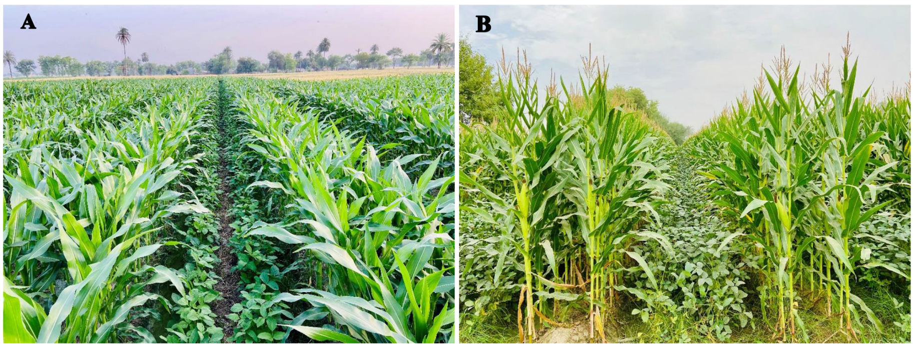

FIGURE 2
Field demonstration of maize/soybean strip intercropping system. **(A)** Intercrops were at the vegetative growth stage, and **(B)** Intercrops were at the reproductive growth stage (Photos: Muhammad Ali Raza). Location: Punjab Province, Pakistan.

TABLE 1 The plant to plant, row to row, strip to strip distances for maize and soybean, and total planting densities of maize and soybean in intercropping and sole cropping systems.

Treatments	Plant distance (cm)		Row distance (cm)		Strip distance ** (cm)	Strips/Rows ($plot^{-1}$)		Total planting density (plants ha^{-1})		
	Maize	Soybean	Maize	Soybean		Maize	Soybean	Maize	Soybean	Total
D_1*	16.7	7.2	40	40	60	06 ***	06	60000	140000	200000
D_2	12.5	7.2	40	40	60	06	06	80000	140000	220000
D_3	10.0	7.2	40	40	60	06	06	100000	140000	240000
M	16.7	–	75	–	–	16	–	80000	–	80000
S	–	14.3	–	50	–	–	24	–	140000	140000

*The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The M refers to the sole cropping system of maize, and the S refers to the sole cropping system of soybean.
**Strip distance between the strips of maize and soybean in maize/soybean strip intercropping system.
***Each strip of maize or soybean in the maize/soybean strip intercropping system contained two rows of maize or two rows of soybean.

were destructively sampled from each plot at each sampling time. The leaf area of all leaves was determined by multiplying the greatest leaf width and length with the crop-specific co-efficient factor of 0.70 for maize and 0.75 for soybean (Gao et al., 2009). Then, the leaf area index (LAI) was calculated using the following equation (Montgomery, 1911).

$$LAI = \frac{\left(Leaf \;\; area \;\; plant^{-1} \times Plant \;\; number \;\; plot^{-1}\right)}{Plot \;\; area}$$

Three maize and five soybean plants from each plot were collected at 45, 65, 85, 105, and 125 DAS for total dry matter production and partitioning analysis. Then, all samples were divided into various plant parts (root, straw (leaves + stem + non-grain parts), and grain) and sun-dried for the next seven to ten days to achieve a constant weight and presented as g $plant^{-1}$. The total dry matter (TDM; g $plant^{-1}$) of maize and soybean was determined from the summation of the dry matter of root, straw, and grain. Additionally, the total dry matter (g $plant^{-1}$) of intercropping treatments was calculated from the summation of the total dry matter of maize and soybean in D_1, D_2, and D_3.

To determine the grain yield of maize and soybean, 24 maize-ears and 40 soybean plants were collected from each plot of D_1, D_2, D_3, M, and S at the maturity of both crops. These samples were used to quantify the yield response of maize and soybean to changing planting density in intercropping. All the harvested samples were sun-dried for the next seven to ten days. Then, the dried samples were manually threshed and weighed to determine the maize and soybean grain yield and converted into kg ha^{-1}. Additionally, the total grain yield of intercropping treatments was calculated from the summation of the grain yield of maize and soybean in D_1, D_2, and D_3.

To calculate the radiation use efficiency of both crops under different treatments, we first determine the daily total incident solar radiation (MJ m^{-2} day^{-1}) using the following equation (Angstrom, 1924).

$$SR = SR_0 \left(a + b \times n/N\right)$$

Where, SR_0 was the extraterrestrial radiation. The a and b were the constants and used for those areas where the data for SR is not available (Allen et al., 1998). The n was the measured sunshine hours and the data for n was obtained from near the weather observatory, and N was the maximum possible sunshine hours.

The fraction of intercepted radiation (Fi) of maize and soybean in sole and intercropping systems was calculated using the exponential equation from their respective LAI values (Monteith and Elston, 1983).

$$F_i = 1 - exp\left(-k \times LAI\right)$$

TABLE 2 Rainfall (mm), irrigation water (mm), and total water use (mm) of maize and soybean under sole and intercropping systems at the experimental site of Sichuan Agricultural University, Bahawalpur, South Punjab, Pakistan.

Years	Rainfall					Irrigation water *					Total water use (rainfall + irrigation) **				
	Feb	Mar	April	May	June	Feb	Mar	April	May	June	Feb	Mar	April	May	June
2018	03	03	04	05	62	60	81	121	121	30	63	84	125	126	92
2019	17	09	18	33	28	40	81	121	91	60	57	90	139	124	88
2020	01	216	18	14	31	60	00	101	121	50	61	216	119	135	81

*All treatments were irrigated with the same amount of irrigation water by differentiating the treatments.
**During the whole cropping season, the total water use by maize or soybean under sole or intercropping systems was 490 mm in 2018, 498 mm in 2019, and 613 mm in 2020.

Where, k was the extinction coefficient for total solar radiation (Monteith, 1977; Muurinen and Peltonen-Sainio, 2006), and the values of k for maize and soybean were 0.70 (Lindquist et al., 2005) and 0.45 (Zhang et al., 2014), respectively.

The total amount of incident photosynthetically active radiation (*Si*) was determined by multiplying the total incident radiation by 0.50 because researchers have concluded that the incident photosynthetically active radiation is equal to half (50%) of the daily total incident radiation (Szeicz, 1974; Sinclair and Muchow, 1999; Tesfaye et al., 2006). Then, the amount of intercepted radiation (*Sa*) for maize and soybean under sole and intercropping systems was calculated using the following equation (Szeicz, 1974).

$$Sa = F_i \times S_i$$

Finally, the radiation use efficiency (RUE) of maize and soybean under sole and intercropping systems were calculated individually using the following equation (Monteith, 1977).

$$RUE = \frac{TDM}{\sum Sa}$$

Where, *TDM* was the total dry matter of maize or soybean, $\sum Sa$ was the cumulative intercepted photosynthetically active radiation of maize or soybean.

For calculating water use efficiency (WUE), we first measured the total water use (TWU) of maize and soybean in different treatments using the simplified water balance equation (Raza et al., 2021b).

$$TWU = P + IW + SWs - SWh$$

Where *P* was the total precipitation (mm) received during the whole growing period (from February to July), *IW* was the total amount of applied irrigation water (mm), *SWs* and *SWh* were the soil water content (mm) at sowing and harvesting of the experiment, respectively. Then, the water use efficiency of both crops was calculated using the following equation (Zhang et al., 1998):

$$WUE = {}^{GY}/_{TWU}$$

Where, *GY* was the grain yield of maize or soybean in intercropping or sole cropping systems, and *TWU* was the total water use calculated using the simplified water balance equation.

Furthermore, we calculated the water equivalent ratio (WER) to estimate the water-use advantage of intercropping over sole cropping system, and the partial WER of maize (WER_{Maize}) and soybean ($WER_{Soybean}$), and total WER was calculated using the following equations (Mao et al., 2012):

$$WER_{Maize} = \frac{WUE_{IM}}{WUE_M}$$

$$WER_{Soybean} = \frac{WUE_{IS}}{WUE_S}$$

$$Total\ \ WER = WER_{Maize} + \ WER_{Soybean}$$

Where, WUE_{IM} and WUE_{IS} were the water use efficiency of intercropped maize and soybean, respectively. The WUE_M and WUE_S were the grain yield of sole cropped maize and soybean, respectively.

We measured the land equivalent ratio (LER) to determine the land use advantage of intercropping over the sole cropping system (Raza et al., 2021b). The partial LER of maize (LER_{Maize}) and soybean ($LER_{Soybean}$), and total LER was calculated using the following equations:

$$LER_{Maize} = \frac{GY_{IM}}{GY_M}$$

$$LER_{Soybean} = \frac{GY_{IS}}{GY_S}$$

$$Total\ \ LER = LER_{Maize} + \ LER_{Soybean}$$

Where, GY_{IM} and GY_{IS} were the grain yield of intercropped maize and soybean, respectively. The GY_M and GY_S were the grain yield of sole cropped maize and soybean, respectively.

Economic analysis

An economic analysis was performed to assess the economic viability of the maize/soybean intercropping system. Total expenditure for maize and soybean production under intercropping and sole cropping system was included; the cost of land rent, maize and soybean grains, land preparation, fertilizer (i.e., Urea, DAP, and SOP), weeding, thinning, irrigation, harvesting, and threshing of crops. Each treatment's total income (gross income) was estimated according to the yearly local market prices for maize and soybean grains in Pakistan. The net profit was calculated by subtracting the total expenditure from the total income (Raza et al., 2018).

Statistical analysis

All data analyses were performed using Statistix 8.1. Significant differences were determined using ANOVA, and the LSD (Least Significance Difference) test was used to compare the means at a 5% probability level. Mean values are presented mean ± SE (standard error), based on the three independent replicates per treatment.

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Results

Growth parameters

The LAI of maize and soybean under different planting systems is shown in Figure 3. At all sampling times, the LAI of maize and soybean were significantly lower under intercropping than sole maize and soybean. In intercropping treatments, at the final sampling time (125 DAS), the average highest soybean (4.2) and maize (4.6) LAI was measured under D_1 and D_2, whereas the average lowest soybean (3.1) and maize (3.5) LAI was recorded in D_3 and D_1, respectively. However, at all sampling times, the total LAI of maize and soybean in intercropping treatments was significantly higher than M and S (Table 3). For instance, at 125 DAS, the total LAI in D_1, D_2, and D_3,

Different treatments significantly affected the total dry matter production of maize and soybean. Across different sampling stages and treatments, maize and soybean plants accumulated higher dry matter in M and S, respectively, than intercropping treatments. In contrast, at the final sampling stage (125 DAS), the average total dry matter (maize dry matter + soybean dry matter; Table 3) of D_2 (361.2 g plant^{-1}) was higher than the corresponding values of dry matter in M (338.7 g plant^{-1}) and S (64.8 g plant^{-1}). In intercropping treatments, maize accumulated the highest (319.9 g plant^{-1}) and lowest (218.6 g plant^{-1}) dry matter under D_2 and D_3, while soybean accumulated the maximum (52.4 g plant^{-1}) and minimum (30.9 g plant^{-1}) dry matter in D_1 and D_3, respectively (Figure 4). In addition, different maize planting density treatments in intercropping not only affected dry matter production of intercrops but also changed dry matter partitioning in various plant parts of maize (Table 4) and soybean (Table 5). For example, across the years, at 125 DAS, treatment D_2 significantly increased dry matter of maize grains by 13% and 46% compared to D_1 and D_3, while treatment D_1 enhanced dry matter of soybean grains by 21% and 47% compared to D_2 and D_3, respectively. Whereas, relative to D_2, the treatment D_3 significantly decreased dry matter of maize and soybean roots (by 29% and 19%), straw (by 32% and 29%), and grains (by 31% and 18%), respectively, indicating that the high maize planting density in intercropping caused a significant reduction in dry matter accumulation and partitioning to economic parts (i. e., grains).

Crop level yields and system-level yield

Grain yield by the intercropped maize and soybean in D_1, D_2, and D_3, compared to sole cropping treatments, is presented in Figure 5. The grain yield of maize and soybean in intercropping treatments ranged from 7376.9 to 9047.5 kg ha^{-1} and 830.9 to 1193.5 kg ha^{-1}, respectively, which were

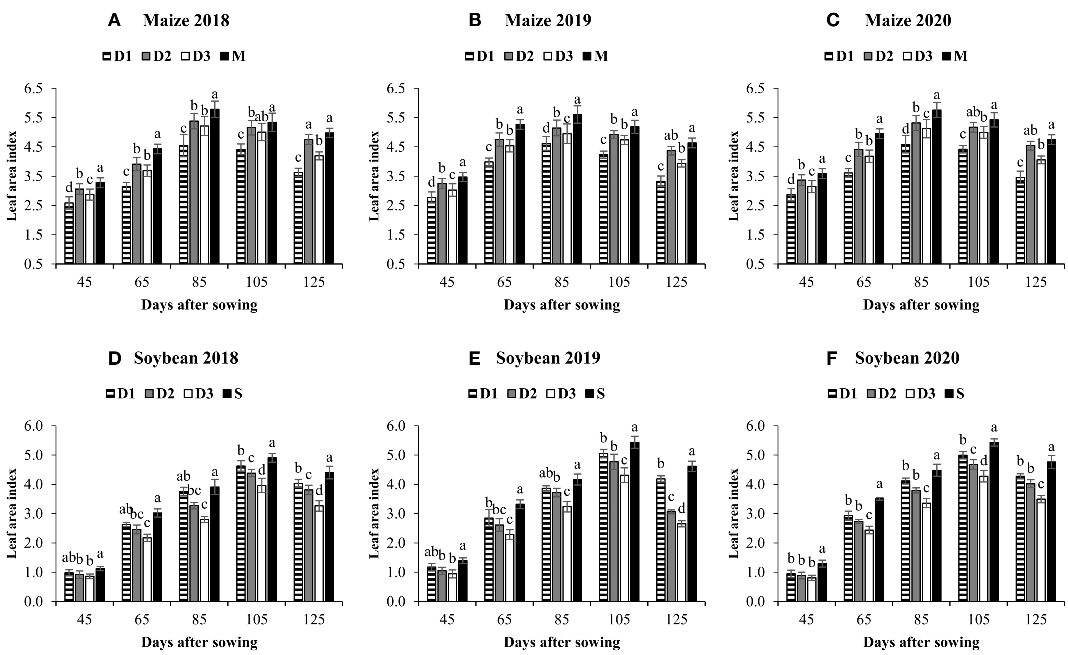

FIGURE 3
Leaf area index of maize **(A–C)** and soybean **(D–F)** in response to different maize planting densities (6 maize plants m^{-2}, low, D_1; 8 maize plants m^{-2}, medium, D_2; and 10 maize plants m^{-2}, high, D_3) under maize/soybean strip intercropping. Bars show ± standard errors (n = 3). The different lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments. The M and S represent the sole maize and soybean, respectively.

TABLE 3 Total leaf area index and total dry matter of maize and soybean at 45, 65, 85, 105, and 125 days after sowing (DAS) under different maize/soybean strip intercropping treatments and sole cropping of maize and soybean.

Year	Treatments	Total leaf area index (Maize leaf area index + soybean leaf area index)					Total dry matter (g plant^{-1}) (Maize dry matter + soybean dry matter)				
		45 DAS	65 DAS	85 DAS	105 DAS	125 DAS	45 DAS	65 DAS	85 DAS	105 DAS	125 DAS
2018	D_1	3.6 ± 0.2b	5.8 ± 0.2a	8.3 ± 0.4ab	9.0 ± 0.1a	7.7 ± 0.2b	23.9 ± 3.4a	117.8 ± 9.6a	201.6 ± 21.7a	278.0 ± 28.3a	315.1 ± 37.4a
	D_2	4.0 ± 0.2a	6.4 ± 0.1a	8.7 ± 0.2a	9.5 ± 0.2a	8.6 ± 0.3a	23.7 ± 2.6a	122.8 ± 7.4a	203.2 ± 10.1a	300.6 ± 12.8a	345.4 ± 22.8a
	D_3	3.7 ± 0.2b	5.9 ± 0.3a	8.0 ± 0.4b	9.0 ± 0.2a	7.5 ± 0.3b	17.5 ± 1.2b	86.2 ± 3.8b	137.9 ± 6.4b	202.0 ± 18.6b	232.5 ± 20.7b
	M	3.3 ± 0.2c	4.4 ± 0.2b	5.8 ± 0.3c	5.3 ± 0.3b	5.0 ± 0.2c	15.7 ± 1.7b	126.7 ± 12.1a	207.1 ± 6.8a	296.0 ± 16.9a	324.2 ± 12.1a
	S	1.1 ± 0.1d	3.0 ± 0.1c	3.9 ± 0.3d	4.9 ± 0.1b	4.4 ± 0.2c	15.1 ± 1.3b	24.5 ± 2.2c	45.1 ± 4.6c	56.6 ± 4.3c	65.2 ± 4.8c
	LSD	0.3	0.6	0.5	0.6	0.6	5.4	22.8	28.5	37.4	42.8
2019	D_1	3.9 ± 0.2a	6.8 ± 0.4a	8.5 ± 0.3ab	9.3 ± 0.1ab	7.5 ± 0.2	30.9 ± 3.2a	151.7 ± 14.2a	235.4 ± 27.7a	325.2 ± 34.2a	375.5 ± 39.7a
	D_2	4.3 ± 0.1a	7.4 ± 0.4a	8.9 ± 0.2a	9.7 ± 0.3ab	7.4 ± 0.2	31.4 ± 3.5a	159.6 ± 11.3a	238.2 ± 21.3a	336.8 ± 23.8a	389.1 ± 29.1a
	D_3	4.0 ± 0.1a	6.8 ± 0.4a	8.2 ± 0.5b	9.1 ± 0.3b	6.6 ± 0.2	23.5 ± 1.3b	113.4 ± 6.5b	172.2 ± 11.2b	236.2 ± 18.8b	270.1 ± 21.2b
	M	3.5 ± 0.2b	5.3 ± 0.2b	5.6 ± 0.3c	5.2 ± 0.2c	4.6 ± 0.2	19.5 ± 1.6b	158.7 ± 12.6a	241.6 ± 12.6a	317.8 ± 18.6a	357.6 ± 21.1a
	S	1.4 ± 0.1c	3.3 ± 0.2c	4.2 ± 0.2d	5.4 ± 0.2c	4.6 ± 0.2	21.2 ± 1.4b	35.1 ± 1.8c	45.4 ± 6.6c	60.1 ± 6.5c	71.2 ± 3.6c
	LSD	0.4	0.8	0.5	0.6	0.5	4.0	25.6	37.1	35.2	46.9
2020	D_1	3.8 ± 0.2b	6.5 ± 0.3a	8.7 ± 0.3ab	9.4 ± 0.1ab	7.7 ± 0.2b	26.1 ± 3.1a	137.3 ± 12.8a	212.7 ± 25.0a	301.8 ± 30.1a	348.1 ± 36.3a
	D_2	4.3 ± 0.1a	7.1 ± 0.3a	9.1 ± 0.2a	9.8 ± 0.3a	8.6 ± 0.3a	26.2 ± 2.8a	146.3 ± 10.2a	214.8 ± 16.1a	305.0 ± 18.1a	350.1 ± 25.6a
	D_3	4.0 ± 0.2ab	6.6 ± 0.3a	8.5 ± 0.5b	9.3 ± 0.3b	7.6 ± 0.2b	18.8 ± 1.4b	102.5 ± 6.7b	155.1 ± 8.9b	212.6 ± 12.7b	245.9 ± 15.8b
	M	3.6 ± 0.2b	4.9 ± 0.2b	5.8 ± 0.3c	5.4 ± 0.2c	4.8 ± 0.2c	18.4 ± 1.3b	151.7 ± 11.6a	224.3 ± 13.8a	311.4 ± 12.8a	334.3 ± 18.7a
	S	1.3 ± 0.1c	3.5 ± 0.0c	4.5 ± 0.2d	5.4 ± 0.1d	4.8 ± 0.2c	16.3 ± 0.4b	26.3 ± 1.9c	41.7 ± 5.2c	54.7 ± 4.9c	57.9 ± 9.5c
	LSD	0.4	0.6	0.5	0.6	0.6	4.3	23.2	34.9	37.4	37.3

The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The M refers to the sole cropping of maize, and the S refers to the sole cropping of soybean. Bars show ± standard errors, (n = 3). The lowercase letters within a bar show a significant difference (p< 0.05) among treatments.

significantly lower than the three-years average grain yield of M (9553.7 kg ha^{-1}) and S (1826.2 kg ha^{-1}). However, across the years, the total grain yield of maize and soybean was significantly higher in D_2 (10122.5 kg ha^{-1}) compared to D_1 (9160.7 kg ha^{-1}) and D_3 (8207.9 kg ha^{-1}), and it was also higher than the grain yield of M and S (Figure 5C). Furthermore, among the intercropping treatments, the grain yield of maize significantly increased with increasing maize density from 6 maize plants m^{-2} (D_1) to 8 maize plants m^{-2} (D_2), while it decreased under 10 maize plants m^{-2} (D_3). Contrarily, soybean grain yield significantly reduced with increasing maize density, and the maximum (1193.5 kg ha^{-1}) and minimum (830.9 kg ha^{-1}) soybean grain yield were obtained in D_1 and D_3, respectively. Overall, in D_1, D_2, and D_3, maize produced 83%, 95%, and 77% of M yield, and soybean produced 65%, 59%, and 45% of S yield, respectively.

Resource (water and radiation) utilization dynamics

The RUE of maize and soybean differed significantly in all treatments, and data are presented in Table 6. Across the years, the partial RUE of intercropped maize (3.5 g MJ^{-1} in D_1, 5.2 g MJ^{-1} in D_2, and 4.3 g MJ^{-1} in D_3) and soybean (2.5 g MJ^{-1} in D_1, 2.1 g MJ^{-1} in D_2, and 1.8 g MJ^{-1} in D_3) were significantly lower than the corresponding values of M (5.9 g MJ^{-1}) and S (3.2 g MJ^{-1}). However, the total RUE of maize and soybean in intercropping was considerably higher than that of the M and S, indicating the advantage of intercropping in utilizing the sunlight than sole systems. Additionally, in intercropping, the RUE of maize was higher than that of soybean, demonstrating the dominance of maize over soybean. On average, D_2 enhanced the total RUE by 20% and 18% compared to D_1 and D_3, respectively.

There were significant differences in WUE of maize and soybean in intercropping and sole cropping treatments, and data are shown in Table 6. Based on average WUE values in three years, the WUE of maize (14.3 kg ha^{-1} mm^{-1} in D_1, 16.2 kg ha^{-1} mm^{-1} in D_2, and 13.3 kg ha^{-1} mm^{-1} in D_3) and soybean (2.1 kg ha^{-1} mm^{-1} in D_1, 1.9 kg ha^{-1} mm^{-1} in D_2, and 1.5 kg ha^{-1} mm^{-1} in D_3) in intercropping treatments was found significantly lower than that of M (17.1 kg ha^{-1} mm^{-1}) and S (3.2 kg ha^{-1} mm^{-1}), respectively. However, the effect of intercropping on WUE was determined using the values of WER because it characterizes whether the total yield of maize and soybean in D_1, D_2, and D_3 will be produced with more water (WER > 1) or less water (WER< 1) in sole maize and soybean treatments, and data are shown in Table 6. In this study, the mean total WER (WER_{Maize} + $WER_{Soybean}$) values of D_1 (1.50), D_2 (1.54),

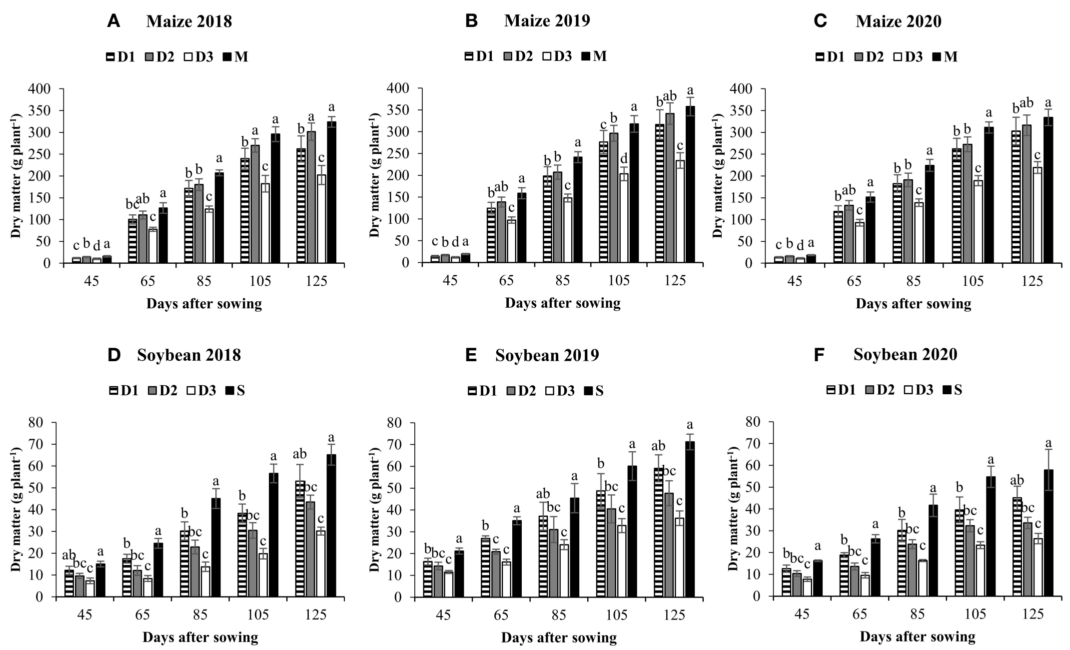

FIGURE 4
Dry matter of maize **(A–C)** and soybean **(D–F)** in response to different maize planting densities (6 maize plants m^{-2}, low, D_1; 8 maize plants m^{-2}, medium, D_2; and 10 maize plants m^{-2}, high, D_3) under maize/soybean strip intercropping. Bars show ± standard errors (n = 3). The different lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments. The M and S represent the sole maize and soybean, respectively.

and D_3 (1.24) were consistently higher than unity, demonstrating the water use advantage of intercropping over sole cropping. Moreover, in intercropping treatments, the partial WER values of maize were consistently higher than the partial WER values of soybean, showing that the maize had a competitive advantage over soybean in using the available water. The maximum WER_{Maize} and $WER_{Soybean}$ were in D_2 and D_1, while the minimum WER_{Maize} and $WER_{Soybean}$ were in D_1 and D_3, respectively.

Land productivity and economic viability

The total LER (LER_{Maize} + $LER_{Soybean}$) of intercropping treatments ranged from 1.22 to 1.55 in the three years of this experiment, and data are given in Table 6. Thus, there was a substantial land-use advantage under intercropping over sole cropping treatments. On average, in intercropping, the total LER was consistently higher in D_2 (1.54) than D_1 (1.50) and D_3 (1.23). Across years and intercropped species, the partial LER values of maize and soybean in intercropping treatments ranged from 0.77 to 0.95 and 0.45 to 0.67, respectively. In intercropping treatments, soybean had the lowest partial LER values, and it decreased with increasing maize planting density. In contrast, maize had the high partial LER values, and it increased from low to medium maize planting density, and then decreased with high maize planting density. Despite the low soybean partial LER values, all the intercropping treatments achieved the high total LER values because the considerable yield of soybean compensated the slight yield loss of maize in D_1, D_2, and D_3 compared to M. Overall, the medium (D_2) maize planting density treatment increased the total LER by 3% and 25% relative to low (D_1) and high (D_3) maize planting density treatments, respectively.

Variations in grain yield directly affected the gross income and net income of D_1, D_2, D_3, M, and S, and data are presented in Table 7. Across the years, the highest gross (2624 US \$ ha^{-1}) and net (1300 US \$ ha^{-1}) income were obtained under treatment D_2, whereas the lowest gross (1539 US \$ ha^{-1}) and net (703 US \$ ha^{-1}) income were noticed in S treatment. Overall, the intercropping treatment D_2, enhanced the net income by 63% compared to M and by 85% compared to S, respectively, indicating that the intercropping had an advantage over M and S in utilizing the available resources, i. e., radiation, water, and land.

Discussion

The combination of maize and soybean as intercropping is a better option for irrigated areas under semi-arid conditions. Our three-year field study proved this, where we recorded high land- and water-equivalent ratios, showing a substantial increase in

TABLE 4 Dry matter partitioning in different plant parts of maize at 45, 65, 85, 105, and 125 days after sowing (DAS) under different maize/soybean strip intercropping treatments and sole cropping of maize.

Year	Treatments	Maize dry matter distribution (g $plant^{-1}$)											
		45 DAS		65 DAS		85 DAS		105 DAS			125 DAS		
		Root	Straw	Root	Straw	Root	Straw	Root	Straw	Grain	Root	Straw	Grain
2018	D_1	1.4 ± 0.1b	10.3 ± 1.5c	11.7 ± 2.1b	88.6 ± 8.6ab	15.9 ± 2.2a	155.6 ± 16.0b	18.3 ± 2.1b	177.4 ± 17.4	44.0 ± 5.4bc	21.4 ± 3.1b	169.1 ± 17.4b	71.5 ± 9.9c
	D_2	1.7 ± 0.2a	12.4 ± 1.4b	14.2 ± 1.5a	96.5 ± 7.8a	17.3 ± 1.7a	163.0 ± 11.7ab	19.3 ± 1.7b	195.1 ± 11.2	55.8 ± 6.7ab	23.6 ± 2.3b	188.9 ± 11.2ab	89.5 ± 9.7b
	D_3	1.1 ± 0.2c	9.0 ± 1.3d	9.4 ± 1.0c	68.4 ± 3.8b	12.0 ± 1.0b	112.2 ± 6.1c	13.4 ± 0.9c	132.4 ± 13.1	36.4 ± 6.2c	16.4 ± 1.5c	127.4 ± 13.1c	58.5 ± 8.2d
	M	1.8 ± 0.1a	13.9 ± 1.6a	16.0 ± 1.5a	110.6 ± 11.8a	19.3 ± 2.6a	187.8 ± 7.8a	21.4 ± 1.9a	206.1 ± 10.4	68.6 ± 9.1a	26.3 ± 2.7a	199.9 ± 10.4a	98.0 ± 8.0a
	LSD	0.25	0.98	2.20	24.91	3.39	25.39	1.95	28.02	13.61	2.56	28.05	5.51
2019	D_1	2.1 ± 0.2c	12.5 ± 1.5c	13.9 ± 2.0c	110.8 ± 11.4bc	19.5 ± 2.0b	178.7 ± 19.5b	22.4 ± 3.1b	201.8 ± 19.3b	52.2 ± 4.4b	26.2 ± 3.6b	193.4 ± 19.3b	96.8 ± 11.1b
	D_2	2.3 ± 0.2b	14.7 ± 1.6b	16.6 ± 1.7b	122.2 ± 9.6ab	20.4 ± 1.5b	186.8 ± 14.8b	24.2 ± 1.7ab	212.4 ± 14.3ab	59.7 ± 5.4ab	28.2 ± 2.6b	206.2 ± 14.3ab	107.0 ± 8.5a
	D_3	1.6 ± 0.2d	10.3 ± 1.5d	11.0 ± 1.4d	86.3 ± 6.0c	14.6 ± 1.0c	133.6 ± 8.1c	17.0 ± 1.4c	145.1 ± 10.2c	41.2 ± 4.8c	20.0 ± 2.3c	140.1 ± 10.2c	73.7 ± 5.7c
	M	2.5 ± 0.2a	17.0 ± 1.4a	19.1 ± 1.6a	139.7 ± 11.9a	23.0 ± 1.4a	218.5 ± 12.0a	26.7 ± 1.5a	221.9 ± 10.9a	69.3 ± 6.9a	31.8 ± 2.8a	217.3 ± 10.1a	108.4 ± 8.9a
	LSD	0.20	1.36	2.24	25.65	1.66	31.20	3.02	17.74	9.81	2.04	18.41	7.64
2020	D_1	2.1 ± 0.2b	11.4 ± 1.3c	12.9 ± 1.7c	105.5 ± 11.4b	18.5 ± 1.6	164.1 ± 18.7b	21.1 ± 3.2b	190.9 ± 17.5b	50.1 ± 3.7b	24.3 ± 3.4b	182.6 ± 17.5b	96.1 ± 10.7a
	D_2	2.2 ± 0.2b	13.5 ± 1.4b	15.4 ± 1.6b	117.4 ± 9.3ab	19.0 ± 1.3	172.0 ± 14.3b	23.2 ± 1.5ab	195.4 ± 13.9b	54.1 ± 3.9ab	26.2 ± 2.4ab	189.1 ± 13.9ab	101.2 ± 7.8a
	D_3	1.7 ± 0.2c	9.3 ± 1.4d	10.2 ± 1.4d	82.7 ± 6.3c	13.7 ± 0.8	125.1 ± 7.9c	16.3 ± 1.4c	134.3 ± 7.3c	38.6 ± 3.5c	18.7 ± 2.3c	129.3 ± 7.3c	71.5 ± 4.2b
	M	2.5 ± 0.2a	15.9 ± 1.1a	17.8 ± 1.4a	133.8 ± 10.8a	21.6 ± 1.5	202.7 ± 12.4a	25.5 ± 1.1a	225.1 ± 10.3a	60.8 ± 4.8a	28.7 ± 3.2a	201.2 ± 13.1a	104.5 ± 3.2a
	LSD	0.21	1.53	2.16	22.67	2.65	30.16	3.53	29.12	7.16	2.49	18.14	14.05

The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The M refers to the sole cropping of maize. Bars show ± standard errors, (n = 3). The lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments.

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TABLE 5 Dry matter partitioning in different plant parts of soybean at 45, 65, 85, 105, and 125 days after sowing (DAS) under different maize/soybean strip intercropping treatments and sole cropping of soybean.

Year	Treatments	Soybean dry matter distribution (g plant^{-1})											
		45 DAS		65 DAS		85 DAS		105 DAS			125 DAS		
		Root	Straw	Root	Straw	Root	Straw	Root	Straw	Grain	Root	Straw	Grain
2018	D_1	0.9 ± 0.1ab	11.3 ± 1.8ab	1.7 ± 0.2ab	15.8 ± 2.0b	4.1 ± 0.5b	26.1 ± 3.9ab	4.7 ± 0.5b	29.2 ± 2.7b	4.3 ± 1.3b	8.5 ± 0.6b	37.1 ± 6.2ab	7.5 ± 0.9b
	D_2	0.8 ± 0.1b	8.8 ± 1.1bc	1.4 ± 0.1bc	10.7 ± 2.2bc	3.4 ± 0.3bc	19.4 ± 3.4bc	3.9 ± 0.5c	22.9 ± 4.5b	3.7 ± 0.6bc	6.7 ± 0.2bc	30.4 ± 2.8bc	6.3 ± 0.7bc
	D_3	0.7 ± 0.1b	6.6 ± 1.4c	1.3 ± 0.2c	7.1 ± 1.5c	2.9 ± 0.3c	10.9 ± 1.9c	3.3 ± 0.4c	14.1 ± 2.5bc	2.4 ± 0.7c	5.2 ± 0.8c	19.8 ± 1.7c	5.1 ± 0.7c
	S	1.0 ± 0.2a	14.1 ± 1.4a	2.1 ± 0.1a	22.5 ± 2.1a	5.3 ± 0.5a	39.7 ± 4.5a	5.6 ± 0.7a	43.3 ± 3.0a	7.6 ± 1.6a	11.9 ± 0.6a	43.6 ± 3.3a	9.7 ± 1.2a
	LSD	0.19	4.53	0.38	6.51	1.00	13.99	0.62	12.79	1.93	2.17	11.55	2.01
2019	D_1	1.2 ± 0.1ab	15.1 ± 1.5b	2.5 ± 0.4a	24.5 ± 1.0b	4.5 ± 0.7b	32.7 ± 5.8ab	5.7 ± 0.9ab	37.5 ± 6.2ab	5.6 ± 0.9b	9.2 ± 1.8ab	41.3 ± 4.2ab	8.6 ± 0.4b
	D_2	1.1 ± 0.1b	13.3 ± 1.7bc	1.9 ± 0.2b	18.9 ± 1.1c	3.9 ± 0.8bc	27.1 ± 5.3bc	4.4 ± 0.9bc	31.2 ± 4.8bc	4.9 ± 1.0bc	7.7 ± 1.7bc	32.9 ± 4.0bc	7.1 ± 0.3c
	D_3	0.9 ± 0.1b	10.7 ± 0.7c	1.4 ± 0.2c	14.7 ± 1.4c	3.2 ± 0.3c	20.9 ± 2.1c	3.9 ± 0.3c	25.2 ± 2.7c	3.7 ± 0.2c	6.4 ± 0.9c	23.8 ± 2.0c	6.0 ± 0.5c
	S	1.6 ± 0.2a	19.5 ± 1.2a	2.8 ± 0.3a	32.3 ± 1.6a	6.1 ± 0.9a	39.3 ± 5.8a	6.9 ± 0.6a	46.0 ± 5.0a	7.3 ± 1.1a	10.7 ± 1.4a	50.3 ± 1.7a	10.1 ± 0.8a
	LSD	0.46	3.71	0.36	5.17	0.96	8.35	1.39	8.99	1.57	2.52	9.23	1.17
2020	D_1	1.0 ± 0.1ab	11.6 ± 1.6ab	2.0 ± 0.3a	16.8 ± 1.1b	4.1 ± 0.5b	26.1 ± 4.5ab	5.0 ± 0.7b	30.0 ± 4.3b	4.6 ± 1.2b	6.8 ± 1.3ab	31.8 ± 3.8ab	6.6 ± 0.5b
	D_2	0.9 ± 0.1b	9.5 ± 1.3bc	1.6 ± 0.2b	12.0 ± 1.6bc	3.5 ± 0.5bc	20.3 ± 1.9bc	4.0 ± 0.6c	24.3 ± 2.7bc	4.1 ± 0.7bc	5.1 ± 1.0bc	23.2 ± 1.6bc	5.4 ± 0.6bc
	D_3	0.8 ± 0.1b	7.1 ± 1.1c	1.3 ± 0.2b	8.3 ± 1.4c	2.9 ± 0.2c	13.4 ± 0.2c	3.4 ± 0.3c	17.0 ± 1.0c	2.9 ± 0.4c	4.1 ± 0.9c	17.9 ± 1.4c	4.3 ± 0.5c
	S	1.2 ± 0.2a	15.0 ± 0.3a	2.3 ± 0.2a	24.0 ± 1.8a	5.3 ± 0.5a	36.4 ± 4.7a	6.0 ± 0.5a	41.5 ± 3.2a	7.2 ± 1.3a	8.9 ± 1.2a	40.5 ± 8.4a	8.5 ± 0.9a
	LSD	0.29	3.58	0.32	5.51	0.81	11.01	0.71	10.24	1.64	2.27	12.84	1.32

The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The S refers to the sole cropping of soybean. Bars show ± standard errors, (n = 3). The lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments.

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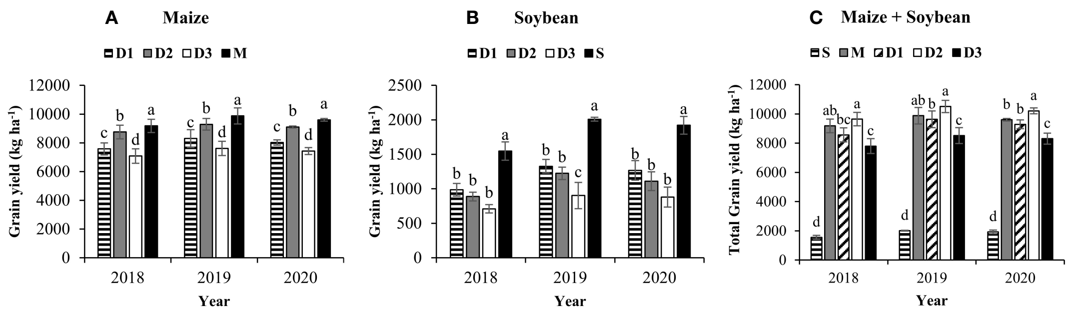

FIGURE 5
Three years average grain yield of maize **(A)**, soybean **(B)**, total grain yield **(C)** in response to different maize planting densities (6 maize plants m^{-2}, low, D_1; 8 maize plants m^{-2}, medium, D_2; and 10 maize plants m^{-2}, high, D_3) under maize/soybean strip intercropping. Bars show ± standard errors (n = 3). The different lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments. The M and S represent the sole maize and soybean, respectively.

land and water use in intercropping treatments over sole cropping systems. Notably, just 50% of the total land was available for maize or soybean in intercropping treatments, while maize or soybean yield in intercropping treatments was higher than half of the sole maize or soybean yield. These results are aligned with the previously observed growth and yield pattern of cereals and legumes under intercropping systems (Li et al., 2020; Raza et al., 2021a). Overall, this shows that the extra yield produced by soybean in intercropping had minor consequences for maize production, and the interaction between maize and soybean was not highly competitive in intercropping treatments. Therefore, the system as a whole (maize + soybean) enhanced the total resource capturing and utilization beyond that of the sole cropping systems due to the complementary resource use of both species in intercropping (Yang et al., 2017; Iqbal et al., 2018; Liu et al., 2018; Ren et al., 2019; Li et al., 2020).

In intercropping, the better growth (measured as leaf area index and total dry matter production) of maize was likely associated with greater light use efficiency (Liu et al., 2018), water use efficiency (Rahman et al., 2017), nutrient accumulation (Ahmed et al., 2018), and plasticity of edge-row plants (Zhu et al., 2016). In contrast, the intercropped soybean growth was significantly lower in intercropping treatments than in sole soybean and this difference was increased with increasing maize density where soybean suffered from heavy maize shading (Yang et al., 2017) and water stress than sole soybean (Raza et al., 2021a). Thus, optimum maize planting density in intercropping (8 maize plants m^{-2}) can increase maize yield with maintained soybean yield by improving the light transmittance at the soybean canopy and reducing the intra-specific competition for available resources, especially for light and water (Zhang 2007; Yang et al., 2015; Feng et al., 2020). Additionally, under semi-arid conditions, maize and soybean growth and yield are easily subjected to water stress (Cui et al., 2020). Therefore, the intercropping of maize with soybean could play a vital role in saving water, especially under semi-arid conditions, because intercropping systems reduce water evaporation due to greater canopy closure, which means that intercrops can produce more grains per mm of water than sole crops (Cooper et al., 1987; Wallace, 2000; Raza et al., 2021b).

Compared to past studies (Gao et al., 2010), the enhanced radiation use efficiency in different maize planting density treatments under maize/soybean intercropping was mainly associated with density and planting arrangement advantage. In this study, we planted both crops using the narrow-wide-row planting arrangement (narrow inter-row distance between maize or soybean rows and wide intra-row distance between maize and soybean strips), which gives the edge row advantage and spatial light distribution advantage. Besides, the total planting density (maize planting density + soybean planting density; Table 1) in intercropping treatments was considerably higher than sole crops (Feng et al., 2019), which resulted in increased radiation use efficiency as it was followed by a high leaf area index (Raza et al., 2021a). Although the individual leaf area index values of intercrop species were lower in intercropping, but the total leaf area index of maize and soybean was relatively higher than sole crops. This might have resulted in an increased light interception in intercropping, which consequently increased the total radiation use efficiency of maize/soybean intercropping than sole maize or sole soybean. Our results are in line with the previous report (Feng et al., 2019), in which they reported greater light interception and radiation use efficiency in maize/soybean intercropping and linked it with an improved leaf area index, light interception, and dry matter production (Liu et al., 2018). However, the partial RUE of intercropped maize or soybean in intercropping was significantly lower than that of sole maize or soybean, indicating the competition for solar radiations between intercrops in intercropping, as reported in many

TABLE 6 Radiation-use-efficiency (RUE), water-use-efficiency (WUE), water equivalent ratio (WER), and land equivalent ratio (LER) of maize and soybean under different maize/soybean strip intercropping treatments and sole cropping of maize and soybean.

Year	Treatments	Radiation use efficiency (g MJ^{-1})			Water use efficiency (kg ha^{-1} mm^{-1})			Water equivalent ratio			Land equivalent ratio		
		Partial RUE		Total RUE	Partial WUE		Total WUE	Partial WER		Total WER	Partial LER		Total LER
		mRUE	sRUE	mRUE + sRUE	mWUE	sWUE	mWUE + sWUE	mWER	sWER	mWER + sWER	mLER	sLER	mLER + sLER
2018	D_1	3.2 ± 0.49d	2.5 ± 0.38b	5.6 ± 0.41b	14.9 ± 1.0b	1.9 ± 0.2b	16.8 ± 1.0b	0.84 ± 0.02b	0.65 ± 0.11^{NS}	1.50 ± 0.13a	0.83 ± 0.01b	0.65 ± 0.11a	1.48 ± 0.11b
	D_2	4.9 ± 0.37b	2.2 ± 0.23bc	7.0 ± 0.24a	16.8 ± 1.6a	1.7 ± 0.2b	18.5 ± 0.7a	0.95 ± 0.01a	0.58 ± 0.04	1.53 ± 0.05a	0.95 ± 0.01a	0.58 ± 0.06ab	1.53 ± 0.06a
	D_3	4.0 ± 0.50c	1.7 ± 0.39c	5.7 ± 0.09b	13.9 ± 1.3b	1.4 ± 0.5b	15.2 ± 1.1c	0.79 ± 0.04b	0.47 ± 0.04	1.25 ± 0.09b	0.77 ± 0.03c	0.46 ± 0.04b	1.23 ± 0.07c
	M	5.7 ± 0.87a	–	–	17.6 ± 0.7a	–	–	–	–	–	–	–	–
	S	–	3.3 ± 0.50a	–	–	2.9 ± 0.3a	–	–	–	–	–	–	–
	LSD	0.05	0.69	0.83	1.12	0.50	1.42	0.08	–	0.18	0.049	0.13	0.13
2019	D_1	3.8 ± 0.41d	2.8 ± 0.34b	6.6 ± 0.33b	15.8 ± 1.1c	2.5 ± 0.3b	18.3 ± 1.0b	0.83 ± 0.02b	0.66 ± 0.05a	1.49 ± 0.05a	0.84 ± 0.02b	0.66 ± 0.06a	1.50 ± 0.06a
	D_2	5.5 ± 0.35b	2.4 ± 0.37bc	7.9 ± 0.38a	17.8 ± 1.6b	2.3 ± 0.5b	20.1 ± 0.8a	0.94 ± 0.01a	0.61 ± 0.04a	1.55 ± 0.03a	0.94 ± 0.01a	0.61 ± 0.05a	1.55 ± 0.05a
	D_3	4.7 ± 0.44c	2.1 ± 0.35c	6.7 ± 0.18b	14.6 ± 1.4d	1.7 ± 0.6c	16.3 ± 1.2c	0.77 ± 0.02b	0.45 ± 0.10b	1.22 ± 0.11b	0.77 ± 0.01c	0.45 ± 0.10b	1.22 ± 0.11b
	M	6.3 ± 0.87a	–	–	18.9 ± 1.7a	–	–	–	–	–	–	–	–
	S	–	3.6 ± 0.44a	–	–	3.8 ± 0.3a	–	–	–	–	–	–	–
	LSD	0.45	0.59	0.53	0.95	0.58	1.61	0.07	0.15	0.18	0.06	0.13	0.14
2020	D_1	3.7 ± 0.32d	2.1 ± 0.31b	5.8 ± 0.27b	12.3 ± 0.4c	2.0 ± 0.3b	14.3 ± 0.5b	0.83 ± 0.01b	0.67 ± 0.11a	1.50 ± 0.12a	0.83 ± 0.01b	0.67 ± 0.11a	1.51 ± 0.12a
	D_2	5.1 ± 0.28b	1.7 ± 0.20b	6.8 ± 0.26a	14.0 ± 1.1b	1.7 ± 0.3b	15.8 ± 0.4a	0.95 ± 0.02a	0.59 ± 0.09a	1.54 ± 0.10a	0.95 ± 0.01a	0.59 ± 0.10b	1.53 ± 0.10a
	D_3	4.4 ± 0.40c	1.5 ± 0.20b	5.9 ± 0.13b	11.4 ± 0.9d	1.4 ± 0.5b	12.8 ± 0.5c	0.77 ± 0.01c	0.47 ± 0.09b	1.24 ± 0.10b	0.77 ± 0.02c	0.47 ± 0.09c	1.24 ± 0.11b
	M	5.9 ± 0.76a	–	–	14.8 ± 0.8a	–	–	–	–	–	–	–	–
	S	–	2.9 ± 0.59a	–	–	3.0 ± 0.3a	–	–	–	–	–	–	–
	LSD	0.42	0.65	0.62	0.57	0.64	0.59	0.05	0.10	0.09	0.05	0.07	0.08

The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The M and S refers to the sole cropping of maize and soybean, respectively. Bars show ± standard errors, (n = 3). The lowercase letters within a bar show a significant difference ($p < 0.05$) among treatments. NS refers to non-significant difference ($p < 0.05$) among treatments.

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TABLE 7 Total expenditure and total net income of maize and soybean under different maize/soybean strip intercropping treatments and sole cropping of maize and soybean.

Treatments	Total Expenditure (US $ ha^{-1})			Total Net Income (US $ ha^{-1})			Average (US $ ha^{-1})	
	2018	2019	2020	2018	2019	2020	Expenditure	Net Income
D_1	1542	1220	1131	896	1464	1185	1298	1182
D_2	1574	1246	1155	1051	1574	1274	1325	1300
D_3	1606	1272	1178	511	969	788	1352	756
M	1415	1120	1038	623	962	810	1191	798
S	993	786	728	559	868	683	836	703

The D_1 (6 maize plants m^{-2}, low, D_1), D_2 (8 maize plants m^{-2}, medium, D_2), and D_3 (10 maize plants m^{-2}, high, D_3) represent the three maize/soybean intercropping treatments differing with maize plant density. The M refers to the sole cropping of maize, and the S refers to the sole cropping of soybean.

previous studies (Gao et al., 2010; Feng et al., 2020; Raza et al., 2020). Therefore, the radiation use efficiency of intercropping systems can be increased by selecting the optimum planting density of intercrop species, especially of tall crops (i. e., maize, millet, sorghum, etc.) because it directly influences the light environment of short stature crops (i. e., soybean, peanut, pea, etc.) in cereal legume intercropping systems.

The data of water equivalent ratio indicated that maize/soybean intercropping considerably increased the water use efficiency. Considering that the intercropping had a 175% planting density in D_1, 200% planting density in D_2, and 225% planting density in D_3, indicating that the intercropped soybean and maize produced more seeds mm^{-1} of water than sole maize or soybean because under intercropping treatments the total available water was halved for soybean and maize. In addition, the different maize planting density treatments significantly affected the water use efficiency of intercropped species. The increasing maize density from 8 to 10 maize plants m^{-2} decreased the water use efficiency and partial water equivalent ratio of maize and soybean, suggesting the competition for water first among maize plants and second between maize and soybean plants, which means that appropriate planting density of intercrop species is critical in achieving high water productivity through resource complementarity (Mao et al., 2012), especially under the scenario of limited water resources (Ren et al., 2016). Interestingly, in all treatments, maize produced more grains mm^{-1} of water than soybean because maize had a competitive advantage over soybean in root growth and development, which ultimately increased the water uptake and used in maize than soybean (Raza et al., 2021b). However, despite this asymmetry in water uptake and use between soybean and maize, all intercropping treatments were still advantageous in translating water into grains, as indicated by total grain yields. This improvement in water use efficiency in D_1, D_2, and D_3 might be caused by: (i) the water use efficiency of maize and soybean in intercropping depends on the selection of appropriate planting density, especially of maize (Ren et al., 2016); (ii) medium planting density of maize (8 maize plants m^{-2}; D_2) increased the water use efficiency of maize and maintained the water use efficiency of soybean under maize/soybean intercropping, which in return increased the total water equivalent ratio (Raza et al., 2021b); and (iii) all intercropping treatments were irrigated with the same amount of water as sole soybean or maize but produced more grains mm^{-1} of water, which might be associated with reduced evapotranspiration from the soil and plant surface due to greater canopy closure in intercropping (Cooper et al., 1987; Wallace, 2000). Another possible reason for high WER is related to complementarity in water uptake lower and upper soil depths by maize and soybean, respectively (Bai et al., 2016). However, more research is needed to understand complementarity in water acquisition from different soil depths by intercrops.

Total economic return (net profit) is the main factor for adopting any new planting method or practice (Piepho, 1998; Raza et al., 2019). Agreeing with previous results (Du et al., 2017; Li et al., 2020), the findings of this study demonstrate high resource (radiation, water, and land) use advantages, crop yield stability, and total net profit of all intercropping treatments over the sole maize and sole soybean under semi-arid conditions with irrigation. Additionally, the higher net profit of intercropping over sole cropping suggested that farmers could plant soybean and maize together in intercropping with a minimal overall yield penalty. The improvement in greater economic returns mainly attributed to an extra yield of soybean with maintained maize yield, especially under D_2, which ultimately increased the total profit by 63% and 85% over sole maize and soybean because, in the local market, the price of soybean is three times expensive than maize price. Therefore, we can conclude that intercropping of soybean with maize, especially at eight maize plants m^{-2}, is the better planting practice to obtain high economic returns with limited resources. Moreover, with appropriate planting configuration and density in maize/soybean strip intercropping, farmers can increase soybean production without decreasing the maize production and area, ultimately improving soil fertility and productivity through nitrogen fixation and release of root exudates (Chen et al., 2017). However, future studies are needed to quantify the resource use mechanism of intercropped maize and soybean in intercropping, especially under the changing climate scenarios. For instance, crops under intercropping may perform differently under low light regions (i. e., Sichuan in China), and farmers need to reduce the overall planting density to avoid the mutual shading effect on intercrops.

Conclusion

The system yield (maize yield + soybean yield), resource utilization (radiation and water), and net income advantages of intercropping over sole cropping were high and consistent over three years, indicating that intercropping is a more effective and profitable planting system than sole systems. Overall, these results indicate that optimizing strip intercropping systems can save 20–50% of water and land, especially under the present scenario of limited resources and climate change. Therefore, we can conclude that intercropping could be a productive and sustainable system to alleviate poverty and drought risk, especially for small landholder farmers in developing countries. However, future studies are required to quantify the resource use mechanism of intercrops in intercropping, particularly in the present climate change scenario. Moreover, intercropping-specific small farm machinery is needed (sowing and haversting specific equipments) to obtain the maximum advantages of intercropping; without resolving this issue, we cannot attain the full benefits of intercropping systems.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

MAR, HSY and HG: design and conceived research and writing original draft; RQ, AMD, MHBK, and SH: writing, reviewing and editing; JW, HGT, AS; reviewing, editing and analysis; AM, ERC, AF, and SA: data curation; FY, MS and WY: project administration and supervision; WY: reviewing and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Muhammad Ali Raza,
Gansu Academy of Agricultural Sciences (CAAS), China

REVIEWED BY
Muhammad Hayder Bin Khalid,
Sichuan Agricultural University, China
Sana Ur Rehman,
Pir Mehr Ali Shah Arid Agriculture University, Pakistan

*CORRESPONDENCE
Arthur Bernardes Cecílio Filho
rutra@fcav.unesp.br

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Greenhouse gas emissions and carbon footprint of collard greens, spinach and chicory production systems in Southeast of Brazil

Breno de Jesus Pereira[1], Arthur Bernardes Cecílio Filho[1]*, Newton La Scala Jr.[1] and Eduardo Barretto de Figueiredo[2]

[1]College of Agricultural and Veterinarian Sciences, São Paulo State University - Sao Paulo State University (UNESP), São Paulo, Brazil, [2]Department of Rural Development, Federal University of São Carlos (UFSCar), São Paulo, Brazil

Food production in sustainable agricultural systems is one of the main challenges of modern agriculture. Vegetable intercropping may be a strategy to mitigate greenhouse gas (GHG) emissions, replacing monoculture systems. The objective is to identify the main emissions sources and to estimate GHG emissions of intercropping and monoculture production of collard greens, New Zealand spinach and chicory. Four scenarios were evaluated: ICS – intercropping collard greens and spinach; MCS – monoculture collard greens and spinach; ICC – intercropping collard greens and chicory; MCC - monoculture collard greens and chicory. The boundaries' reach from "cradle-to-gate" and the calculation of GHG emissions were performed using IPCC methodology and specific factors (Tier 2). The total GHG emitted was standardized as CO_2 equivalent (CO_2eq). The GHG emissions in ICS and ICC scenarios were approximately 31% lower than in MCS and MCC scenarios. Carbon footprint in ICS (0.030 kg CO_2eq kg^{-1} vegetables year^{-1}) and ICC (0.033 kg CO_2eq kg^{-1} vegetables year^{-1}) scenarios were also lower than in MCS (0.082 kg CO_2eq kg^{-1} vegetables year^{-1}) and MCC (0.071 kg CO_2eq kg^{-1} vegetables year^{-1}) scenarios. Fertilizers, fuel (diesel) and irrigation were the main contributing sources for total GHG emitted and carbon footprint in all evaluated scenarios. The results suggest that intercropping systems may reduce GHG emissions associated with the production of vegetables evaluated as compared with monoculture.

KEYWORDS

vegetables, global warming potential, intercropping, direct and indirect emissions, carbon footprint

Introduction

In the last few decades, the production of leafy vegetables has been rising and standing out in a world agricultural context (FAO, 2019), consequence of the increasing demand for food and changing in feeding habits (Vico et al., 2020). The accelerated increase of population and the need to produce food for eight billion people lead to a huge environmental impact, mainly on climate change/global warming, since conventional agricultural system (monoculture) is characterized by intense exploration of natural resources (soil and water) and large use of inputs, materials and fuel (fertilizers, pesticides, diesel, plastic etc.), increasing direct and indirect greenhouse gas emissions (GHG) (Notarnicola et al., 2017).

Improving food production systems aiming at making them more sustainable is the main challenge of agriculture in the current century (Foteinis and Chatzisymeon, 2016). Compared with conventional systems, sustainable agricultural systems are characterized by reduced use of chemical fertilizers, pesticides, fuel and lower impact on natural resources (soil and water) (Jeswani et al., 2018; Seo et al., 2019) and GHG emissions (Pereira et al., 2021). These changes applied in the vegetable production sector may directly contribute to achieve some of the main goals proposed by United Nations (UN) aiming at the sustainable development, such as development of sustainable agriculture, responsible consumption and production, and climate action (UN, 2015).

Vegetable production, performed mainly in monoculture systems, contributes directly climate change/global warming due to greenhouse gas (GHG) emissions generated by intensive soil tillage and use of fertilizers and fuels (Martin-Gorriz et al., 2020; Pereira et al., 2021). The challenges brought by climate change/ global warming will require strategies of adaptation to meet consumers' demands and to ensure high standards for food safety (Bisbis et al., 2018). One alternative to food production in monoculture is the intercropping systems of vegetables because, in addition to agroeconomic viability (Cecílio Filho et al., 2017; Carlos et al., 2021), this system has a potential to mitigate GHG emissions (Pereira et al., 2021).

The main agronomic advantage of intercropping is better use of agricultural area since two species are simultaneously cultivated in the same area, increasing diversity of species in the system and reducing the use of inputs, materials and fuel (Nascimento et al., 2018). This system's agronomic variability depends on temporal and/or spatial complementarity of the species cultivated, as demonstrated in cultivation of collard greens and New Zealand spinach by Cecílio Filho et al. (2017), and in cultivation of collard greens and chicory by Carlos et al. (2021). Therefore, vegetable intercropping is a viable technology to meet the rising demands for food production and reduce the impact on climate change/global warming (Pereira et al., 2021).

Studies identifying the main sources of GHG emissions and the impact of vegetable production systems on climate change/ global warming have been published for many countries in the last few years (Clavreul et al., 2017; Ntinas et al., 2017; Pishgar-Komleh et al., 2017; Seo et al., 2017; Tasca et al., 2017; Zarei et al., 2019; Lo-Iacono-Ferreira et al., 2020). In Brazil, the only study found in the literature evaluating the impacts of vegetable cropping systems was published by Pereira et al. (2021). The authors demonstrated the potential of intercropping of vegetables to mitigate GHG as compared to monocultures, cultivating vegetables such as tomato, cucumber and lettuce, in greenhouse. However, no publications were found about the potential of mitigation of GHG emissions in the intercropping of other vegetables, such as collard greens (*Brassica oleracea* var. *acephala*), New Zealand spinach (*Tetragonia expansa*) and chicory (*Cichorium intybus*), which are addressed in this study.

Collard greens are leafy vegetables cultivated at about 71.279 agricultural farms in Brazil, which produce approximately 161.986 tons per year (IBGE, 2017). Therefore, evaluating the impact on climate change/global warming to produce this vegetable, intercropped with New Zealand spinach or chicory, and compared to their respective monocultures, will be important to suggest agricultural practices with lower GHG emissions and higher yield. As demonstrated by Pereira et al. (2021), intercropping of vegetables may reduce GHG emissions by 35% in comparison to monocultures. In addition, the authors demonstrated that carbon footprint may be five times lower in the intercropping when compared to monocultures.

In this context, our study aimed to calculate GHG emissions and carbon footprint in two production systems (intercropping and monoculture) of collard greens, New Zealand spinach and chicory, arranged in four scenarios: 1) intercropping of collardgreens and New Zealand spinach; 2) monocultures of collard greens and New Zealand spinach; 3) intercropping of collard greens and chicory; 4) monocultures of collard greens and chicory; and to identify the main sources of GHG emissions, suggesting to mitigation practices. Our hypothesis is that intercropping systems to produce collard greens, New Zealand spinach and chicory are responsible to lower greenhouse gas emissions and lower carbon footprint when compared to monoculture systems.

Material and methods

Description of production scenarios

GHG emissions and carbon footprint were evaluated in four scenarios of production of collard greens and New Zealand spinach (Cecílio Filho et al., 2017) and collard greens and chicory (Carlos et al., 2021), in Jaboticabal city, São Paulo state, Brazil. The scenarios were defined as follows: 1) ICS – intercropping of collard greens and New Zealand spinach; 2) MCS – monocultures of collard greens and New Zealand spinach; 3) ICC – intercropping of collard greens and chicory; 4) MCC – monocultures of collard greens and chicory (Figure 1).

ICS and MCS scenarios consisted of collard greens and New Zealand spinach cultivation in intercropping (ICS) and monocultures (MCS), in open field, during one agricultural year, considering one cycle of cultivation for collard greens (cycle of 12 months) and two cycles for New Zealand spinach (5 months each cycle), with three harvests per cycle of spinach (Figure 1) (Cecílio Filho et al., 2017). According to the authors, for ICS, collard greens cv. 'Top Bunch' and New Zealand spinach cv. 'New Zealand' were planted simultaneously in the same area, in beds with two rows of collard greens (double rows – spaced by 0.50 × 0.50 m) and three rows of New Zealand spinach (spaced by 0.40 × 0.30 m). For MCS, using the same species and spacing of planting, the plants were cultivated in different areas (Figure 1).

For ICC and MCC scenarios, one year of cultivation of collard greens and chicory in intercropping (ICC) and monoculture (MCC) systems, in open field, were considered, one cycle of collard greens (cycle of 12 months) and three cycles of chicory (4 months each cycle), with two harvests per cycle of chicory (Figure 1) (Carlos et al., 2021). According to the authors, for ICC, collard greens cv. 'HS-20' and chicory cv. 'Pão de Açúcar' were cultivated in the same bed, with two rows of collard greens (double rows – spaced by 0.70 × 0.40 m) and five rows of chicory (spaced by 0.25 × 0.20 m). For MCC, using the same species and spacing of planting, the plants were cultivated in different areas (Figure 1).

Functional units

Aiming to compare inputs and outputs for each scenario, threes functional units were defined to be used in this study: one kilogram of vegetables (kg vegetables year^{-1}), one kilocalorie of vegetables (kcal vegetables year^{-1}), produced during one year of cultivation, and one hectare of cultivation (ha vegetables year^{-1}). GHG emissions were calculated using the methodology of Intergovernmental Panel on Climate Change (IPCC, 2006) and other specifics factors (Tier 2). All factors used can be found in Supplementary material – Table 1. Total GHG emissions were calculated in CO_2 equivalent (CO_2eq), considering Global

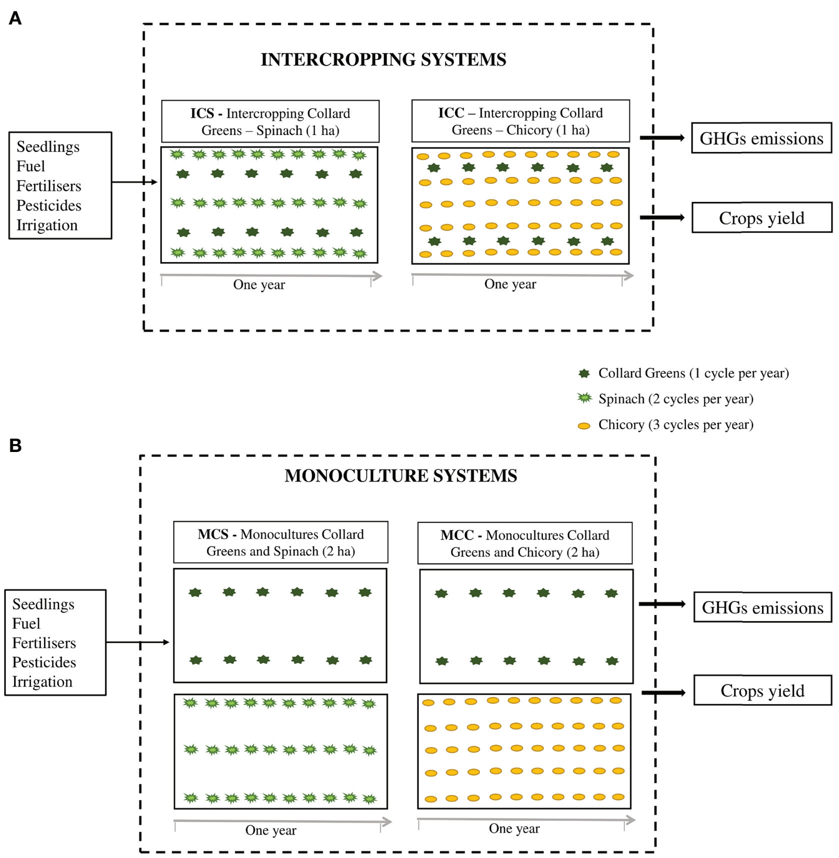

FIGURE 1
Vegetable production scenarios evaluated: **(A)** intercropping: ICS – intercropping of collard greens and spinach (area of 1 ha); ICC – intercropping of collard greens and chicory (area 1 ha); **(B)** monocultures: MCS – monocultures of collard greens and spinach (2 ha); MCC – monocultures of collard greens and chicory (2 ha); and their respective sources of GHG emissions during one agricultural year of production.

Warming Potential equal to 1 for carbon dioxide (CO_2), equal to 28 for methane (CH_4) and equal to 268 for nitrous oxide (N_2O) over a given period of 100 year (IPCC et al., 2013).

Boundaries established for the study

The boundaries established for this study include agricultural phase of production of collard greens, New Zealand spinach and chicory and transportation of seedlings and fertilizers (cradle-to-gate analyses) for each scenario (Figure 1). Sources of GHG emissions in the boundaries established were classified into five categories: seedling production (polypropylene trays and greenhouse structure); fuel (diesel used in the transportation of seedlings and fertilizers, and in the operations using tractor); fertilizers (NPK, limestone and organic fertilizers); pesticides (insecticides and fungicides); irrigation (PVC tubes, sprinklers, and electricity) (Figure 1).

In ICS and ICC scenarios there is an overlap of cultivation area, that is, secondary crops (New Zealand spinach and chicory) are cultivated between the main crop (collard greens) rows, using the same spacing of planting as the monocultures. In MCS and MCC scenarios the crops are cultivated individually in two different areas (one area for each species present in the intercropping) because this is the principle of the monoculture. However, both systems (intercropping and monoculture) have the same number of plants because the same spacing of planting was used, that is, one hectare of intercropping has the same number of plants for each species as two hectares of monoculture (Figure 1).

Thus, aiming to portray the real condition of each cultivation system, the estimates of GHG emissions in the production scenarios were made by comparing one hectare of intercropping with two hectares of monoculture, being one hectare of monoculture for each species present in the intercropping (Figure 1). Carbon footprint to produce one kilogram of vegetables was determined by dividing total GHG emissions in each production scenario (kg CO_2eq ha^{-1} year^{-1}) by the total yield for each crop (kg vegetables ha^{-1} year^{-1}), adding the partials to obtain the total in each scenario (Figure 1 and Table 2). To calculate carbon footprint in kilocalories (kcal), total crop yield was converted into kcal using values of caloric composition in 100 g of fresh vegetables, equal to 27 kcal 100 g^{-1} collard greens, 16 kcal 100 g^{-1} New Zealand spinach and 18 kcal 100 g^{-1} chicory (TACO, 2011). After the total of kcal was calculated, carbon footprint to produce one kcal of vegetables was determined by dividing total GHG emissions in each production scenario (kg CO_2eq ha^{-1} year^{-1}) by the total energy yield of each crop (kcal vegetables ha^{-1} year^{-1}), adding the partials to obtain the total in each scenario (Figure 1 and Table 2). In this study, the CO_2 absorbed by plants was disregarded.

NPK fertilizers, liming and organic fertilizers

The amounts of N used in fertilization at planting, in ICS and ICC scenarios, were defined by establishing an average value of the recommendation for collard greens, and for side dress, by adopting an average value of the recommendation for each species (Cecílio Filho et al., 2017; Pereira et al., 2021). In MCS and MCC scenarios an average value of the fertilization recommendation for each species at planting and side dress was adopted (Trani et al., 2018). Total values of the amount of N used (kg N ha^{-1} ano^{-1}) are shown in Table 1.

Total of P fertilizer (superphosphate 17 – 18% P_2O_5), K fertilizer (potassium chloride 58 – 60% K_2O), limestone and organic fertilization (manure) used in each scenario were based on the recommendations of Trani et al. (2018) and are described in Table 1. Total amounts of limestone for each scenario were divided by three years, considering this the time needed to perform a new application (Table 1). Indirect emissions attributed to the manufacturing process of NPK and limestone fertilizers were estimated by factors used in the EBAMM and GREET models, adapted by Macedo et al. (2008), and the IPCC (2006) factor was used to calculate direct emissions associated with the limestone application. The average N content of the manure was 1.7%, and the emission factor used in the calculations of the direct emission was according to Lessa et al. (2014).

Pesticides

To control pests and diseases, the use of insecticide (Akito – 10% of active ingredient Beta-Cypermethrin), insecticide/acaricide (Oberon – 2% of active ingredient Spiromesiphen) and fungicide/bactericide (Kasumin – 2% of active ingredient Kasugamycin) was considered in all evaluated scenarios in this study. The amount was determined according to the recommendation for each crop (Table 1). The use of herbicides was not considered in any of the evaluated scenarios. The factors adapted by Macedo et al. (2008) and by Do Carmo et al. (2016) were used to calculate the indirect emission associated with the manufacturing of insecticides and fungicide, respectively.

Irrigation – indirect emission

In all evaluated scenarios, the use of sprinkler irrigation system using 75-mm-diameter PVC tubes in the lateral lines and 100-mm-diameter PVC tubes in the main line of the system was considered. The material weight was based on the manufacturer's information, calculated from the weight of a 6-m-long pipe. The use of 50 sprinklers (12 m x 18 m spacing),

TABLE 1 Amount of inputs and materials used for one year of production of collard greens (1 cycle per year), New Zealand spinach (2 cycles per year) and chicory (3 cycles per year) in intercropping and monoculture scenarios.

Source	Unit	ICS [a]	MCS [b]	ICC [c]	MCC [d]
N fertiliser	kg ha^{-1} year^{-1}	350.0	410.0	410.0	500.0
P fertiliser (P_2O_2)	kg ha^{-1} year^{-1}	420.0	600.0	600.0	780.0
K fertiliser (K_2O)	kg ha^{-1} year^{-1}	280.0	400.0	310.0	490.0
Limestone	kg ha^{-1} year^{-1}	500.0	1,000.0	500.0	1,000.0
Manure	t ha^{-1} year^{-1}	40.0	80.0	40.0	80.0
Fungicides (i.a [e])	kg ha^{-1} year^{-1}	0.19	0.29	0.29	0.38
Insecticides (i.a [e])	L ha^{-1} year^{-1}	1.27	1.91	1.91	2.54
Diesel	L ha^{-1} year^{-1}	213.5	411.5	259.5	545.5
Electricity	kwh ha^{-1} year^{-1}	1,093.95	2,187.9	1,093.95	2,187.9
Irrigation pipes	kg ha^{-1} year^{-1}	159.20	318.40	159.20	318.40
Irrigation sprinkler	kg ha^{-1} year^{-1}	4.17	8.33	4.17	8.33
Seedling trays	kg ha^{-1} year^{-1}	135.74	135.74	264.0	264.0
Iron [f]	kg ha^{-1} year^{-1}	87.50	87.50	87.50	87.50
Film Plastic [f]	kg ha^{-1} year^{-1}	53.33	53.33	53.33	53.33

[a]ICS – intercropping collard greens - spinach.
[b]MCS – monoculture collard greens and spinach.
[c]ICC – intercropping collard greens - chicory.
[d]MCC – monoculture collard greens and chicory.
[e]Active ingredient.
[f]Seedling greenhouse.

weighing 250 g each and mostly manufactured using low-density polyethylene – LDPE, was designed. Lifespans of five years for PVC pipes and three years for sprinklers were considered. Emission factors used in calculating the emissions associated with the manufacture of PVC pipes and sprinklers were according to Posen et al. (2017).

For system operation, the use of a DANCOR cast iron pump (10 hp/7293 Watts), sufficient to irrigate 1 hectare using the adopted irrigation system, was designed. Total electricity consumption was determined by assuming a 30-min daily watering, for a period of 10 months, resulting in 150 hours per year in all scenarios (Table 1), using the following equation:

$$Consumption\ (kWh) = (Pp \times h\ /1000) \times tc$$

where Pp pump power (Watts); h hours of operation per month; tc time of the crop cycle (in months).

To calculate CO_2eq emissions associated with energy consumption in irrigation, the average value of emission factors for electricity generation in the Brazilian National Interconnected System according to the Ministry of Science, Technology and Innovations (MCTI, 2020) was used.

Seedling production

For seedling production, the use of a 624-m^2 greenhouse, structured with galvanized iron and covered with transparent polyethylene film, 15-mm thick with additives against ultraviolet rays, was considered. Lifespans of 40 years for the iron used in the structure and 3 years for the plastic film were considered. The amount of iron and plastic film used are shown in Table 1. However, the emissions referring to the time of use for seedling production, corresponding to two months per year, in all evaluated scenarios were calculated. For sowing of vegetable seedlings, we considered the use of plastic trays with 200 cells of 0.018 dm^3 each. The trays are manufactured with low-density polyethylene, weighing 1.100 g each. The number of trays used for seedling production was calculated based on the planting spacing of the crops, considering the total of plants in one hectare (Table 1). The adopted lifespan of the trays was 5 years.

The GHG emissions to manufacture the iron and the plastic film were calculated according to IPCC (2006) and Cheng et al. (2011), respectively. Emissions associated with tray manufacturing were calculated using a factor according to Posen et al. (2017).

Diesel – direct and indirect emission

The total diesel consumed in the evaluated scenarios includes diesel consumed in the transportation of seedlings and fertilizers over a distance of 50 km to the cultivation area, transported by a Mercedes Artego semi-heavy truck. In the area of vegetable cultivation, we considered diesel used for ploughing, harrowing, construction of beds, limestone application, cattle manure application and harvest transportation from the field to the shed (established distance of 1 km) (Tables 1 and

Supplementary Material – Table 2), using a MF 275 tractor (77 hp). In MCS and MCC scenarios, the operations of ploughing, harrowing and construction of beds are performed at each new cycle in the cultivation areas of spinach and chicory. However, in ICS and ICC scenarios those operations are performed only one time (before the first cultivation), because from the second cycle of spinach or chicory, the collard greens are already growing in the area, making it impossible to perform those operations; therefore, no-till planting of spinach and chicory must be carried out (Supplementary Material – Table 2).

Direct CO_2eq emissions associated with fuel combustion were calculated using the emission factor established by the São Paulo State Environmental Company (CETESB, 2018). For indirect emissions, associated with diesel extraction and production, the factor according to Macedo et al. (2008) was used.

Variations in soil carbon stock

Changes in soil carbon stock were estimated based on IPCC (2006) factors for a 20-year period. Land use change (F_{LU}), soil management (F_{MG}) and crop residue deposition (F_I) factors were defined according to the specific climate, classified as tropical humid, in the São Paulo state (CEPAGRI, 2006) and considering a high soil management intensity, with values of F_{LU} = 0.83, F_{MG} = 1.00 and F_I = 0.92. The reference carbon stock value (C_{ref}) used was 38 t C ha^{-1}, i.e., the IPCC standard value for clay soils (dark red Oxisol), considering a soil depth of 0–30 cm. Thus, the estimates were made using the following equation:

$$\Delta C_{soil} = (C_{ref} \times F_{LU} \times F_I \times F_{MG}) - C_{ref}$$

where ΔC_{soil} change in soil carbon stock over 20 years (t C ha^{-1}); C_{ref} reference carbon stock for Oxisols (t C ha^{-1}); F_{LU} factor associated with land use change (dimensionless); F_I factor associated with crop residue deposition (dimensionless); F_{MG} factor related to the adopted soil management practices (dimensionless).

After determining the total soil carbon accumulation/loss, the value found was converted from carbon (C) into carbon dioxide (CO_2) by multiplying it by the ratio of 44/12, i.e., 1 t of C corresponds to 3.67 t of CO_2.

Results

GHG emissions and soil carbon

Total of direct and indirect GHG emissions associated with collard greens and spinach production in ICS scenario were 4,953 kg CO_2eq ha^{-1} year^{-1} while in MCS were 7,093 kg CO_2eq ha^{-1} year^{-1}. In ICC scenario, total emissions reached 5,900 kg CO_2eq ha^{-1} year^{-1} while in MCC scenario they reached 8,587 kg CO_2eq ha^{-1} year^{-1} (Figure 2). These results show a reduction of about 31% in GHG emissions when the vegetables evaluated are cultivated in intercropping as compared to monocultures. Such reductions in GHG emissions in ICS and ICC scenarios are mainly related to decrease in fertilizer use, as the species present in intercropping system have a synergy as to fertilizers uptake applied at planting; in fuel (diesel) consumption, as the operations for soil tillage are performed in only one area of

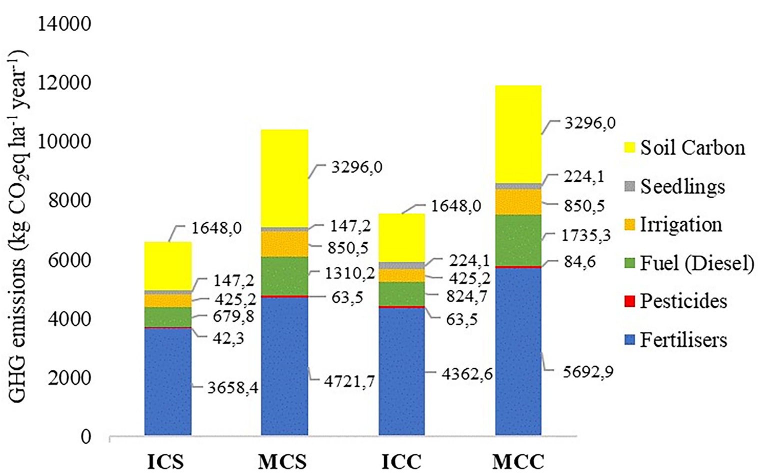

FIGURE 2
Total GHG emissions (kg CO_2eq ha^{-1} year^{-1}), associated with changes in soil carbon stock and GHG emission sources defined according to the boundaries adopted in the vegetable production scenarios evaluated: ICS – intercropping of collard greens and New Zealand spinach; MCS – monocultures of collard greens and New Zealand spinach; ICC – intercropping of collard greens and chicory; MCC – monocultures of collard greens and chicory.

cultivation; and in the material cultivation use such as irrigation equipment and electricity consumption, as ICS and ICC scenarios require smaller irrigation system, needed to cover half of the cultivation area when compared to that required in MCS and MCC scenarios, demonstrating a great competitive advantage in reducing GHG emissions in intercropping systems.

When adding the estimates of changes in soil carbon stock due to land use change and soil management, the ICS and ICC scenarios (1,648.0 kg CO_2 ha^{-1} $year^{-1}$) result in lower carbon losses compared with MCS and MCC scenarios (3,296.0 kg CO_2eq ha^{-1} $year^{-1}$) (Figure 2). The 50% reduction in losses is related to the use of 50% of the cultivated area, since, in intercropping, the two crops grow together in the same area (1 ha), while in monocultures two cultivation areas are needed (2 ha). Thus, ICS and ICC scenarios may be options for production systems with a potential to mitigate GHG emission, as in addition to reducing CO_2 emissions associated with inputs, there is also a reduction in CO_2 emissions from losses in soil carbon stock, since it is possible to optimize the production in the same area by using intercropping systems.

In all analyzed scenarios, fertilizer use was the main responsible for GHG emissions, representing about 74% of total emissions in each intercropping scenario (ICS: 3,658.4 kg CO_2eq ha^{-1} $year^{-1}$ and ICC: 4,362.6 kg CO_2eq ha^{-1} $year^{-1}$), and about 66% of total emissions in each monoculture scenario (MCS: 4,721.7 kg CO_2eq ha^{-1} $year^{-1}$ and MCC: 5,692.9 kg CO_2eq ha^{-1} $year^{-1}$). Among the fertilizers used, nitrogen fertilizer was the major contributor, mainly due to direct (from 20 to 25% of total in the evaluated scenarios) and indirect (from 23 to 28% of total in the evaluated scenarios) emissions associated with this input. In addition to fertilizers, fuel (diesel) accounted for about 14% of total emissions in ICS and ICC scenarios, and for about 18 and 20% in MCS and MCC scenarios, respectively (Figure 2).

Analyzing the emissions associated with each individual species, in MCS and MCC scenarios, it is possible to observe that chicory production emits 5,102.59 kg CO_2eq ha^{-1} $year^{-1}$, while the production of New Zealand spinach and collard greens emits 3,608.20 kg CO_2eq ha^{-1} $year^{-1}$ and 3,484.86 kg CO_2eq ha^{-1} $year^{-1}$, respectively (Figure 3). The highest emissions in chicory cultivation are related to the greater number of crops established during the year, making it possible to carry out three cycles of chicory, two of New Zealand spinach and one of collard greens. In addition, the use of fertilizers, diesel in the operations and transport, pesticides and electricity for chicory cultivation is greater, increasing the emissions associated with these sources.

Carbon footprint

Concerning carbon footprint to produce one kilogram of vegetables, in MCS scenario it was 0.082 kg CO_2eq kg^{-1} vegetables $year^{-1}$ while in ICS scenario it was 0.030 kg CO_2eq kg^{-1} vegetables $year^{-1}$, which represents a 64% reduction of carbon footprint in ICS scenario when compared with the MCS (Figure 4). Carbon footprint was equal to 0.071 kg CO_2eq kg^{-1} vegetables $year^{-1}$ in MCC scenario and equal to 0.033 kg CO_2eq kg^{-1} vegetables $year^{-1}$ in ICC scenario, representing approximately 54% of reduction (Figure 4). When analyzing the carbon footprint values in kilograms of CO_2eq per kilocalories of produced vegetables, we observed that the reductions from the MCS scenario to ICS and from the MCC to ICC were 61 and 48%, respectively (Figure 4). The reductions of carbon footprint in intercropping scenarios (ICS and ICC)

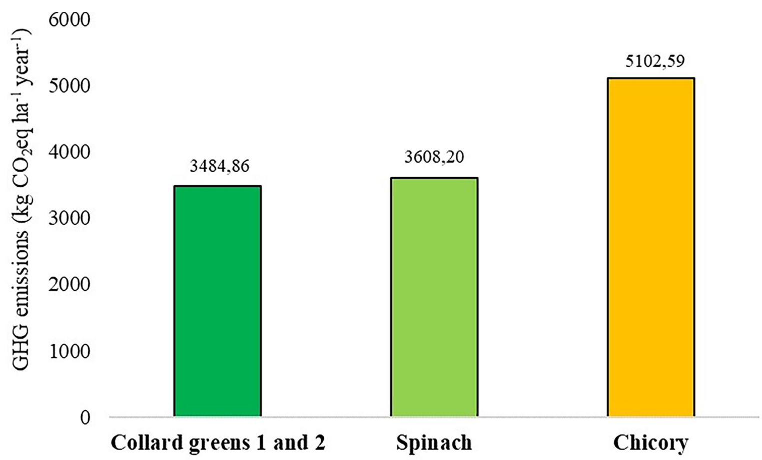

FIGURE 3
Total GHG emissions (kg CO_2eq ha^{-1} $year^{-1}$) for individual production of each species in monoculture scenarios: MCS – monocultures of collard greens 1 and New Zealand spinach and MCC – monocultures of collard greens 2 and chicory.

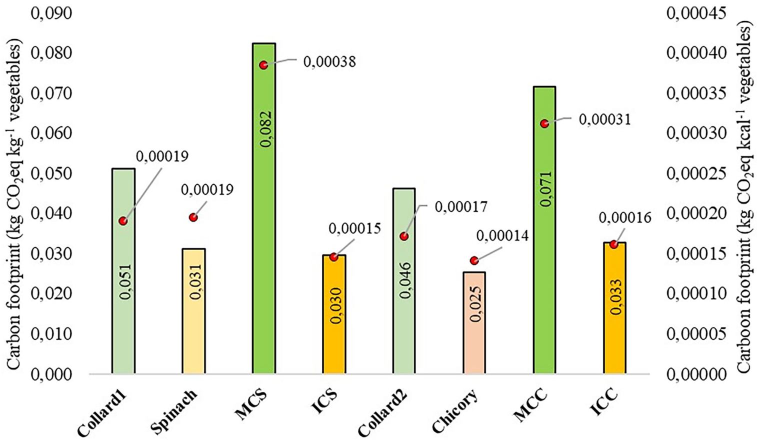

FIGURE 4
Carbon footprint (Colored bars = kg CO_2eq kg^{-1} vegetable $year^{-1}$; Red points = kg CO_2eq $kcal^{-1}$ vegetables $year^{-1}$) for each vegetable species produced and for each system in the evaluated production scenarios: ICS – intercropping of collard greens and New Zealand spinach; MCS – monocultures of collard greens and New Zealand spinach; ICC – intercropping of collard greens and chicory; MCC – monocultures of collard greens and chicory.

compared to monoculture scenarios (MCS and MCC) is mainly related to the reduction in GHG emissions when vegetables are cultivated in intercropping, since this system has better efficiency in land use and requires less use of fertilizers, fuels and electricity consumption with the irrigation system (Figure 2).

In MCS and MCC scenarios it is possible to analyze the carbon footprint for individual crops. In the MCS, the carbon footprint of collard greens was 0.051 kg CO_2eq kg^{-1} collard greens $year^{-1}$, and for spinach it was 0.031 kg CO_2eq kg^{-1} spinach $year^{-1}$. However, when analyzing the carbon footprint per kilocalorie of energy produced, it was observed that the values were the same for both species, that is, 0.00019 kg CO_2eq $kcal^{-1}$ spinach $year^{-1}$ and 0.00019 kg CO_2eq $kcal^{-1}$ collard greens $year^{-1}$ (Figure 4). These results were due to the yield and energy capacity of these species, since despite producing less fresh mass, collard greens (27 kcal 100 g^{-1}) are more energetic than spinach (16 kcal 100 g^{-1}) (TACO, 2011). In MCC scenario, the values were 0.046 kg CO_2eq kg^{-1} collard greens $year^{-1}$ (or 0.00017 kg CO_2eq $kcal^{-1}$ collard greens $year^{-1}$) for collard greens monoculture and 0.025 kg CO_2eq kg^{-1} chicory $year^{-1}$ (or 0.00014 kg CO_2eq $kcal^{-1}$ chicory $year^{-1}$) for chicory monoculture (Figure 4). The difference in the carbon footprint in collard greens monocultures 1 and 2 are associated with the different yields (Table 2), since GHG emission for collard greens is the same in both monoculture scenarios (MCS and MCC) (Figure 3). Among the evaluated vegetables, chicory was the one which showed lower carbon footprint (Figure 4), but had the highest GHG emission (Figure 3). Such result is related the number of cultivation cycles of this vegetable in one year (3 cycles), that is, despite the highest total GHG emission, there is also a higher yield for this vegetable during one year (Table 2). Therefore, the higher the crop yield, the smaller carbon footprint per kilogram (Pishgar-Komleh et al., 2017).

Discussion

Studies assessing GHG emissions and carbon footprint in the production of collard greens, New Zealand spinach and chicory were not found in literature. However, when assessing the impact of GHG emissions in vegetable production systems in intercropping and monoculture in Brazil, Pereira et al. (2021) found that the intercropping may reduce GHG emissions by 35% in comparison to monoculture production. As in the present study, the authors also found that the decrease in fertilizer use promoted by intercropping was one of the main responsible for reducing GHG emission. It is important to highlight that in the cited study, the authors evaluated the production systems with different vegetable species (cucumber, tomato and lettuce) from those evaluated in this study; however, the results obtained corroborate those of the present study about intercropping efficiency as compared to monocultures in GHG emission mitigation associated with vegetable production sector.

Assessing the impact of other leafy vegetables (lettuce and escarole) in Spain, which have a similar form of cultivation to that of the species evaluated in this study, Romero-Gámez et al. (2014) observed that fertilizers, mainly nitrogen fertilizers, were the main contributor sources to the GHG emissions associated with monoculture production of lettuce and escarole, as observed in the results obtained in the present study. In Greece, Foteinis and Chatzisymeon (2016) found that lettuce

TABLE 2 Vegetable yield (t fresh mass ha^{-1} $year^{-1}$ and kcal ha^{-1} $year^{-1}$) of each species (collard greens – one cycle per year; New Zealand spinach – two cycles per year; chicory – three cycles per year) within the cropping systems evaluated and total yield of each cropping system.

Crop systems	Fresh yield	Energy yield
ICS		
Collard greens + New Zealand Spinach	(67.09 [a] + 100.16 [b]) = 167.25	(18,114,300[a] + 16,026,240[b]) = 34,140.5
MCS		
Collard greens	68.06	18,376,200
New Zealand Spinach	115.78	18,524,160
ICC		
Collard greens + Chicory	(47.87 [c] + 132.24 [c]) = 180.07	(12,924,900[d] + 23,796,720[d]) = 36,721.6
MCC		
Collard greens	75.39	20,353,680
Chicory	201.99	36,358,200

[a]Collard greens yield (Cecílio Filho et al., 2017).
[b]New Zealand Spinach yield (Cecílio Filho et al., 2017).
[c]Collard greens yield (Carlos et al., 2021).
[d]Chicory yield (Carlos et al., 2021).

production, in conventional and organic systems, emitted about 1,893 and 1,603 kg CO_2eq ha^{-1} $cycle^{-1}$, respectively, with irrigation being the main contributor source with about 57.3 and 58.7% of GHG emissions, respectively. In the present study, irrigation was the third major contributor, accounting for 8 to 13% in all evaluated scenarios. This difference is mainly due to the electricity source used for the operation of the irrigation system. While in Greece electricity has high GHG emissions associated with manufacturing and consumption, due to its origin from fossil and non-renewable sources, in Brazil, most of the electricity (about 75%) comes from renewable sources (IPEA, 2019), resulting in lower GHG emissions associated with production and consumption during the use of the irrigation system, when compared to the production of other vegetables in Europe.

Concerning carbon footprint, Pereira et al. (2021) showed that, in Brazil, intercropping vegetables reduced the carbon footprint by up to 80% compared to monocultures. The results obtained in this study corroborate those found by Pereira et al. (2021) and confirm the intercropping as a more sustainable system for vegetable production than monocultures, when considering the ratio between yield and emissions per kilogram or kilocalorie of produced vegetables. The main challenge of modern agriculture is to reduce the environmental impacts generated by cropping systems, but without compromising crop yield. Thus, the results obtained in this study and the economic efficiency of intercropping demonstrated by Cecílio Filho et al. (2017) and Carlos et al. (2021) show that this cropping system meets environmental (climate changes) and economic aspects, which makes it an excellent alternative to the traditional monoculture production system of these vegetables.

In a literature review, Clune et al. (2017) reported that the carbon footprint to produce spinach varied from 0.51 to 0.54 kg CO_2eq kg^{-1} spinach. Seo et al. (2017) verified that carbon footprint for organic spinach (*Spinacia oleracea*) production in Japan was 0.049 kg CO_2eq 0.100 kg^{-1} spinach. The authors observed that fuel consumed in transportation contributed with 90% of the carbon footprint. It is necessary to highlight that this difference might be related to the boundaries established; while in the cited study the boundaries include fuel used in agricultural production phase and transportation of produce to the distribution center, in the present study the boundaries included the transportation over a distance of 1 kilometer inside the farm to the shed and, therefore, there is less fuel consumption.

When comparing the carbon footprint of Chinese kale (Brassicaceae) production in conventional and organic systems in Thailand, Yuttitham (2019) estimated values equal to 0.402 ±0.47 kg CO_2eq kg^{-1} Chinese kale for conventional system and to 0.195±0.122 kg CO_2eq kg^{-1} Chinese kale for organic system. As in the present study, the authors identified that in conventional system the main contributor sources were the use of fertilizers, fuel and irrigation. Nevertheless, the difference in the carbon footprint values when compared to those obtained in this study are related to higher GHG emissions due the fossil fuel used to generate the electricity consumed and the boundaries adopted, which also included the transportation to the distribution center.

As shown in our results, fertilizer use was the main responsible for the impact on GHG emissions associated with collard greens, spinach and chicory production, in open field, in the different evaluated scenarios. Reducing the use of synthetic fertilizers and increasing the efficiency of use of this input in the production of these vegetables may contribute to the mitigation of GHG emissions from this sector, especially in the state of São Paulo, Brazil. This state is the second major GHG emitter associated with the use of synthetic fertilizers

(SEEG, 2020), with vegetable production being an important contributor; therefore, mitigation proposals for this sector should be more widely studied and implemented, such as use of organic fertilizers and N-fixing species, which generate less impact. For example, completely replacing N synthetic fertilizer with organic N fertilizers may reduce by 28% indirect GHG emissions associated with the manufacturing of N synthetic fertilizer in all evaluated scenarios. Additionally, crop rotation using N-fixing species such as *Crotalaria juncea*, *Cajanus cajan* and *Canavalia ensiformis* would take in about, respectively, 183.4, 143.6 and 169.4 kg N ha^{-1} in four years (equivalent to 45.8, 35.9 and 42.3 kg N ha^{-1} $year^{-1}$), in dark red Oxisol (Silva et al., 2002), values that would represent reductions from 9 to 13% of the total synthetic N used in ICS and ICC scenarios (1 ha) and from 14 to 22% of the total in MCS and MCC scenarios (2 ha) (Table 1), varying according to N-fixing species used. Furthermore, it is important to highlight that in ICS and ICC scenarios the reduction in GHG emissions associated with the amount of fertilizers was about 25% compared to those of MCS and MCC scenarios, proving that intercropping of vegetables is also a promising technology for mitigating GHG emissions associated with synthetic fertilizers.

Fuel (diesel) consumption is also a great contributor to GHG emissions associated with vegetable production in the evaluated systems. A few alternatives aiming to reduce GHG emissions associated with this input would be to reduce the intensity and frequency of soil tillage with each new production cycle, starting to adopt reduced tillage practices, such as no-tillage, hence reducing diesel consumption. Looking ahead to future changes in the types of machines and engines used in agriculture, another mitigation alternative would be the use of hybrid tractors or tractors fully powered by fuels from non-fossil and more sustainable sources such as ethanol and renewable electricity (Hoy et al., 2014). Additionally, it is important to emphasize that production in intercropping system is also an efficient practice for mitigating GHG emissions associated with fuels, as in the results of this study, the reduction of GHG emissions associated with diesel, in intercropping scenarios, varied from 48 to 52% when compared to monoculture scenarios.

There are some limitations for a large-scale implementation of intercropping in vegetable production, as for vegetable production using this system to be economically viable, it is necessary to have temporal and spatial complementarity between the associated species. Thus, regional studies, such as those published by Cecílio Filho et al. (2017) and Carlos et al. (2021), should be carried out in order to define the proper management and synergy between species, since factors such as climate, competition for water, light and nutrients, especially temperature, may affect the speed of growth and development of the intercropped species and, consequently, influence yield.

A few factors such as the methodology (tiers) used in studies estimating GHG emission and carbon footprint, functional unit and boundaries adopted, may imply variations in calculating total GHG emitted or obtaining exact values of the impact on climate change/global warming (Bartzas et al., 2015; Notarnicola et al., 2017; Adewale et al., 2018) and, therefore, such limitations should be considered in the results obtained in this study. However, despite this issue, this methodology is considered the one that best suits this type of analysis, being widely accepted and used (Adewale et al., 2018).

The results presented in this study provide information about the contribution of these vegetables to GHG emissions from agriculture in Brazil and may help in future studies with broader projections of the impact of the vegetable production sector on Brazilian GHG emissions. In addition, this study demonstrates that the intercropping of collard greens, New Zealand spinach and chicory is an excellent alternative to monocultures of these vegetables, which may be part of the implementation strategies of more integrated and sustainable systems in Brazil and contribute to meet the global objectives for food safety of population and support the sustainable development of agriculture.

Conclusions

The scenarios of vegetable cultivation in intercropping for collard greens, New Zealand spinach and chicory based on the parameters of this study accounted for 32% lower GHG emissions when compared to monoculture production scenarios for the same species of vegetables, during one year of cultivation in open field. The use of fertilizers, fuel (diesel), and electricity and materials used in irrigation are the main contributor sources to GHG emissions and carbon footprint, in all evaluated scenarios. The carbon footprint (in kg CO_2eq kg^{-1} vegetables) in intercropping production scenarios of collard greens and spinach (ICS – 0.030 kg CO_2eq kg^{-1} vegetables $year^{-1}$) and collard greens and chicory (ICC – 0.033 kg CO_2eq kg^{-1} of vegetables $year^{-1}$) was 63 and 54% lower than in the scenarios of their respective monocultures (MCS – 0.082 kg CO_2eq kg^{-1} of vegetables $year^{-1}$ and MCC – 0.071 kg CO_2eq kg^{-1} of vegetables $year^{-1}$), respectively. Strategies aiming to reduce the impact of the production of these vegetables on GHG emissions should prioritize reducing the use of fertilizers, mainly nitrogen ones, through practices such as crop rotation with N-fixing species and greater use of organic fertilizers; reduce fuel consumption (diesel), by reducing soil tillage operations; and opt for more integrated cultivation systems such as intercropping, which promote lower GHG emissions compared to monocultures, in addition to being possible to obtain greater yield in these systems.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

BP: investigation, data curation, writing-original draft. AC: conceptualization, methodology, writing-review and editing, supervision. NL: methodology, data curation, writing-review and editing, supervision. EF: methodology, data curation, writing-review and editing, validation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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EDITED BY
Michele Pisante,
University of Teramo, Italy

REVIEWED BY
Renato De Mello Prado,
São Paulo State University, Brazil
Akbar Hossain,
Bangladesh Wheat and Maize Research Institute, Bangladesh

*CORRESPONDENCE
Taiwen Yong
yongtaiwen@sicau.edu.cn

†These authors have contributed equally to this work

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Straw incorporation and nitrogen reduction effect on the uptake and use efficiency of nitrogen as well as soil CO_2 emission of relay strip intercropped soybean

Benchuan Zheng[1,2†], Ping Chen[1†], Qing Du[1], Huan Yang[1], Kai Luo[1], Xiaochun Wang[1], Feng Yang[1], Taiwen Yong[1*] and Wenyu Yang[1]

[1]College of Agronomy, Sichuan Agricultural University/Sichuan Engineering Research Center for Crop Strip Intercropping System/Key Laboratory of Crop Ecophysiology and Farming System in Southwest, Ministry of Agriculture, Chengdu, China, [2]Crop Research Institute, Sichuan Academy of Agricultural Sciences/Scientific observing and experimental Station of oil crops in the upper Yangtze River, Ministry of Agriculture, Chengdu, China

Intercropping can increase crop N uptake and reduce carbon emissions. However, the effects of straw incorporation and N reduction on N use and carbon emissions in intercropping are still unclear. We explored the mechanism of N uptake, N use efficiency, and CO_2 emissions in the wheat-maize-soybean relay strip intercropping system. A two-year field experiment was conducted with two straw managements, i.e., wheat straw incorporation (SI) and straw removal (SR), and four N application levels of soybean, i.e., 60 (N60), 30 (N30), 15 (N15), and 0 kg N ha^{-1} (N0). We assessed soil properties, CO_2 emissions, and characteristics of roots, nodules, and aboveground N uptake of intercropped soybean. Results showed that geometry mean diameter of aggregate, soil porosity, soil total N, and soil urease activity were notably greater in SI than in SR. N input reduced from N60 to N30 did not significantly affect the soil total N content and urease activity in SI. The root length, root surface area, root volume, root biomass, root bleeding intensity, and inorganic N content of bleeding sap were greater in SI than in SR. In the SI, although the root length and surface area peaked at N60, the root biomass and inorganic N content of bleeding sap were insignificant between N60 and N30. The nodule number, nodule dry weight, nodule nitrogenase activity, and nodule nitrogen fixation potential in SI were notably increased compared with SR. The nodule nitrogen fixation potential in SI notably increased with the decrease of N input at the R3 stage, but it peaked in N30 at the R5 stage. On average, the aboveground N uptake and nitrogen recovery efficiency (RE) was notably higher by 43.7% and 76.8% in SI than in SR. SI+N30 achieved the greatest aboveground N uptake and RE. The CO_2 emission and accumulated CO_2 emission were notably greater in SI than in SR, and the accumulated CO_2

emission of SI was the lowest with N30 input. In conclusion, SI+N30 promoted N uptake and utilization efficiency with reduced CO_2 emissions during the soybean cropping season. It provides a potential strategy for sustainable agricultural development in intercropping systems.

KEYWORDS

straw incorporation, nitrogen uptake, relay strip intercropping, soybean, carbon dioxide emission

Introduction

Food security has become a severe issue with the world's population and food consumption growth. Modern strengthened agriculture production can meet food security to some degree, while it largely depends on high chemical fertilizer input, e.g., nitrogen (Sinclair and Rufty, 2012). High nitrogen (N) input leads to severe environmental risks, e.g., greenhouse gas emissions, water pollution, and soil acidification (Goldblatt et al., 2009). Sustainable agricultural production management strategies are urgently needed to achieve food security. Intercropping has been recognized as a sustainable agricultural development model globally due to the yield advantages, efficient use of resources, and maintain soil fertility (Nyawade et al., 2019; Du et al., 2020; Fan et al., 2020). The wheat-maize-soybean relay strip intercropping system is a mainly planting pattern in southwestern regions of China. It has the advantages of reducing N fertilizer input, eliminating environmental pollution, and boosting the system yield (Yong et al., 2012; Yong et al., 2015). However, considerable wheat straws were burned or removed, and the overuse of N fertilizer for soybean in the system resulted in resource waste and environmental pollution. Straw incorporation could increase farmland productivity by improving soil properties, e.g., increasing soil organic matter (SOM) and nutrients (Chen et al., 2018; Dong et al., 2018; Ma et al., 2019), enhancing soil aggregates formation (Song et al., 2019; Bu et al., 2020), and optimizing microbial community diversity and composition (Zhao et al., 2016; Cong et al., 2020). Especially, straw incorporation can protect soil from water erosions in areas with heavy rainfall or wind erosions in sandy-sloping farmland (Li et al., 2018). Straw removed from the field can be used for burning or bioenergy due to the unrenewable fossil energy, but reusing crop straw may result in environmental pollution (Zhao et al., 2019). Therefore, straw incorporation provides a potential way to reduce chemical fertilizer input and decrease the risk of environmental pollution (Asten et al., 2005; Ma et al., 2019; Latifmanesh et al., 2020).

The root system is the main organ through which plants use soil nutrients and water. Thus, crop growth and yields affect by proliferating roots in nutrient-enriched regions and increasing soil nutrient uptake (James et al., 2008; Chilundo et al., 2017; Ramamoorthy et al., 2017). To clear the root growth in the soil and the relationship between root, soil structure, and soil nutrients are in favor of increasing grain yield and the efficiency of fertilizer use (Xu et al., 2018; Zheng et al., 2021; Zheng et al., 2022). Agriculture management, e.g., straw incorporation and N application, can improve the structure and function of the root system by regulating soil structure, increasing soil structure stability, and affecting the spatial distribution of soil nutrients (Yu et al., 2017; Xu et al., 2018; Yu et al., 2018). Straw incorporation favors decreasing soil bulk density and increasing soil total porosity, which improves soil ventilation conditions and promotes root penetration in deep soil (Xu et al., 2018). Besides, organic matter in the straw is decomposed and released, which enhances the content of available N, phosphorus, and potassium (Yang et al., 2019). However, the straw decomposition is limited by cereal straws' high C: N ratio (Shaukat et al., 2011). A reasonable N input balances the C: N ratio and promotes straw decomposition by soil microorganisms (Huang et al., 2019; Li et al., 2021). Moreover, straw incorporation affected the vertical distributions of available nutrients (Yu et al., 2018), which promoted root proliferation. N input can encourage the growth of the root system (Zheng et al., 2022). However, there may be an optimal N rate for root growth, above which root growth may be suppressed. In the ridge film mulching and furrow planting pattern, taproot length, taproot diameter, taproot dry weight, and lateral root mass density of winter oilseed rape peaked at 240 kg N ha^{-1} when N input ranging from 0 to 300 kg N ha^{-1} (Gu et al., 2019). The improved root system favors promoting soil N uptake and increasing N use efficiency (Zheng et al., 2021). Xu et al. (2018) found that straw incorporation coupled with low N input can promote root distribution in deep soil, then increase nitrogen partial factor productivity and agronomic nitrogen use efficiency of winter wheat. However, the effects of straw incorporation and reduced N application on crop root growth in the intercropping system are unclear.

The cereal-legume intercropping system has attracted much attention because of legume symbiotic nitrogen fixation (Zhang et al., 2017; Zheng et al., 2022). The nodulation and N fixation of the legume not only meets its own N needs but also provides additional N sources for the soil. The soil N content affects legumes' nodulation and nitrogen fixation (Zheng et al., 2022). The biological N fixation (BNF) of legumes is promoted by increasing nodulation when soil N is deficient; in contrast, sufficient N resources will suppress the BNF of legumes (Hu et al., 2016). The interspecific competitive use of N between cereal and legumes strengthens legume's nodulation and N fixation (Li et al., 2001). Although the legume BNF is suppressed by soil compactness (Siczek A, 2011), straw incorporation can promote BNF by alleviating soil compactness and increasing soil porosity (Xu et al., 2018). Moreover, soybean nodulation and N fixation are promoted by reducing N input in the maize-soybean relay strip intercropping system (Du et al., 2020). However, the effects of straw incorporation and reduced N input on the nodulation and N fixation of soybean in the intercropping system are unclear.

Straw incorporation and N application increased crop production and produced adverse environmental effects, e.g., increased carbon dioxide (CO_2) emissions (Bhattacharyya et al., 2012; Shao et al., 2014). Then, mitigation of CO_2 emissions will be an essential task for the sustainable development of agriculture. Chai et al. (2013) indicated that maize intercropped with rape, pea, and wheat can decrease carbon emissions per unit area compared with monoculture maize in arid irrigation areas. Similarly, wheat-maize intercropping can reduce soil carbon emission in contrast to monoculture maize, and zero tillage with straw mulching has the lowest soil respiration rate Hu et al. (2015). Accordingly, the carbon emission is reduced by 12.4% compared to tillage without straw retention. Zhang et al. (2020) observed that reduction N application significantly decreased CO_2 emissions in contrast to conventional N application under the straw incorporation treatment in Huang-Huai-Hai wheat-maize rotation areas of China. Hence, intercropping and N reduction application provides possible ways to reduce carbon emissions. However, the effects of straw incorporation and reduced N input on soil CO_2 emissions in intercropping systems are still unclear and need better understood.

Our previous study indicated that maize-soybean relay strip intercropping could increase N uptake by changing spatial root distribution, promoting the BNF of soybean (Zheng et al., 2022). Further, reduced Ninput can enhance BNF by strengthening interspecific competitive use N (Du et al., 2020). However, it is unclear whether or not straw incorporation coupled with reduced N application can improve N uptake and utilization, and the mechanisms of N utilization and environmental effects are still clouded in wheat-maize-soybean relay strip intercropping. We hypothesized that wheat straw incorporation coupled with reduced N application would promote N use in an environment-friendly way by improving soil properties, strengthening soybean root growth and N fixation, and decreasing soil CO_2 emissions in the wheat-maize-soybean relay strip intercropping system. The objectives of this study were to (1) clarify the influence of straw incorporation coupled with reduced N application on the soil properties, root growth, nodulation, and N uptake and use efficiency of soybean and (2) evaluate the characteristic of CO_2 emissions in the wheat-maize-soybean relay strip intercropping system.

Materials and methods

Site description

The experimental site is located in Renshou County (30° 16'N, 104°00'E), Sichuan Province, Southwest China, in the 2018-2019 and 2019-2020 growing seasons. The climate of this region is subtropical monsoon humidity, with an average annual precipitation of 1110.7 mm and a temperature of 17.9°C. The precipitation and temperature during the soybean cropping seasons are shown in supplementary Figure1. The soil in this region is anthrosol with a clay loam texture. The soil organic matter, total N, total P, total K, and pH were 7.85 g kg^{-1}, 0.61 g kg^{-1}, 0.84 g kg^{-1}, 22.66 g kg^{-1}, and 8.21, respectively. The wheat-maize-soybean relay strip intercropping system was the main planting pattern in this region.

Experimental design and field management

The experiment site was set up on a fallow field. A two-factor split-plot experimental design was carried out with three replicates. The main factor was straw management with full straw incorporation (SI) and complete straw removal (SR). The sub-factor was N application rates of intercropped soybean, including conventional N employed by local farmers (N60, 60 kg N ha^{-1}), reduced N by 50% (N30, 30 kg N ha^{-1}), reduced N by 75% (N15, 15 kg N ha^{-1}), and zero N (N0). In the SI treatment, all wheat straw was crushed into pieces (0.05 m) and incorporated into the soil by rotary tillage (about 20 cm depth) after wheat harvest every year. In the SR treatment, all wheat straws were removed from the field, and the crops stubble less than 5 cm in height.

In the wheat-maize-soybean relay strip intercropping system, a wide-narrow row planting was adopted (1.6 m and 0.4 m for wide and narrow rows), and a total ratio of wheat-to-maize-to-soybean rows was 4:2:2. Wheat was planted in the wide rows with row spacings of 0.25 m as a first crop. Then, maize was sown in narrow rows with row spacings of 0.4 m. The distance was 0.425 m between wheat and maize rows. After the wheat harvest, two rows of soybean were sown in the wheat strips with row spacings of 0.4 m, which was 0.6 m between maize and soybean (supplementary Figure 2). The plot size was 5 m × 6 m. The planting density of wheat, maize, and soybean was 2,000,000 plants ha^{-1}, 58,863 plants ha^{-1}, and 117,726 plants ha^{-1}, respectively. The N, P, and K fertilizers were applied as urea (46% N), superphosphate (12% P_2O_5), and potassium chloride (60% K_2O), respectively. N fertilizer for wheat (150 kg N ha^{-1}) and soybean was applied as basal fertilizer, while N for maize was divided into two parts, i.e., 120 kg N ha^{-1} as basal fertilizer and 120 kg N ha^{-1} as topdressing. The P and K fertilizers were applied as base fertilizers for each crop, i.e., 36 kg P_2O_5 ha^{-1} and 54 kg K_2O ha^{-1} for wheat, 120 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} for maize, and 60 kg P_2O_5 ha^{-1} and 52.5 kg K_2O ha^{-1} for soybean. Wheat fertilizers were broadcast in the planting strips and incorporated into the topsoil (20 cm) by a rotary tiller. Maize fertilizers were strip placed in the middle of two maize planting rows at a distance of 20 cm from maize rows. Soybean fertilizers were hole placed at 10 cm from the soybean. The fertilizers for maize and soybean were hand-placed at a depth of 10 cm. In the 2018-2019 growing season, wheat was sown on November 15, 2018 and harvested on May 14, 2019, maize was sown on April 9, 2019 and harvested on July 27, 2019; soybean was sown on June 8, 2019 and harvested on November 3, 2019. In the 2019-2020 growing season, wheat was sown on November 14, 2019 and harvested on May 8, 2020, maize was sown on April 5, 2020 and harvested on July 29, 2020, soybean was sown on June 9, 2020 and harvested on October 28, 2020. The cultivars of wheat (*Triticum aestivum* L.), maize (*Zea mays L.*), and soybean (*Glycine max* L. *Merr.*) were Zhongkemai-138, Denghai-605, and Nandou-25, respectively.

Sampling and measurement

Soil samples of soybean were collected from each plot at a depth of 0-20 cm using soil anger (2 cm diameter and 20 cm depth) at the fifth trifoliolate stage (V5, July 17, 2019 and July 21, 2020), the beginning seed stage (R5, August 29, 2019 and September 8, 2020), and the full-maturity stage of soybean (R8, November 3, 2019 and October 28, 2020). Three individual samples were collected per plot, then thoroughly mixed and sieved through a 2 mm mesh to remove plant tissues, roots, and rocks. The fresh soil samples were stored at -80°C for the soil urease activity analysis (Zheng et al., 2022). The soil samples were air-dried to investigate the total nitrogen (TN) content (Liu et al., 2021).

Soil samples for soil aggregates and bulk density (BD) assessment were collected after the soybean harvest. Soil clods were collected at 0-20 cm soil depth. Within each plot, five individual soil samples were collected. The fresh soil was gently stripped into 10-12 mm clods along the natural planes of weakness, then air-dried for soil aggregation analysis. Soil aggregate separation was performed according to Guo et al. (2020). Two undisturbed soil cores from each plot at 0-10 cm and 10-20 cm depths with a volume of 100 cm^3 were collected for soil BD measurement. Soil samples were oven-dried at 105°C for 24 h, long enough to reach constant weight for weighting and BD calculation (Xu et al., 2018).

Plant samples of soybean were collected at the fifth trifoliolate stage (V5, July 17, 2019 and July 21, 2020), the beginning flowering stage (R1, July 31, 2019 and August 5, 2020), the beginning pod stage (R3, August 15, 2019 and August 24, 2020), the beginning seed stage (R5, August 29, 2019 and September 8, 2020), and the full-maturity stage (R8, November 3, 2019 and October 28, 2020). In each treatment, six soybean plants were cut from the first internode, and the aboveground samples were dried at 85°C to constant weight and weighting, then ground and passed through a 60-mesh sieve (0.25 mm) for plant N content measurement (Zheng et al., 2021). The underground roots use a traditional excavation method to obtain 0.20 m × 0.20 m × 0.20 m soil clods (Zheng et al., 2021). Soybean roots were scanned at a 300 dpi resolution (Epsom expression 10000 XL (Japanese) Co., Ltd). The scanned root images were analyzed using Win-RHIZO™ software (Régent Instruments Inc., Canada). Then the root samples were dried at 85 °C to a constant weight.

Root bleeding sap samples were collected at the beginning-pod stage (R3) and the beginning-seed stage (R5) of soybean. The collection method was modified from Zheng et al. (2021). Namely, soybeans were cut 10 cm above the soil surface. Then, skimmed cotton was put into a self-sealing bag. The weighed self-sealing bag was placed on the soybean stalk and fixed with a rubber band. The bleeding sap in the skimmed cotton was collected and weighed after 12 h (6:00 pm - 6:00 am). The bleeding sap intensity was calculated as bleeding sap weight per plant per 12h (g $plant^{-1}$ $12h^{-1}$). The ammonium-N and nitrate-N content of bleeding sap was measured using a Cleverchem Anna Random Access Analyzer (DeChem-Tech.GmbH-Hamburg, Germany).

Nodule samples were collected at the V5, R1, R3, and R5 stages of soybean. In each plot, six representative plants were dug out to investigate the nodules' number and weight according to the early study (Yong et al., 2018). In 2020, the nodule nitrogenase activity was measured by acetylene reduction assay (C_2H_2) at the R3 and R5 stages of soybean (Siczek A, 2011). The nodule nitrogen fixation potential was evaluated according to Yong et al. (2018).

To explore the effects of straw incorporation and N application rates on the environment. The soil CO_2 emission rate was measured by a soil carbon flux meter (Brooke soilbox-

343 portable soil respiration system, Germany) at the V5, R1, R3, R5, and R8 stages of soybean in 2020. The measuring site was set at the interspecific rows between maize and soybean, with a distance of 20 cm from the soybean row.

Calculations and statistics

The soils' total porosity was evaluated according to soil bulk density and calculated as follows (Yang et al., 2021):

$$P = 100 \times \left(1 - \frac{B}{S}\right) \quad (1)$$

Where P is total soil porosity (%), B is soil bulk density (g cm^{-3}), and S is soil density (2.65 g cm^{-3}).

The soils' macroaggregate mechanical stability was evaluated using Mean weight diameter (MWD) and Geometry mean diameter (GMD), and calculated as follows (Kihara et al., 2012):

$$\text{MWD (mm)} = \frac{\sum_{i=1}^{n} X_i W_i}{\sum_{i=1}^{n} W_i} \quad (2)$$

$$GMD \quad (mm) = Exp\left[\frac{\sum_{i=1}^{n} W_i \ln x_i}{\sum_{i=1}^{n} W_i}\right] \quad (3)$$

Where X_i is the average diameter of grade i aggregates (mm), and W_i is the mass ratio of grade i aggregates (%).

The N use efficiency (NUE) of different treatments was evaluated using recovery efficiency (RE), and calculated according to Zheng et al. (2021).

$$RE\,\% = \frac{U_N - U_O}{F_N} \quad (4)$$

Where U_N is the aboveground N uptake with N, U_0 is the aboveground N uptake without N, and F_N is the N application rates, respectively.

The cumulative CO_2 emission (CE) was calculated according to Hu et al. (2015)

$$\text{CE(kg ha}^{-1}) = \sum\left[\frac{F_{n+1} + F_n}{2} \times (T_{n+1} - T_n)\right] \times 60 \times 24 \times \frac{1}{100} \times \frac{B}{A} \quad (5)$$

Where the F_n and F_{n+1} were the CO_2 emission rate of the n and n+1 sampling times (mg m^{-2} min^{-1}), T_n and T_{n+1} were sampling times of n and n+1(d), 60 and 24 were the conversion of min^{-1} to d^{-1}, B is the relative molecular mass ratio (C: CO_2), and A is warming potential coefficient (1).

Statistical significance was performed with the two-way analysis of variance (ANOVA), and the multiple comparisons were conducted with the least significant difference test (LSD, α=0.05) to determine the differences between individual treatment means. The analyses were performed with SPSS v.22 (IBM Corp., Armon, NY Inc, USA) and Microsoft Excel. SigmaPlot v.14.0 (Systat Software Inc. USA) and Origin 2017 (OriginLab Corporation, USA) were used to draw the figures.

Results

Soil physicochemical properties

As shown in Figure 1, the MWD, GMD, and soil porosity were insignificant among N application rates, but the GMD and soil porosity were notably greater in SI than in SR (Figures 1B, C-E,F). Straw incorporation and N application significantly influenced soil chemical properties (Table 1). The soil TN content and urease activity were markedly higher in SI than in SR under different N application rates at the various growth stages of soybean. Independent with N application rates, the soil TN content was significantly greater by 8.9%~16.7% in SI than in SR at the R8 stage of soybean. There were no significant differences in soil TN content and urease activity between N60 and N30 under the SI treatment at the different growth stages of soybean. But, those were significantly lower in N15 and N0 than in N30 and N60. Compared with N60, the soil TN and urease activity significantly decreased with the decrease of N inputs under the SR treatment at the different growth stages of soybean.

Root configuration and root biomass

The root length, surface area, and volume were significantly affected by straw incorporation and N application at the different growth stages of soybean (Tables 2–4). Independent with N application rates, those were significantly higher in SI than in SR at different growth stages of soybean (Tables 2–4). Compared with N60, the root length and surface area were increased in N30 but significantly decreased in N15 and N0 under the SI treatment at the different growth stages of soybean (Tables 2–3). However, the root length and surface area were considerably reduced in N30, N15, and N0 in contrast to N60 under the SR treatment (Tables 2–3). Compared with SR, the root biomass was notably increased in SI (Figure 2). On average, the root biomass of SI significantly increased by 21.6%, 27.4%, 19.9%, and 18.3% compared with SR under the N60, N30, N15, and N0 treatments at the R8 stage (Figures 2E-J). With the decrease of N, although root biomass was insignificant between N60 and N30 in SI, it was significantly decreased in N15 compared with N30. Similarly, root biomass was notably reduced with the decrease of N in SR treatment.

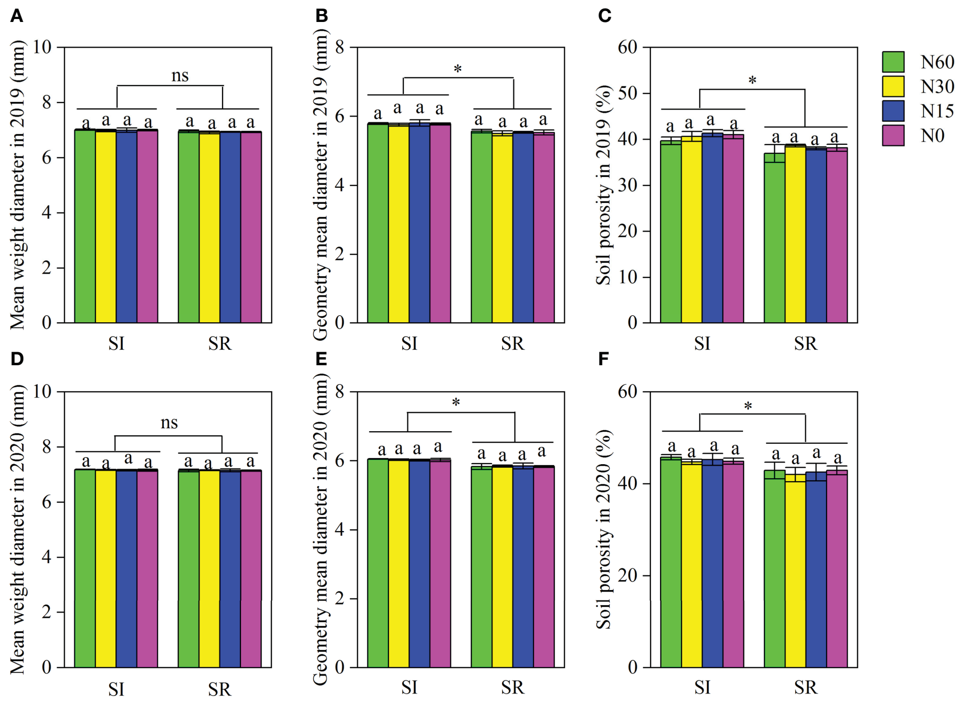

FIGURE 1
Soil physical properties of intercropped soybean at full maturity stage. The same lower-case letters indicate insignificant differences under different N application rates (*LSD*, *P*>0.05). Data were shown as mean ± S.D. (n=3). The asterisk "*" and "ns" indicate significant differences (*P*<0.05) and insignificant differences (*P*>0.05) between straw incorporation and straw removal, respectively. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}). **(A)**, mean weight diameter in 2019; **(B)** geometry mean diameter in 2019; **(C)** soil porosity in 2019; **(D)**, mean weight diameter in 2020; **(E)** geometry mean diameter in 2020; **(F)** soil porosity in 2020.

Root bleeding intensity and inorganic nitrogen content of sap

Straw incorporation and N application significantly affected the root bleeding intensity and inorganic N content of bleeding sap (Figure 3). Compared with SR, the averaged root bleeding intensity in SI was significantly increased by 38.5% at the R3 stage and by 25.8% at the R5 stage, respectively (Figures 3A, B). Compared with N60, no significant differences in root bleeding intensity in N30 were observed under the SI treatment at the R3 stage. However, it significantly decreased in N15 and N0. With the decrease of N, the root bleeding intensity of SR was notably decreased (Figure 3A); in contrast, the highest root bleeding intensity of SI was observed in N30 at the R5 stage (Figure 3B). The averaged ammonium-N and nitrate-N contents of root bleeding sap were significantly greater in SI than in SR, by 49.0% and 86.4%, respectively, at the R3 stage and by 36.5% and 67.0% at the R5 stage (Figures 3C-F). The ammonium-N and nitrate-N contents of bleeding sap in SI showed no significant differences between N60 and N30 (Figures 3C-F). In contrast, those were significantly higher in N60 than in N30 under the SR treatment (Figures 3C-F).

Nodule Number and Nodule Dry Weight

Straw incorporation and N application significantly affected the number and dry weight of root nodules at different growth stages of soybean (Figures 4 and 5). Those were markedly greater in SI than in SR under different N application rates at most of the growth stages, except for the V5 stage in 2019. Compared with N60, the number and dry weight of nodules were significantly increased in N30, N15, and N0 under both SI and SR treatments at different growth stages of soybean. With the decrease of N, nodules' number and dry weight were decreased. The number and dry weight of nodules were greater at the R5 stage than at other stages. On average, the nodules number and dry weight of SI were significantly greater by respectively 11.4%, 12.5%, 12.1%, and 14.5%, 12.7%, 8.8% in N30, N15, and N0 than in N60 at the R5 stage (Figures 4D-H, 5D-H). The nodules number and dry weight of SR were notably increased by 9.3%, 13.3%, and 1.9%, respectively, and by 16.2%, 13.3%, 7.3% (Figures 4D-H, 5D-H). Compared with SR, the number and dry weight of nodules were significantly increased by 10.8%, 13.1%, 9.9%, and 11.0% in SI, respectively, under the N60, N30, N15, and N0 treatments at the R5 stage of soybean (Figures 4D-H, 5D-H).

TABLE 1 Soil chemical properties of intercropped soybean at different growth stages.

Years	N application rates	Total nitrogen content (TN, g kg^{-1})						Soil urease activity (μg d^{-1} g^{-1})			
		V5-stage		R5-stage		R8-stage		V5-stage		R5-stage	
		SI	SR	SI	SR	SI	SR	SI	SR	SI	SR
2019	N60	0.83 ± 0.01 a	0.77 ± 0.01 a	0.80 ± 0.03 a	0.74 ± 0.01 a	0.83 ± 0.01 a	0.75 ± 0.01 a	344.7 ± 4.8 a	317.6 ± 2.6 a	274.6 ± 4.2 a	251.1 ± 3.7 a
	N30	0.82 ± 0.01 a	0.72 ± 0.01 b	0.77 ± 0.04 a	0.68 ± 0.00 b	0.82 ± 0.01a	0.70 ± 0.01b	343.6 ± 6.3 a	295.0 ± 7.2 b	263.2 ± 9.3 a	223.2 ± 4.9 b
	N15	0.70 ± 0.01 b	0.66 ± 0.02 c	0.70 ± 0.01 b	0.63 ± 0.01 c	0.76 ± 0.00 b	0.65 ± 0.01c	292.4 ± .6 b	274.9 ± 6.4 c	231.9 ± 4.3 b	199.0 ± 10.4 c
	N0	0.67 ± 0.02 c	0.64 ± 0.04 c	0.64 ± 0.00 c	0.57 ± 0.00 d	0.70 ± 0.01 c	0.60 ± 0.01 d	287.4 ± 0.9 b	249.8 ± 1.3 d	192.2 ± 7.6 c	178.0 ± 2.6 d
2020	N60	0.86 ± 0.02 a	0.79 ± 0.03 a	0.78 ± 0.00 a	0.73 ± 0.01 a	0.82 ± 0.00 a	0.75 ± 0.01 a	354.0 ± 5.9 a	325.0 ± 7.4 a	418.9 ± 7.1 a	386.7 ± 5.3 a
	N30	0.84 ± 0.03 a	0.74 ± 0.02 b	0.78 ± 0.01 a	0.66 ± 0.01 b	0.80 ± 0.02 a	0.70 ± 0.01 b	346.5 ± 4.0 a	291.3 ± 10.1 b	415.7 ± 4.6 a	355.4 ± 1.9 b
	N15	0.76 ± 0.00 b	0.70 ± 0.02 bc	0.68 ± 0.00 b	0.63 ± 0.03 c	0.72 ± 0.00 b	0.64 ± 0.00 c	296.6 ± 3.0 b	280.9 ± 4.9 b	382.6 ± 6.9 b	346.9 ± 11.1 bc
	N0	0.74 ± 0.01 b	0.68 ± 0.01 c	0.63 ± 0.01 c	0.57 ± 0.01 d	0.66 ± 0.01 c	0.60 ± 0.01 d	297.1 ± 15.4 b	280.4 ± 6.5 b	367.2 ± 4.7 c	329.6 ± 17.4 c
ANOVA (*F*-value)											
Year (Y)		51.2*		3.5^{ns}		25.8*		19.2*		4554.7*	
Straw management (S)		154.0*		217.4*		1047.3*		264.4*		245.7*	
N application rate (N)		134.7*		207.2*		582.3*		199.6*		170.6*	
Y×S		2.1^{ns}		0.1^{ns}		15.8*		0.9^{ns}		9.8*	
Y×N		2.3^{ns}		0.1^{ns}		1.9^{ns}		5.3*		5.7*	
S×N		6.5*		4.4*		10.7*		15.4*		6.2*	
Y×S×N		0.7^{ns}		0.8^{ns}		1.6^{ns}		2.5^{ns}		1.2^{ns}	

Different lower-case letters indicate significant differences under different N application rates (LSD, P< 0.05). Data were shown as mean ± S.D. (n=3); The asterisk "*" and "ns" indicate significant difference (P< 0.05) and insignificant difference (P >0.05), respectively; SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R5, the beginning seed stage of soybean; R8, the full-maturity stage of soybean.

Nodule nitrogenase activity and nodule nitrogen fixation potential

Nodules' nitrogenase activity and nitrogen fixation potential were notably affected by straw incorporation and N input at the R3 and R5 stages (Figure 6). Compared with SR, the averaged nitrogenase activity and nitrogen fixation potential of nodules were significantly higher in SI by 7.6%~37.0% and 24.0%~50.3% at the R3 stage (Figures 6A-C) and by 13.0%~181.9% and 25.3%~223.2% at the R5 stage (Figure 6B-D), respectively. The nitrogenase activity and nitrogen fixation potential of nodules were markedly increased in N30, N15, and N0 compared to N60 under both SI and SR treatments at the R3 and R5 stages. Those were significantly increased with the decrease of N input at the R3 stage and significantly decreased at the R5 stage (Figure 6).

Nitrogen uptake and NUE

Compared with SR, the aboveground N uptake per plant in SI was considerably increased during the growing season (Figure 7). On average, the aboveground N uptake in SI was notably greater by 34.1%, 54.8%, 33.2%, and 32.5%, respectively, than in SR under the N60, N30, N15, and N0 treatments (Figure7E-J). Although the aboveground N uptake decreased with the decrease of N in SI and SR, it was insignificant between N60 and N30 in IS. In contrast, the aboveground N uptake was markedly reduced when the N input was lower than 15 kg N ha^{-1} compared with the N input greater than 30 kg N ha^{-1}. Similarly, straw incorporation and N application significantly influenced RE (Figure 8). Compared with SR, the average RE in SI significantly increased by 44.3%, 109.2%, and 85.7% under the N60, N30, and N15, respectively. The RE in N30 was markedly greater than in N60 under both SI and SR. The RE in N15 remarkably increased in 2019 and significantly decreased in 2020 compared with N60.

Carbon dioxide emission

Independent with N input, the soil CO_2 emission rate was more remarkable in SI than in SR during the cropping season

TABLE 2 Effects of straw incorporation and nitrogen application on root length of intercropped soybean at different growth stages (cm plant^{-1}).

Years	N application rates	V5-stage		R1-stage		R3-stage		R5-stage	
		SI	SR	SI	SR	SI	SR	SI	SR
2019	N60	462.4 ± 29.5a	388.6 ± 20.8a	930.1 ± 5.3b	862.1 ± 8.5a	1173.1 ± 8.2b	1013.0 ± 7.3a	1576.8 ± 89.6a	1325.9 ± 92.1a
	N30	468.5 ± 31.7a	387.9 ± 23.0a	957.1 ± 10.8a	729.9 ± 123.4b	1232.7 ± 33.1a	949.3 ± 26.1b	1591.9 ± 2.7a	1122.3 ± 69.4b
	N15	385.1 ± 50.8b	280.0 ± 20.5b	847.3 ± 33.9c	719.8 ± 51.2b	1060.8 ± 56.8c	843.3 ± 22.1c	1335.1 ± 41.0b	1066.3 ± 26.3bc
	N0	355.0 ± 61.4b	277.3 ± 25.8b	836.8 ± 30.2c	705.1 ± 15.8b	1002.2 ± 53.3c	583.4 ± 51.7d	1074.0 ± 89.1c	994.6 ± 9.0c
2020	N60	646.3 ± 32.2ab	563.7 ± 38.1a	1363.7 ± 5.7b	1245.0 ± 43.8a	2453.3 ± 13.6a	2244.6 ± 9.6a	1349.8 ± 71.7a	1199.5 ± 56.5a
	N30	660.6 ± 60.3a	543.2 ± 22.5a	1536.5 ± 51.0a	1143.2 ± 18.2b	2588.4 ± 97.6a	2105.1 ± 4.9b	1413.6 ± 70.7a	1116.8 ± 19.6ab
	N15	561.4 ± 36.4b	411.9 ± 17.1b	1113.7 ± 45.5c	923.2 ± 98.6bc	1953.1 ± 74.2b	1743.2 ± 8.5c	1214.1 ± 1.8b	1014.8 ± 99.4bc
	N0	406.8 ± 71.0c	318.2 ± 30.5 b	942.5 ± 19.4d	838.2 ± 7.5c	1851.4 ± 80.1b	1680.0 ± 16.4c	1108.5 ± 4.6b	913.5 ± 26.4c
ANOVA (*F*-value)									
Year (Y)		96.6*		220.4*		2141.9*		27.6*	
Straw management (S)		47.4*		64.4*		129.4*		175.4*	
N application rate (N)		37.4*		41.1*		90.4*		73.3*	
Y×S		0.8^{ns}		2.2^{ns}		0.0^{ns}		2.5^{ns}	
Y×N		4.9 *		16.0*		15.8*		3.1*	
S×N		0.6^{ns}		5.3*		3.6*		8.4*	
Y×S×N		0.1^{ns}		0.9^{ns}		3.9*		2.9^{ns}	

Different lower-case letters indicate significant differences under different N application rates (LSD, P< 0.05); Data were shown as mean ± S.D. (n=3); The asterisk "*" and "ns" indicate significant difference (P< 0.05) and insignificant difference (P >0.05), respectively; SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean.

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TABLE 3 Effects of straw incorporation and nitrogen application on root surface area of intercropped soybean at different growth stages (cm^2 $plant^{-1}$).

Years	N application rates	V5-stage		R1-stage		R3-stage		R5-stage	
		SI	SR	SI	SR	SI	SR	SI	SR
2019	N60	105.0 ± 4.1a	91.9 ± 4.9a	258.6 ± 34.2a	216.3 ± 10.3a	398.3 ± 19.3b	361.5 ± 11.1a	372.7 ± 40.1ab	318.0 ± 21.4a
	N30	108.9 ± 5.9a	94.9 ± 3.2a	270.4 ± 23.0a	187.6 ± 12.4b	460.1 ± 11.8a	305.6 ± 17.4ab	384.3 ± 55.3a	281.0 ± 28.6ab
	N15	93.5 ± 5.6b	73.1 ± 7.2b	202.1 ± 9.3b	171.7 ± 3.8bc	324.2 ± 36.8c	292.7 ± 39.4ab	302.7 ± 11.4bc	261.5 ± 15.1b
	N0	84.2 ± 1.3c	72.8 ± 1.2b	191.1 ± 7.8b	162.7 ± 12.4c	272.6 ± 77.1c	240.9 ± 86.1b	274.8 ± 27.6c	257.1 ± 28.0b
2020	N60	113.2 ± 12.7a	92.8 ± 2.5a	359.1 ± 28.7a	236.8 ± 14.3a	536.8 ± 14.2a	494.5 ± 4.7a	430.4 ± 4.9a	400.9 ± 1.2a
	N30	117.4 ± 7.9a	82.4 ± 7.6ab	388.1 ± 2.2a	211.0 ± 3.8b	554.1 ± 26.4a	444.8 ± 49.8b	436.9 ± 16.6a	382.1 ± 4.5b
	N15	90.2 ± 5.8b	66.2 ± 17.2bc	210.7 ± 5.9b	189.0 ± 11.6c	403.2 ± 39.8b	366.2 ± 30.4c	366.8 ± 27.4b	331.1 ± 20.3c
	N0	71.3 ± 14.2b	61.5 ± 6.2c	187.1 ± 11.9b	144.7 ± 21.8d	392.4 ± 60.2b	326.8 ± 8.0c	303.1 ± 7.3c	276.2 ± 28.3d
ANOVA (*F*-value)									
Year (Y)		2.5^{ns}		51.7*		83.9*		66.6*	
Straw management (S)		63.8*		219.2*		34.1*		39.0*	
N application rate (N)		38.6*		112.9*		35.2*		44.1*	
Y×S		2.7^{ns}		23.6*		0.1^{ns}		1.4^{ns}	
Y×N		2.3^{ns}		17.6*		1.1^{ns}		2.8^{ns}	
S×N		1.8^{ns}		26.8*		5.2*		2.7^{ns}	
Y×S×N		1.1^{ns}		7.3*		0.2^{ns}		0.7^{ns}	

Different lower-case letters indicate significant differences under different N application rates (LSD, P< 0.05); Data were shown as mean ± S.D. (n=3); The asterisk "*" and "ns" indicate significant difference (P< 0.05) and insignificant difference (P >0.05), respectively; SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean.

TABLE 4 Effects of straw incorporation and nitrogen application on root volume of intercropped soybean at different growth stages (cm^3 $plant^{-1}$).

Years	N application rates	V5-stage		R1-stage		R3-stage		R5-stage	
		SI	SR	SI	SR	SI	SR	SI	SR
2019	N60	7.2 ± 1.4a	5.6 ± 1.2a	15.2 ± 1.7a	12.2 ± 0.3a	32.6 ± 0.5a	26.8 ± 0.5a	34.0 ± 1.2a	24.9 ± 0.9a
	N30	6.7 ± 0.1a	5.5 ± 0.4a	14.4 ± 0.3a	9.8 ± 0.5b	30.1 ± 1.0a	23.6 ± 0.7b	30.4 ± 3.8a	18.8 ± 2.7b
	N15	5.7 ± 0.1b	4.2 ± 0.1b	11.0 ± 1.3b	9.2 ± 1.8b	24.1 ± 4.3b	18.1 ± 1.0c	25.8 ± 1.3b	15.9 ± 1.8b
	N0	5.6 ± 0.2b	4.0 ± 0.2b	10.10.2 ± b	8.2 ± 0.4b	18.4 ± 1.1c	17.5 ± 2.3c	19.2 ± 0.2c	15.2 ± 2.8b
2020	N60	4.2 ± 0.6a	3.3 ± 0.2a	14.7 ± 4.2a	11.4 ± 0.2a	31.7 ± 0.4a	28.0 ± 0.7a	32.2 ± 0.6a	27.2 ± 1.4a
	N30	4.0 ± 0.6a	2.6 ± 0.2b	14.2 ± 2.1a	9.3 ± 0.8b	30.3 ± 0.3a	25.4 ± 0.6b	26.8 ± 1.2b	23.3 ± 0.4b
	N15	2.4 ± 0.1b	2.1 ± 0.2c	10.6 ± 1.9b	8.5 ± 0.1bc	23.6 ± 1.7b	22.2 ± 1.2c	22.3 ± 0.1c	20.0 ± 3.2bc
	N0	2.3 ± 0.4b	2.0 ± 0.3c	10.2 ± 0.3b	7.0 ± 1.8c	21.2 ± 0.3c	17.9 ± 4.3c	20.1 ± 2.4c	18.5 ± 1.1c
ANOVA (*F*-value)									
Year (Y)		310.7*		1.6^{ns}		4.9*		2.1^{ns}	
Straw management (S)		52.3*		49.5*		61.0*		113.4*	
N application rate (N)		26.2*		21.5*		92.4*		79.5*	
Y×S		5.5*		0.4^{ns}		2.0^{ns}		25.6*	
Y×N		0.0^{ns}		0.0^{ns}		0.5^{ns}		0.6^{ns}	
S×N		0.6^{ns}		2.0^{ns}		2.3^{ns}		3.8*	
Y×S×N		1.2^{ns}		0.1^{ns}		1.8^{ns}		1.5^{ns}	

Different lower-case letters indicate significant differences under different N application rates (LSD, P< 0.05); Data were shown as mean ± S.D. (n=3); The asterisk "*" and "ns" indicate significant difference (P< 0.05) and insignificant difference (P >0.05), respectively; SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean.

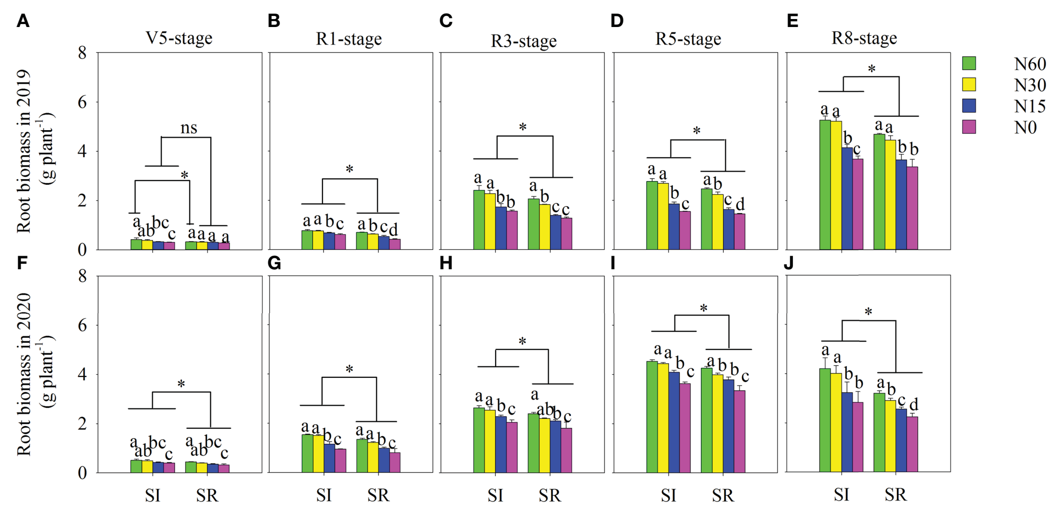

FIGURE 2
Effects of straw incorporation and nitrogen application on root biomass of intercropped soybean at the different growth stages. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P*< 0.05). Data were shown as mean ± S.D. (n=3). The asterisk "*" and "ns" indicate significant differences (*P*< 0.05) and insignificant differences (*P* >0.05) between straw incorporation and straw removal, respectively. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean; R8, the full-maturity stage of soybean. **(A)**, root biomass at the V5 stage in 2019; **(B)**, root biomass at the R1 stage in 2019; **(C)**, root biomass at the R3 stage in 2019; **(D)**, root biomass at the R5 stage in 2019; **(E)**, root biomass at the R8 stage in 2019; **(F)**, root biomass at the V5 stage in 2020; **(G)**, root biomass at the R1 stage in 2020; **(H)**, root biomass at the R3 stage in 2020; **(I)**, root biomass at the R5 stage in 2020; **(J)**, root biomass at the R8 stage in 2020.

(Figure 9A). With the decrease of N input, the lowest soil CO_2 emission rate was observed in N30 under both SI and SR during the cropping season. The soil CO_2 emission rate increased from the V5 stage to the R3 stage and decreased from the R3 stage to the R5 stage (Figure 9A). Similarly, the accumulated CO_2 emission was significantly higher in SI than in SR during the cropping season (Figures 9B–E). Compared with SR, the accumulated CO_2 emission in SI was notably increased by 43.2%~60.0% from the V5 to R1 stages (Figure 9B), by 18.6% ~40.8% from the R1 to R3 stages (Figure 9C), by 8.1%~40.4% from the R3 to R5 stages (Figure 9D), by 28.2%~57.1% from the R5 to R8 stages (Figure 9E), respectively. The lowest accumulated CO_2 emission was observed in N30 under both SI and SR treatments (Figures 9B-E).

Discussion

Numerous studies have reported that straw incorporation and N application can enhance crops' N uptake and use efficiency (Baker and Blamey, 1985; Malhi et al., 2011; Xia et al., 2018; Zheng et al., 2021). However, there are also debates. Shan et al. (2012) found that independent of N application rates, the aboveground N uptake and N recovery rates of winter wheat are significantly decreased by straw incorporation in contrast to straw removal. Malunga et al. (2017) reported that the N uptake of intercropped maize is increased by N addition, but the N use efficiency decreased with increasing N input in the maize-legume intercropping. In our present study, the N uptake and recovery efficiency (RE) of soybean in SI was significantly increased in contrast to SR. There was no significant differences in N uptake between N60 and N30 under the straw incorporation treatment. However, it was significantly decreased in N30 in contrast to N60 under the SR treatment. In addition, we found that the averaged RE was significantly increased by 79.7% under the SI treatment in comparison to SR treatment. Compared with N60, the RE in N30 was significantly increased under both SI and SR treatments. However, the RE in N15 was significantly increased in 2019 and significantly decreased in 2020, in contrast to N60. This indicated that the reduced application of excessive N fertilizer is not conducive to the N uptake and utilization of soybean in the wheat-maize-soybean relay strip intercropping system. But, straw incorporation can alleviate the nutrient absorption and yield loss caused by no N application or excessive N reduction. The following aspects regulated the aboveground N uptake increase and intercropped soybean utilization efficiency.

Firstly, the aboveground N uptake increase and intercropped soybean utilization results from the rise of N sources in the soil. In our present study, the soil total N content was significantly higher in SI than in SR under different N application rates during the cropping season. Moreover, soil total N content of SI was

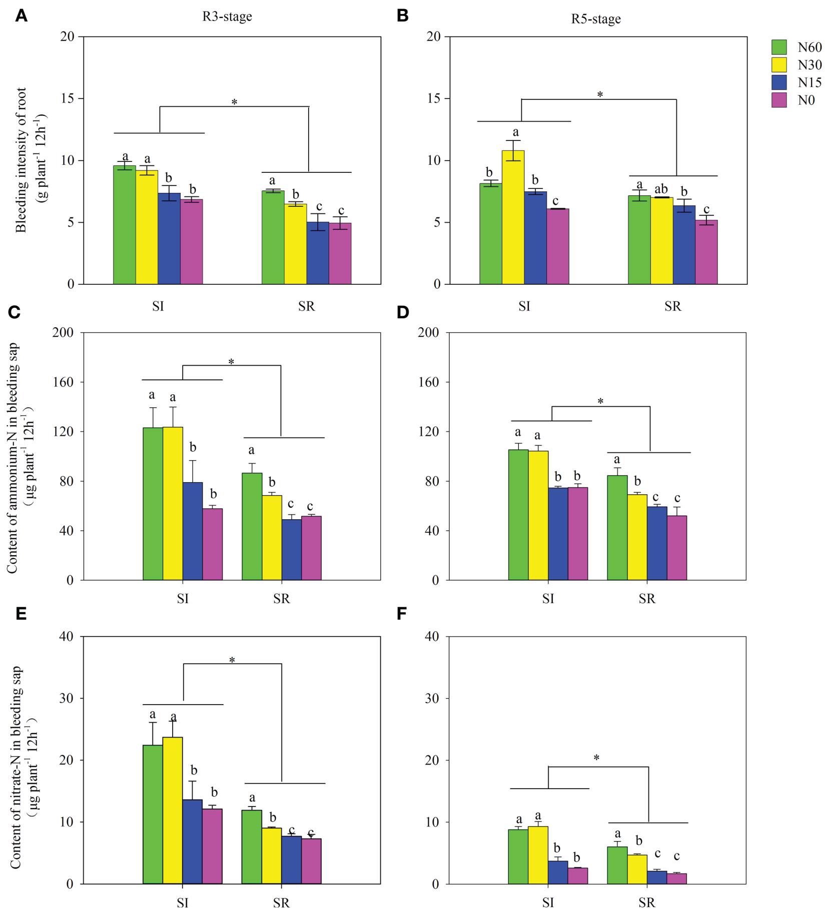

FIGURE 3
Effects of straw incorporation and nitrogen application on root bleeding intensity and N content at different growth stages. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P* < 0.05). Data were shown as mean±S.D. (n=3). The asterisk "*" indicate significant differences (*P* < 0.05) between straw incorporation and straw removal, respectively. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha-1); N30, reduced N by 50% (30 kg N ha-1); N15, reduced N by 75% (15 kg N ha-1); N0, zero N (0 kg N ha-1); V5, the fifth trifoliolate stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean. **(A)**, bleeding intensity of soybean root at the R3 stage; **(B)**, bleeding intensity of soybean root at the R5 stage; **(C)**, content of ammonium-N in bleeding sap at the R3 stage; **(D)**, content of ammonium-N in bleeding sap at the R5 stage; **(E)**, content of nitrate-N in bleeding sap at the R3 stage; **(F)**, content of nitrate -N in bleeding sap at the R5 stage.

insignificant between N60 and N30, but that of SR was remarkably decreased in N30 in contrast to N60. On the one hand, straw incorporation and reduced N application accelerated straw decomposition and promoted the release of nutrients from crop straw by increasing the activity of soil bacteria and fungi (Wang and Bakken, 1997; Moran et al., 2005). On the other hand, the increased soil N content is due to the increased soil urease activity (Zheng et al., 2022). Compared with SR, the soil urease activity was significantly increased in SI. Besides, the highest soil urease activity in SI was obtained in N60 and N30. The increase in urease activity promoted urea hydrolysis and N release.

Secondly, intercropped soybean's aboveground N uptake and utilization efficiency are related to root growth and absorption capacity (Zheng et al., 2021; Zheng et al., 2022). Root growth affects crop growth, nutrients, and water uptake, and a well-developed fine root system accounts for N uptake and utilization (Zheng et al., 2021). On average, the root biomass of intercropped soybean was significantly increased by 21.8% in SI compared with the SR. This finding is consistent with previous findings (Xu et al., 2018). Crops can increase soil nutrient uptake *via* root proliferation in nutrient-enriched regions (Chilundo et al., 2017). In our present study, the root length, surface area, and volume of intercropped soybean were significantly greater in SI than in SR. The changed root configuration enhanced the root absorption range of soybean in SI treatment. The optimized root configuration help to efficiently use the potential soil resource, and a higher root surface denotes the high efficiency of acquiring (Zheng et al., 2022). This is probably the

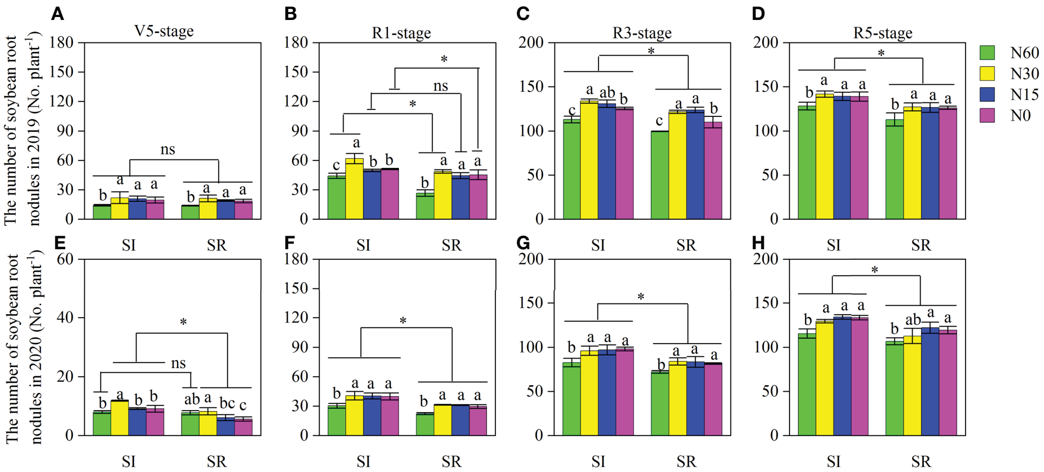

FIGURE 4
Effects of straw incorporation and N application on root nodule number per plant of intercropped soybean at different growth stages. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P*< 0.05). Data were shown as mean ± S.D. (n=3). The asterisk "*" and "ns" indicate significant differences (*P*< 0.05) and insignificant differences (*P* >0.05) between straw incorporation and straw removal, respectively. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean. **(A)**, the number of soybean root nodules at the V5 stage in 2019; **(B)**, the number of soybean root nodules at the R1 stage in 2019; **(C)**, the number of soybean root nodules at the R3 stage in 2019; **(D)**, the number of soybean root nodules at the R5 stage in 2019; **(E)**, the number of soybean root nodules at the V5 stage in 2020; **(F)**, the number of soybean root nodules at the R1 stage in 2020; **(G)**, the number of soybean root nodules at the R3 stage in 2020; **(H)**, the number of soybean root nodules at the R5 stage in 2020.

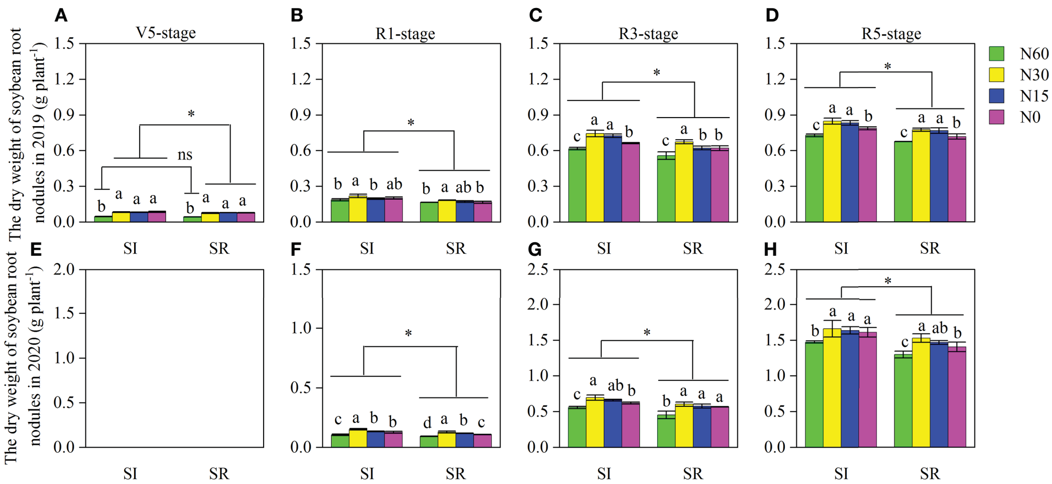

FIGURE 5
Effects of straw incorporation and N application on root nodule dry weight of intercropped soybean at different growth stages. Due to the small number of nodules per plant and small nodule diameter at the V5 stage in 2020, the nodule weight was not measured. Different lower-case letters indicate significant differences under different N application rates (LSD, *P*< 0.05). The asterisk "*" and "ns" indicate significant differences (*P*< 0.05) and insignificant differences (*P* >0.05) between straw incorporation and straw removal, respectively. Data were shown as mean ± S.D. (n=3). SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean. **(A)**, the dry weight of soybean root nodules at the V5 stage in 2019; **(B)**, the dry weight of soybean root nodules at the R1 stage in 2019; **(C)**, the dry weight of soybean root nodules at the R3 stage in 2019; **(D)**, the dry weight of soybean root nodules at the R5 stage in 2019; **(E)**, the dry weight of soybean root nodules at the V5 stage in 2020; **(F)**, the dry weight of soybean root nodules at the R1 stage in 2020; **(G)**, the dry weight of soybean root nodules at the R3 stage in 2020; **(H)**, the dry weight of soybean root nodules at the R5 stage in 2020.

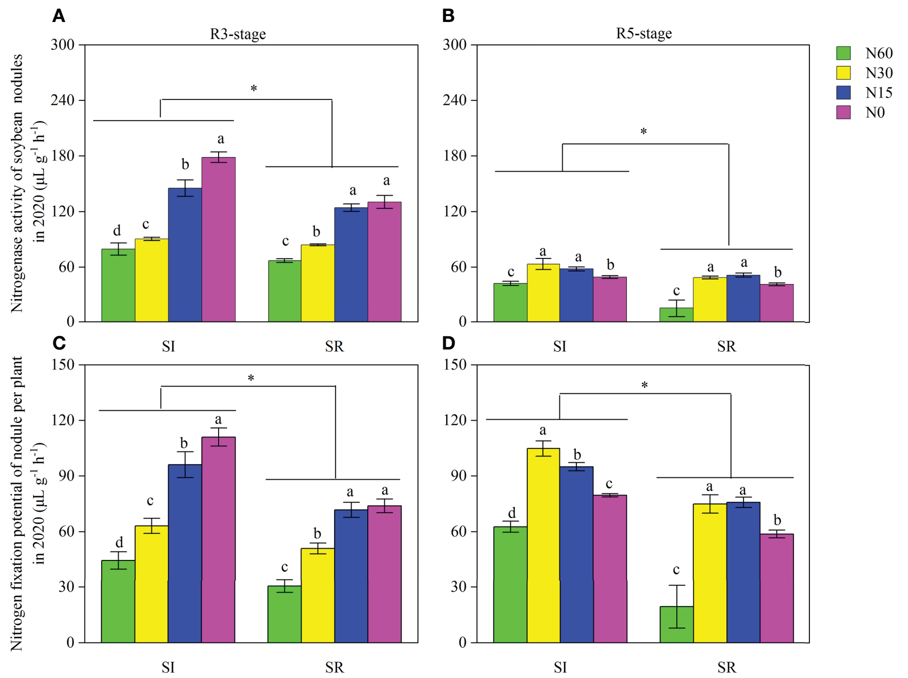

FIGURE 6
of straw incorporation and N application on nodule nitrogenase activity and nitrogenase fixation potential of intercropped soybean at different growth stages (in 2020). Different lower-case letters indicate significant differences under different N application rates (LSD, $P< 0.05$). The asterisk "*" indicate significant differences ($P< 0.05$) between straw incorporation and straw removal. Data were shown as mean ± S.D. (n=3). SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean. **(A)**, nitrogenase activity of soybean nodules at the R3 stage in 2020; **(B)**, nitrogenase activity of soybean nodules at the R5 stage in 2020; **(C)**, nitrogen fixation potential of soybean nodule per plant at the R3 stage in 2020; **(D)**, nitrogen fixation potential of soybean nodule per plant at the R5 stage in 2020.

reason why N is efficiently used in SI. Xu et al. (2018) found that straw incorporation coupled with low N input can promote root growth and deep root. In our study, the root growth parameters of intercropped soybean, e.g., root length and surface area, were greater in N30 than in N60 under the SI. In contrast, the root length and surface area of SR significantly decreased in N30 compared to N60. The improved root growth and configuration may be due to enhanced soil physical properties. Xu et al. (2018) found that straw incorporation coupled with low N application promoted root proliferation and growth in deep soil by decreasing soil bulk density. In our present study, SI significantly increased the soil macroaggregates' stability and porosity, but significantly reduced soil bulk density compared with the SR. This increases the soil permeability and the ability of water and fertilizer conservation, which is beneficial to the root growth of intercropped soybean. A well-developed root growth and distribution can promote root uptake capacity (Zheng et al., 2021; Zheng et al., 2022). The root bleeding sap intensity is an important indicator of root activity, and the components of bleeding sap reflect the nutrients of root absorption and transport (Zheng et al., 2021). In our study, we found that the root bleeding intensity of soybean in SI was notably increased compared with SR. Then, the ammonium-N and nitrate-N contents of bleeding sap were significantly increased in SI at the R3 and R5 stages. The ammonium-N and nitrate-N contents of soybean bleeding sap were greater in SI when combined with N input, especially N input exceeding 30 kg N ha^{-1}.

In addition, the increase in aboveground N uptake and utilization efficiency of intercropped soybean is related to soybean nodulation and N fixation (Zheng et al., 2022). Almost half of the N demand in the lifespan for legume growth can be met by biological N2 fixation (BNF) (Salvagiotti et al., 2008). The BNF of legumes not only meets its own growth needs but also provides additional N sources for soil. The BNF of legumes can be inhibited by N application (Hu et al., 2016). The nodulation and N fixation of soybean can be promoted by reducing N input in contrast to conventional N input (Du et al., 2020). With the increase of N input, although the nitrogenase activity and nitrogen fixation potential of soybean nodules were remarkably decreased at the R3 stage, those peaked in N30 at the R5 stage (Figures 6A-C). In contrast, the aboveground N uptake was notably increased with the increase of N input (Figure 7). In maize-soybean intercropping systems, increasing N input will promote maize

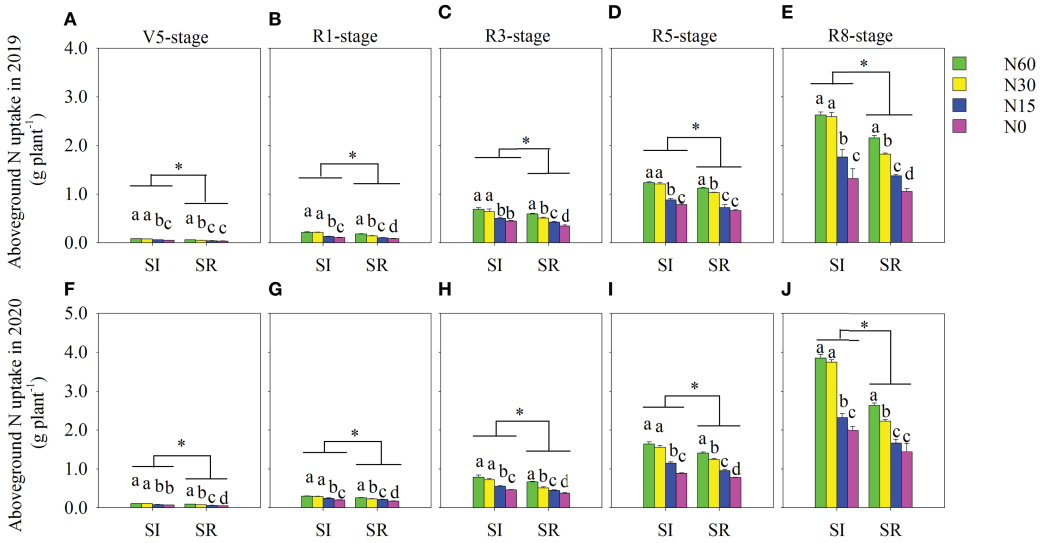

FIGURE 7
Effects of straw incorporation and nitrogen application on aboveground N-uptake of intercropped soybean at different growth stages. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P*< 0.05). Data were shown as mean ± S.D. (n=3). The asterisk "*" indicates significant differences (*P*< 0.05) between straw incorporation and straw remove. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean; R8, the full-maturity stage of soybean. **(A)**, aboveground N uptake at the V5 stage in 2019; **(B)**, aboveground N uptake at the R1 stage in 2019; **(C)**, aboveground N uptake at the R3 stage in 2019; **(D)**, aboveground N uptake at the R5 stage in 2019; **(E)**, aboveground N uptake at the R8 stage in 2019; **(F)**, aboveground N uptake at the V5 stage in 2020; **(G)**, aboveground N uptake at the R1 stage in 2020; **(H)**, aboveground N uptake at the R3 stage in 2020; **(I)**, aboveground N uptake at the R5 stage in 2020; **(J)**, aboveground N uptake at the R8 stage in 2020.

growth, then strengthen the interspecific competitive use of resources (Zheng et al., 2021). Thus legumes acquire more N from the soil than symbiosis nitrogen fixation due to resource limitation because symbiosis nitrogen fixation is an extremely energy-consuming process (Tjepkema and Winship, 1980). Moreover, we found that the nodule number, nodule dry weight, nodule nitrogenase activity, and nodule N fixation potential of soybean were significantly increased with the decrease of N application rate under both SI and SR treatments. Compared with SR, the nodulation and N fixation

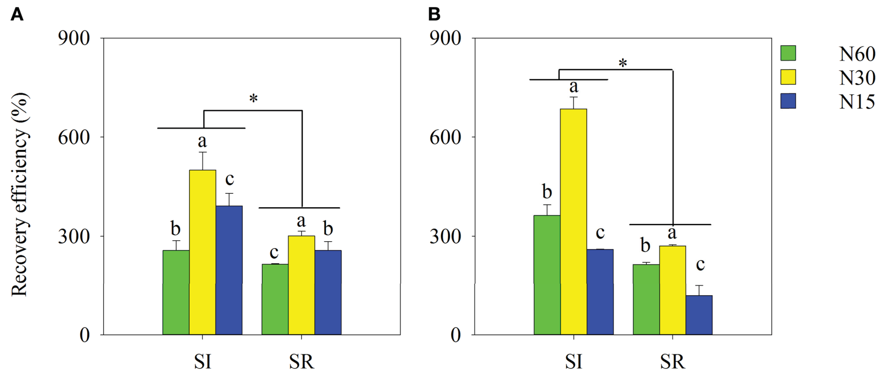

FIGURE 8
Effects of straw incorporation and nitrogen application on N use efficiency of intercropped soybean at the full-maturity stage. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P*< 0.05). Data were shown as mean ± S.D. (n=3). The asterisk "*" indicates significant differences (*P*< 0.05) between straw incorporation and straw removal. SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}). **(A)**, the recovery efficiency of soybean in 2019; **(B)**, the recovery efficiency of soybean in 2020.

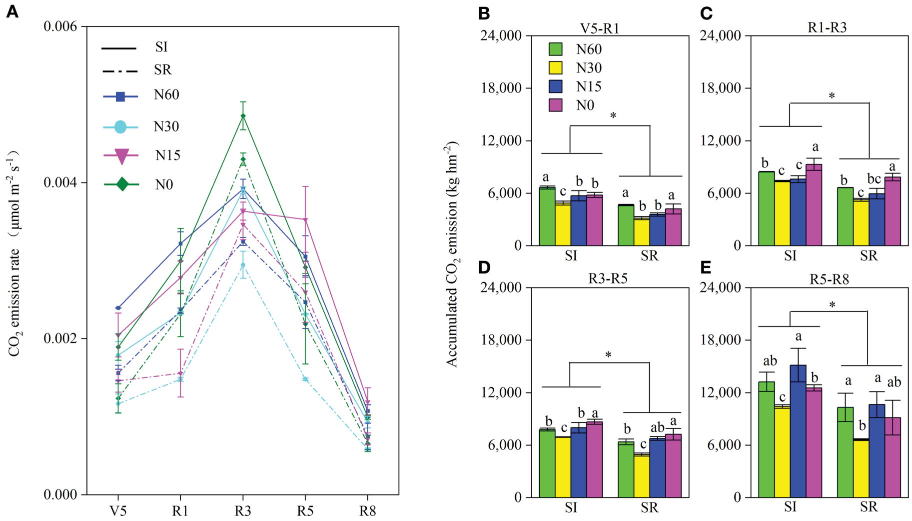

FIGURE 9
Effects of straw incorporation and nitrogen application on soil CO2 emissions properties. Different lower-case letters indicate significant differences under different N application rates (*LSD*, *P*< 0.05). Data were shown as mean ± S.D. (n=3). SI, straw incorporation; SR, straw removal; N60, convention N (60 kg N ha^{-1}); N30, reduced N by 50% (30 kg N ha^{-1}); N15, reduced N by 75% (15 kg N ha^{-1}); N0, zero N (0 kg N ha^{-1}); V5, the fifth trifoliolate stage of soybean; R1, the beginning flowering stage of soybean; R3, the beginning pod stage of soybean; R5, the beginning seed stage of soybean; R8, the full-maturity stage of soybean; V5-R1, the growth stage from V5 to R1; R1-R3, the growth stage from R1 to R3; R3-R5, the growth stage from R3 to R5; R5-R8, the growth stage from R5 to R8. The asterisk "*" indicate significant differences (P < 0.05) between straw incorporation and straw removal. **(A)**, the CO2 emission rate at the different growth stages of soybean; **(B)**, the accumulated CO2 emissions from V5 to R1; **(C)**, the accumulated CO2 emission from R1 to R3; **(D)**, the accumulated CO2 emission from R3 to R5; **(E)**, the accumulated CO2 emission from R5 to R8.

capacity of soybean were significantly enhanced by SI. Because competitive use of N between microorganisms and crops will decrease soil N when the straw is incorporated (Wang and Bakken, 1997). Then, the decrease of N content in soybean rhizosphere soil promotes soybean nodulation. Furthermore, Siczek A (2011) indicated that soybean's nodulation and N fixation ability could be enhanced by reducing soil compactness. Although straw incorporation significantly reduced soil bulk density, the soil macroaggregates stability and porosity notably increased. Finally, it was beneficial to nodulation and N fixation of soybean.

Straw incorporation not only increased N uptake and crop yield but also increased soil carbon emissions (Bhattacharyya et al., 2012; Zhao et al., 2019). In this study, straw incorporation significantly increased soil CO_2 emission rates and accumulated CO_2 emissions in contrast to straw removal under different N application rates. With the advance of the soybean growth process, the soil CO_2 emission rate increased at first and then decreased, and the highest CO_2 emission rate was observed in the R3-stage. Straw incorporation promoted soil microorganisms' activity by increasing soil C and N sources and improving soil physical properties (Li et al., 2018). In addition, the increase in CO_2 emission is related to the growth of the root system. This is probably due to the root exudates promoting microbial respiration (Badri and Vivanco, 2009). A previous study indicated that straw incorporation coupled with high N input could increase CO_2 flux (Pan et al., 2006). On the contrary, Zhang et al. (2020) found that straw incorporation coupled with reduced N input significantly decreased CO_2 emission in contrast to conventional N. Zhou et al. (2021) indicated that reduced N application combined with long-term reduce/zero tillage could significantly decrease soil C emissions. In this study, the soil CO_2 emission rates and accumulated CO_2 emissions significantly decreased in N30 compared to N60 under the SI treatment.

Conclusions

Our results indicated that the soil GMD of macroaggregates, soil total porosity, soil total N content, and soil urease activity were greater in SI than in SR. However, the soil bulk density in SI significantly decreased. Compared with SR, the root length, root surface area, volume, and biomass of soybean in SI were notably significantly increased. The root length and surface area were greater in N30 than in N60. Furthermore, soybean nodulation and N fixation in SI was markedly higher than in SR. Compared with convention N input, reduced N input significantly increased nodulation and N fixation of soybean. The aboveground N uptake and RE of soybean in SI significantly increased in contrast to SR. The highest RE of soybean was observed in SI with N30 treatment. Besides, the soil CO_2 emission rate and accumulated CO_2 emissions were significantly higher in SI than in SR. But, those were considerably decreased in N30 in contrast to N60. In conclusion, the increased N uptake and utilization were due to the improved soil properties, root N uptake capacity, and enhanced nodulation and N fixation of soybean. Straw incorporation coupled with 30 kg N ha^{-1} input was a sustainable strategy in the wheat-maize-soybean relay strip intercropping system. It significantly increases N uptake and utilization of soybean and reduces soil CO_2 emissions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

BZ, PC, and TY conceived and designed the experiment. BZ and PC performed the statistical analyses. BZ, PC, QD, HY, and KL were involved in field data collection. All authors contributed to writing the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1036170/full#supplementary-material

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EDITED BY
Junfei Gu,
Yangzhou University, China

REVIEWED BY
Anshu Rastogi,
Poznan University of Life Sciences, Poland
Fei Wang,
Huazhong Agricultural University, China
Ghulam Abbas,
Islamia University of Bahawalpur, Pakistan

*CORRESPONDENCE
Xiaochun Wang
xchwang@sicau.edu.cn

†These authors have contributed equally to this work

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Comparative analysis of farmer practices and high yield experiments: Farmers could get more maize yield from maize-soybean relay intercropping through high density cultivation of maize

Guopeng Chen[1,2†], Yongfu Ren[3†], Atta Mohi Ud Din[1,4,5], Hina Gul[6], Hanlin Chen[7], Bing Liang[1,2], Tian Pu[1,2], Xin Sun[1,2], Taiwen Yong[1,2], Weiguo Liu[1,2], Jiang Liu[1,2], Junbo Du[1,2], Feng Yang[1,2], Yushan Wu[1,2], Xiaochun Wang[1,2*] and Wenyu Yang[1,2]

[1]College of Agronomy, Sichuan Agricultural University, Chengdu, China, [2]Sichuan Engineering Research Center for Crop Strip Intercropping System, Key Laboratory of Crop Ecophysiology and Farming System in Southwest China (Ministry of Agriculture), Chengdu, China, [3]Agriculture Technology Extension Station, Liangzhou County Bureau of Agriculture and Rural Affairs, Wuwei, China, [4]Key Laboratory of Crop Physiology Ecology and Production Management, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, China, [5]National Research Center of Intercropping, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, [6]National Center of Industrial Biotechnology, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Shamsabad, Pakistan, [7]Agriculture Technology Extension Station, Pingchang County Bureau of Agriculture and Rural Affairs, Bazhong, China

Intercropping is a high-yield, resource-efficient planting method. There is a large gap between actual yield and potential yield at farmer's field. Their actual yield of intercropped maize remains unclear under low solar radiation-area, whether this yield can be improved, and if so, what are the underlying mechanism for increasing yield? In the present study, we collected the field management and yield data of intercropping maize by conducting a survey comprising 300 farmer households in 2016-2017. Subsequently, based on surveyed data, we designed an experiment including a high density planting (Dense cultivation and high N fertilization with plough tillage; DC) and normal farmer practice (Common cultivation; CC) to analyze the yield, canopy structure, light interception, photosynthetic parameters, and photosynthetic productivity. Most farmers preferred rotary tillage with a low planting density and N fertilization. Survey data showed that farmer yield ranged between 4-6 Mg ha^{-1}, with highest yield recorded at 10-12 Mg ha^{-1}, suggesting a possibility for yield improvement by improved cropping practices. Results from high density experiment showed that the two-years average yield for DC was 28.8% higher than the CC. Compared to CC, the lower angle between stem and leaf (LA) and higher leaf area index (LAI) in DC resulted in higher light interception in middle canopy and increased the photosynthetic productivity under DC. Moreover, in upper and lower canopies, the average activity of phosphoenolpyruvate (PEP) carboxylase was 70% higher in DC than CC. Briefly, increase in LAI and high Pn improved both light interception and photosynthetic productivity, thereby mediating an increase in the maize yield. Overall, these results indicated that farmer's yields on average can be increased by 2.1 Mg ha^{-1} by increasing planting density and N fertilization, under plough tillage.

KEYWORDS

intercropping, leaf angle, leaf area index, photosynthetic rate, yield

Introduction

Ever-increasing global population is a continuous challenge, especially for the densely populated countries like China, causing food security problems (Gandhi and Zhou, 2014). One of the key solutions of this problem is to improve the existing crop yield from cultivated lands. Multiple cropping systems like cereal-legume intercropping have been proven to play important role in improving land utilization as compared to mono-cropping system (Li et al., 2016). Therefore, these methods have been widely adopted worldwide in countries like China, America, India, and Africa to increase the crop productivity (Yang et al., 2015). In China, half of the total grain yield is produced through multiple cropping systems and intercropping is practiced on more than 2.8×10^7 ha of the arable land. Traditionally, Chinese farmers have intercropped soybean with wheat, maize, millet, cotton, etc. (Knörzer et al., 2009; Li et al., 2013) but the maize-soybean intercropping is considered the most productive in terms of resource use efficiency and land equivalent ratios. The success of cereal-legume intercropping system profoundly depends on the temporal and spatial complementarity of resource utilization (Xue et al., 2016). Therefore, several studies have been carried out on the critical aspects of intercropping such as varietal breeding and screening, planting pattern (Yang et al., 2015), lodging resistance (Luo et al., 2015), fertilizer management (Yong et al., 2014), water use efficiency and water distribution (Rahman et al., 2017), relative crowding coefficient, competitive ratio, actual yield loss, intercropping advantage indices, growth improvement and light irradiance (Yang et al., 2014). Such studies helped to understand the scientific basis to improve the intercropping systems, however, the knowledge about the actual intercropping practices performed by the farmers is still limited. Therefore, study about the common intercropping practices in farmer's field could help the researchers to address the yield disparity within farmers which will bring uniformity in the productivity of intercropping systems in the country.

Maize-soybean strip intercropping contains two major systems including traditional strip intercropping and relay-strip intercropping. In maize-soybean relay strip intercropping systems (MSR), the narrow-wide planting pattern is adopted and maize is usually sown either at the end of march or at the beginning of April and harvested in July-August (Yang et al., 2014). Later on, soybean is sown between the wide spaces of maize strips at the beginning of June and harvested in late October (Yang et al., 2014). Therefore, relay intercropping help to grow both crop species during one season, in areas like Sichuan where the growing season is too short for the double cropping (Yang et al., 2015). In recent years, maize-soybean relay strip intercropping system has been popularized in the Southwestern China (Yan et al., 2010) and provided considerable economic and social benefits for small-land hold farmers. Importantly, the southwest China is one of the most densely populated agricultural regions where farmers possess relatively small pieces of cultivated area (some plots less than 500 m^2 per farmer), thus farmers adopt different cultivation patterns and practices (Chen et al., 2015; Yan et al., 2018; Zhou et al., 2019a). This phenomenon has generated a wide variation in methods used for fertilization, tillage, and varietal choosing (Liu et al., 2021a). Previously, Gou et al., 2017 evaluated yield potential under the intercropping system in Northwest China under abundance solar radiation, more than 6000 MJ m^{-2} per year. They found that the potential yield of intercropped maize was 12.0 t ha^{-1}, with an actual yield of 10.1 t ha^{-1} in farmer's field. Notably, the maize yield increased after input of N- and P-fertilizers, reaching 17.1 t ha^{-1} (Li et al., 2001). The yield increase was largely attribute to the complementarity effect, nutrient input, choosing compact cultivars, and adequate irrigation (Gou et al., 2017; Chen et al., 2019; Li et al., 2020). However, in southwest China which have comparatively lower solar radiation, little is known about the actual yield of maize farmers adopting the relay intercropping system.

In present study, we hypothesized that maize yield under the intercropping system in Southwest China can be improved by adjusting field management and increase in the light interception as well as photosynthetic productivity. Therefore, we collected and analyzed field management and yield data from 300 farmers in Sichuan province over two-years. Subsequently, we designed a high yield experiment for two years, to analyze canopy structure, light interception, photosynthetic parameters, photosynthetic productivity, and yield. The findings of this study provide new insights into the common intercropping practices by the farmers, which could help the future studies to propose a uniform intercropping system in terms of yield and productivity.

Materials and methods

Assessment of commonly used farmer practice

We selected and visited 300 farmers for survey in Sichuan province between 2016 and 2017 to assess the commonly used farmer practice for MSR in the Sichuan province. Three counties were randomly selected from Sichuan province. For each county, 10 villages were randomly selected, with each village providing 10 households. All the surveyed farmers were involved in MSR. Data collected from these farmer fields included maize grain yield from intercropped fields, planting density, tillage methods, and N fertilization. For more details about the survey data, please see the Supplementary-Survey data.

Site and experimental design

Maize (*Zea mays* L.) variety Zhongyu-3 (with a small angle between stem and leaf, and an average of 19 leaves per plant, resistant to ear rot) and soybean (*Glycine max* L.), variety Nandou-12 (shade-tolerant soybean) were used in the present study. The two varieties occupy the largest local planting area under maize and soybean cultivation. Field experiments were carried out at Modern Agriculture Expert Compound Renshou County, Sichuan Province, China (29°60′ N, 104°00′ E). The study site had an average annual air temperature of 17.4 °C, precipitation of 1009.4 mm, sunshine of 1196.6 h, and lower solar radiation of 3580 MJ m^{-2} (Figure 1) (Gajipra, 2015; Zhou et al., 2019b). Details on solar radiation of maize at key stages, namely V6, V14, R2, are shown in Figure 1B (Tang et al., 2015).

Based on the information obtained from the farmer field survey, we designed a field experiment to assess the response of maize yield components to high density planting (Dense cultivation; DC) as compared to normal farmer practice (Common cultivation; CC). In addition, we adopted plough tillage for DC, and added more nitrogen to compensate the competition within maize plants. The CC was designed on the basis of highest frequency from surveyed data (Figures 2C-H) and the intercropped maize was planted with the density of five plants m^{-2} and N fertilizer applied at a rate 240 kg N ha^{-1} under rotary tillage (Figure 2). DC was designed with high density approach in which intercropped maize was planted at a density of 6.75 plants m^{-2} (the highest density from surveyed data) and N fertilizer applied at a rate of 270 kg N ha^{-1}, under plough tillage. The experiments were conducted in a randomized block design, with three replicate blocks and a total of six plots (2 treatments × 3 blocks). Each plot had an area 267 m^2 (6 m × 44.5 m). Importantly, both CC and DC have same configuration of MSR, i.e., 2M2S (two-rows of soybean were relay-intercropped with two rows of maize after 60 ± 10 days of maize sowing) in which the strip of maize and soybean each had 40 cm width, with 60 cm of space between the strips of maize and soybean (Figure S1). The distance of the plant to plant in CC and DC were 20 cm and 15 cm, respectively. Fertilizer, superphosphate, was applied at a rate of 600 kg ha^{-1} (containing 12% P_2O_5), and 150 kg ha^{-1} of potassium chloride (containing 60% KCl) for maize in CC and DC. Maize was sown on April 9, 2018, and April 5, 2019, while soybean was sown on June 17 of each year. Manual weeding was performed as per requirement under the rainfed agriculture. Maize harvesting was done on August 5, 2018, and August 9, 2019. Soybean harvesting was done on October 26, 2018, and October 28, 2019. Soybean was planted with the density of 12 plants m^{-2}; N fertilizer was applied at a rate of 30 kg N ha^{-1}, 30 kg ha^{-1} of potassium chloride (containing 60% KCl), and 30 kg ha^{-1} of superphosphate (containing 12% P_2O_5).

Analysis of plant morphology

LAI (leaf area index), which refers to the leaf area of the unit land area, was calculated using the ratio of leaf area to the maize and soybean planting areas (Liu et al., 2018b). Total LAI at V6 (sixth leaf), V14 (fourteen leaf), R1 (silking), R2 (blister), and R6 (maturity) stages was measured from five randomly selected plants of intercropped maize. Furthermore, the upper (above the three-ear leaves), middle (three-ear leaves), and lower (below the three-ear leaves) canopies LAI were calculated at R1 stage, respectively. Similarly, the other morphological parameters including plant height, ear height, stem diameter, leaf angle (LA, the angle between leaf and stem), and leaf orientation value (LOV) were also measured from five randomly selected plants at R1 stage. Ear height was the distance from the ground to the uppermost ear bearing node. Leaf area of individual leaves was calculated using the following formula according to a method by (Gao et al., 2016).

$$\text{Leaf area} = \text{length x width x } 0.75$$

A protractor was used to measure the upper canopy LA (the average LA of above three-ear leaves); the middle canopy LA (the average LA of three-ear leaves); the lower canopy LA (the average LA of three-ear leaves).

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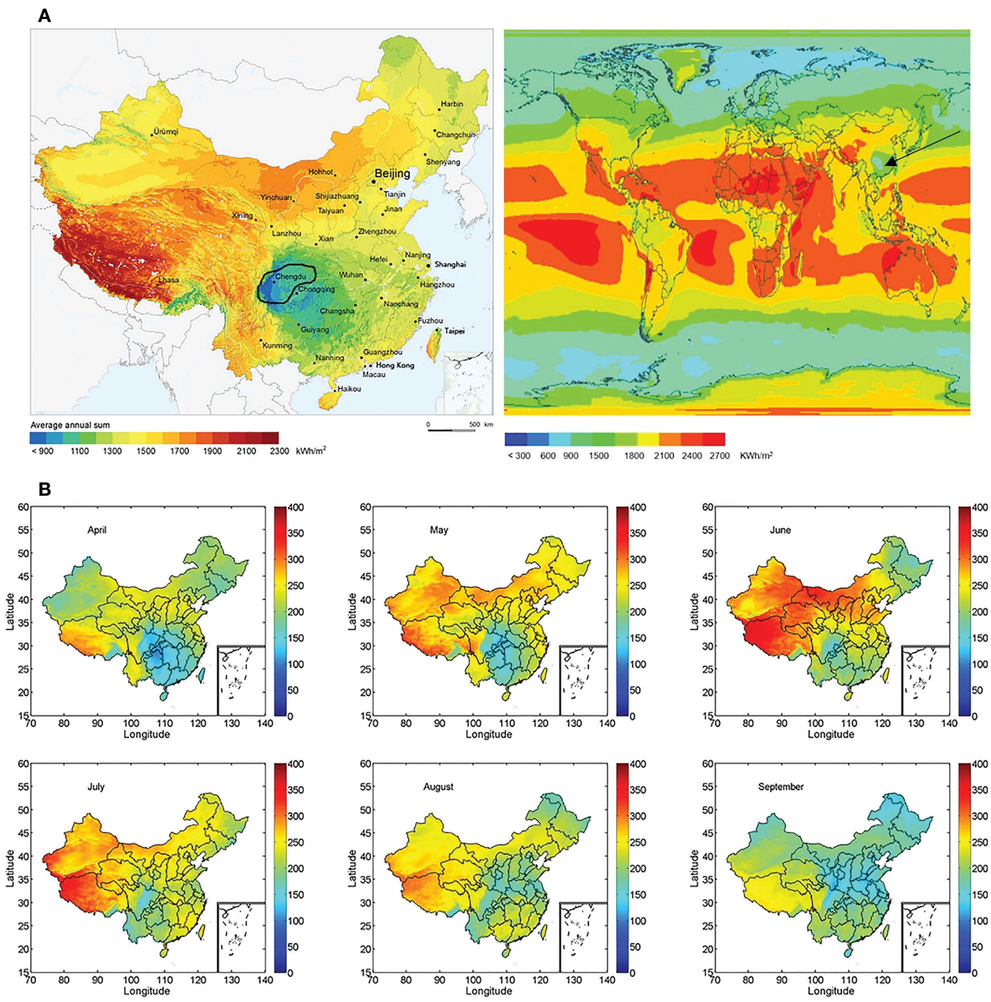

FIGURE 1
Solar radiation in various regions of China (2021 Copyright Sun Reign Ltd). **(A)**: The black circle indicates Sichuan province. Southwest China (includes the Sichuan, Yunnan, Guizhou provinces, and Chongqing city) are lower solar radiation than surrounding countries. **(B)**: Solar radiation during the maize growth period, the unit of solar radiation is W m^{-2}.

LOV was calculated using the following formula, as previously described (Pepper et al., 1977; Lu et al., 2018):

$$\mathrm{LOV} = \sum_{i=1}^{n} \frac{(90 - \theta i)\left(\frac{Lf}{L}\right)}{n}$$

Where θ_i is the angle between stem and leaf, L denotes leaf length, Lf represents the spatial distance between the leaf collar and leaf tip, whereas n indicates the number of measured leaves. For instance, there are three leaves in middle canopy, middle canopy LOV = $(90 - \theta_1)(Lf_1/L_1)/3 + (90 - \theta_2)(Lf_2/L_2)/3 + (90 - \theta_3)(Lf_3/L_3)/3$. LOV of the upper and lower canopies were calculated using a similar approach for the middle canopy.

Determination of light distribution, transmittance, and light interception rate

The measurements were taken on a sunny and cloudless day, between 10:00 AM and 12:00 PM. PAR was measured using a 1-m line quantum sensor (LI COR Inc., Lincoln, NE, USA) and an LI-1400 data logger. Measurements in the canopy were performed at a 30-cm and 20-cm intervals in the vertical and horizontal direction, respectively, at R1 stage in 2018 and 2019 (Figure S1). Light transmittance in respective canopies were calculated as follows: upper canopy = I_u/I_t ×100%; middle canopy = I_m/I_t ×100%; lower canopy = I_l/I_t ×100%. On the other hand, light interception rates in respective canopies were calculated as

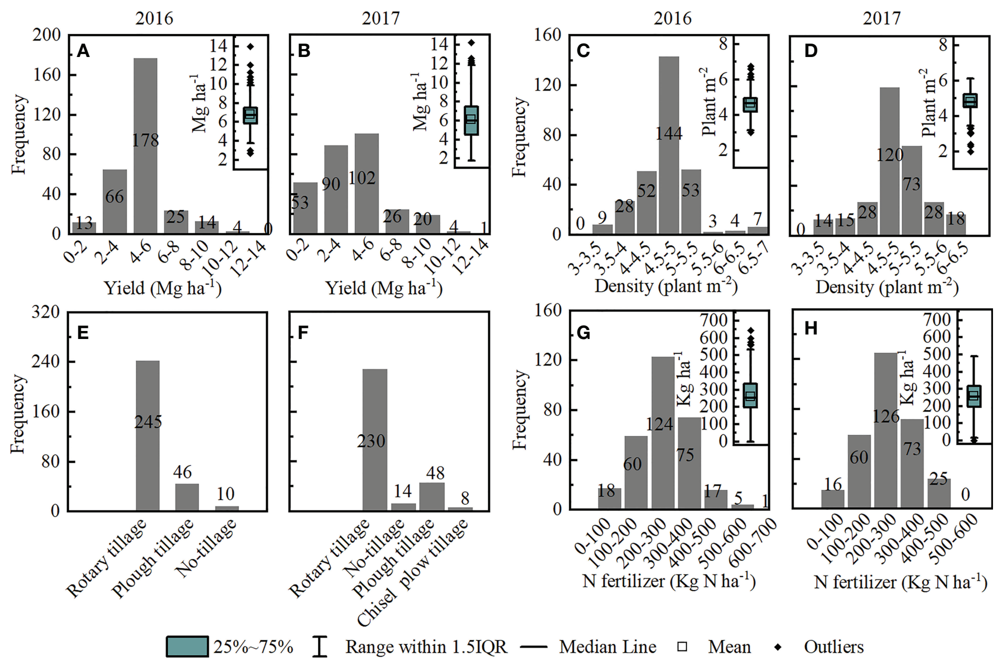

FIGURE 2
Statistics for yield, planting density, tillage methods, and nitrogen (N) fertilizer survey data of relay intercropping maize from farmers. **(A, B)**: grain yield in 2016-2017. **(C, D)**: the frequency distribution histogram and boxplot of planting density in 2016 and 2017. **(E, F)**: the frequency distribution histogram of tillage methods in 2016 and 2017. **(G, H)**: the histogram and boxplot of N fertilizer in 2016 and 2017; n = 300.

follows: upper canopy = (1-I_u/I_t)×100%; middle canopy = (1-I_m/I_u)×100%; lower canopy = (1-I_l/I_m)×100%. I_t is PAR of the top canopy; I_u, Im, and I_l denote PAR of the upper, middle, and lower canopies, respectively (Liu et al., 2018a) (Figure S1).

Analysis of key enzyme activities involved in photosynthesis

Five plants in each plot were randomly selected at the R1 stage, and the activities of Rubisco and PEP carboxylase enzymes was assayed in the upper canopy (fourth leaf above the ear leaf), middle canopy (ear leaf), and lower canopy (fourth leaf below the ear leaf). All leaf samples were immersed in liquid nitrogen and immediately stored at -80 °C for measuring the enzyme activities. Then, we extracted crude enzyme, and measured Rubisco and PEP carboxylase activities according to the previously published methods (Wang et al., 2008; Sui et al., 2017), with slight modifications. 100 mg leaf sample was ground with extraction buffer. Then were centrifuged at 12,000 ×g at 4 °C for 15 minutes. Supernatants were used as crude extract for total activity assays. Activation was performed in a 100 µl mixture solution at 28 °C for 15 minutes. Initial Rubisco activity was determined. The change in the absorbance of NADH was measured at 340 nm within one min. PEP carboxylase activity was measured spectrophotometrically at 340 nm by coupling the PEP carboxylase reaction to the malate dehydrogenase (MDH) reaction, using a buffer with 50 mM bicine (pH 8.2), 2 mM DTT, 5 mM $MgCl_2$, 1 mM $NaHCO_3$, 1 mM Na_4EDTA, 0.25 mM glucose-6-phosphate, 0.15 mM NADH, 2 units MDH and 2 mM PEP and enzyme extract. The reaction was initiated by the addition of PEP.

Determination of the photosynthetic rate and productivity

Photosynthetic activity was measured on a clear and cloudless day, between 10:00 AM and 12:00 PM, at R1 stage. Five plants in each plot were randomly selected and Pn of the upper canopy (fourth leaf above the ear leaf), middle canopy (ear leaf), and lower canopy (fourth leaf below the ear leaf) were determined using LI-6400-XT photosynthetic apparatus (Lincoln, USA). The tests were performed under the following conditions: leaf chamber temperature was set at 25 °C, PAR of 1000 µmol m^{-2} s^{-1}, and a CO_2 concentration maintained at 400 μmol mol^{-1}. Photosynthetic productivity was calculated using the Baig formula (Baig et al., 1998) as follows:

$$\text{Photosynthetic productivity} = \text{Pn x LAI}.$$

Analysis of yield and yield components

An area of 30 m^2 was selected and effective ear at maturity counted. Twenty ears were selected to determine grain number per ear, and 1000-grain weight (1000-GW), with the yield recorded as follows:

$$\text{Yield} = \text{effective ear x grain number per ear x 1000-GW}$$

(Chen et al., 2019).

Statistical analysis

Statistical analyses were performed using SPSS software (SPSS 22, SPSS Inc., USA), and difference among groups was determined using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) multiple-range test. Data followed by $P < 0.05$ was considered statistically significant. Correlation analysis was performed using the Pearson correlation coefficient test, while figures were generated using Origin Pro (version 2019, Origin Lab).

Results

Yield, planting density, tillage methods, and N fertilizer survey data

Results from the survey, comprising about 300 farmer's households showed that most of the intercropping grain yields were 4-6 Mg ha^{-1} in two years. Notably, in 2016 and 2017, 59.3% and 34.5%, respectively, of the surveyed fields had a yield value 4-6 Mg ha^{-1}. The average yields were 6.8 and 6.1 Mg ha^{-1} in 2016 and 2017, respectively (Figures 2A, B). Most farmers preferred a planting density of 4.5-5 plants m^{-2}, with 48.0% and 40.5% of the surveyed field maintaining this planting density in 2016 and 2017, respectively. The average planting density for 2016 and 2017 was 4.7 and 4.8 plants m^{-2} (Figures 2C, D). In addition, most of the surveyed farmers practiced rotary tillage (Figures 2E, F). Annual N fertilizer usage ranged from 200-300 kg ha^{-1} in 2016 and 2017, with average of 240.9 and 251.9 kg ha^{-1}, respectively (Figures 2G, H).

Grain yield and yield components under field experiments

Our DC's enhanced field management increased grain yield (Table 1). Notably, yields under DC increased by 10.7% and 46.8% in 2018 and 2019, respectively, compared to the CC. We found no statistical significance in 1000-KW between DC and CC. We recorded significantly higher effective ear number under DC than that under CC, while the grain number per ear decreased. The effective ear is a critical determinant of maize yield under DC.

LAI of different layers in the canopy and total LAI

All LAI across different canopies under DC were higher than those recorded under CC across the two years (Figures 3A, B). The average two-year LAI in the upper (0.8), middle (0.3) and lower canopies (0.7) under DC significantly higher than that in CC. Similarly, the total LAI recorded in DC was significantly higher than CC at all the studied growth stages (V6, V14, R1, R2 and R6) (Figures 3C, D).

Morphology of maize plants, LA and LOV

The average plant height and ear height under DC was 8.5% and 11.1% higher than those under CC, across 2018 and 2019, respectively. However, stem diameter was lower under DC compared to CC across both years (Figures 4A–F). Next, we determined the LA and LOV across different canopies, and found that the LA of DC decreased under upper and middle canopy, while LOV increased in 2018 and 2019, compared to CC (Table 2). DC had reduced stem diameter and LA and increased plant height, ear height, and high LOV.

TABLE 1 Grain yield and grain yield components of CC and DC.

Year	Treatment	Effective ear ($\times 10^3$ ear·ha^{-1})	Grain number per ear	1000-GW (g)	Yield (Mg·ha^{-1})
2018	CC	51.69b	626.40a	251.60a	8.15b
	DC	63.92a	559.77b	252.15a	9.02a
2019	CC	46.02b	550.79a	281.83a	7.14b
	DC	65.87a	534.86a	297.39a	10.48a

Values are the average of three replicates. DC, Dense cultivation; CC, Common cultivation; 1000-GW, 1000 grains weight. Statistical analysis was carried out using the one-way ANOVA test in 2018 and 2019, respectively. Different letters denote significant differences (P < 0.05).

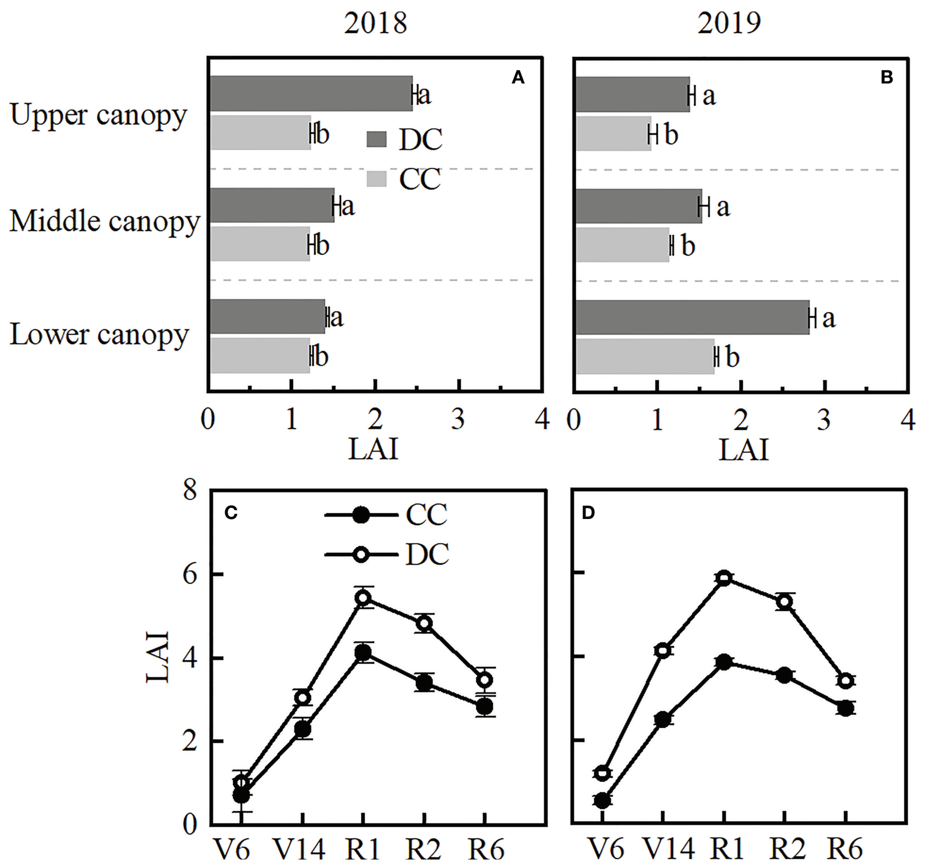

FIGURE 3
LAI of different layers of canopy, and total LAI in different stages. **(A, B)**: LAI of the upper, middle, and lower canopy at the R1 stage in 2018 and 2019. **(C, D)**: total LAI of different stages in 2018 and 2019. Upper canopy: above the three-ear leaves. Middle canopy: three-ear leaves. Lower canopy: below the three-ear leaves. DC, Dense cultivation. CC, Common cultivation. Different letters denote significant differences (P < 0.05), error bars show standard error of mean.

Light distribution, transmittance, and interception rate

Results from light distribution tests revealed lower PAR in DC than CC, within 0-175 cm vertical and 0-40 cm horizontal area of the canopy, respectively. Particularly, PAR within vertical 75 cm was lower under DC, compared to CC (Figures 5A, B). Compared to CC, we noticed significantly lower transmittance in lower and middle canopies of DC, but there was no statistical difference between DC and CC with regards to transmittance and light interception in the upper canopy (Table 3). Moreover, it is worthy to notice that light interception rate of middle canopy in DC was significantly higher than CC.

Activities of PEP carboxylase and Rubisco

In comparison to CC, PEP carboxylase activity was significantly higher in the upper and lower canopies of DC. On average, the activity was 6.1% and 7.8% higher in 2018 and 2019 in DC as compared to CC, respectively (Figures 6A, B). Similarly, the Rubisco activities in both upper and lower canopy leaves were higher in DC than in CC (Figures 6C, D). DC field management not only improved PEP carboxylase activities in the upper leaves but also the Rubisco activities of upper and lower canopy leaves.

Pn and photosynthetic productivity

In the upper canopy, Pn was significantly greater in DC compared to CC (Table 4). However, no significant differences were observed between DC and CC with regards to Pn in the middle and lower canopies. DC recorded higher photosynthetic productivity in the upper, middle, lower, and total canopies were higher than CC in 2018 and 2019. The DC had a higher photosynthetic productivity across all canopies.

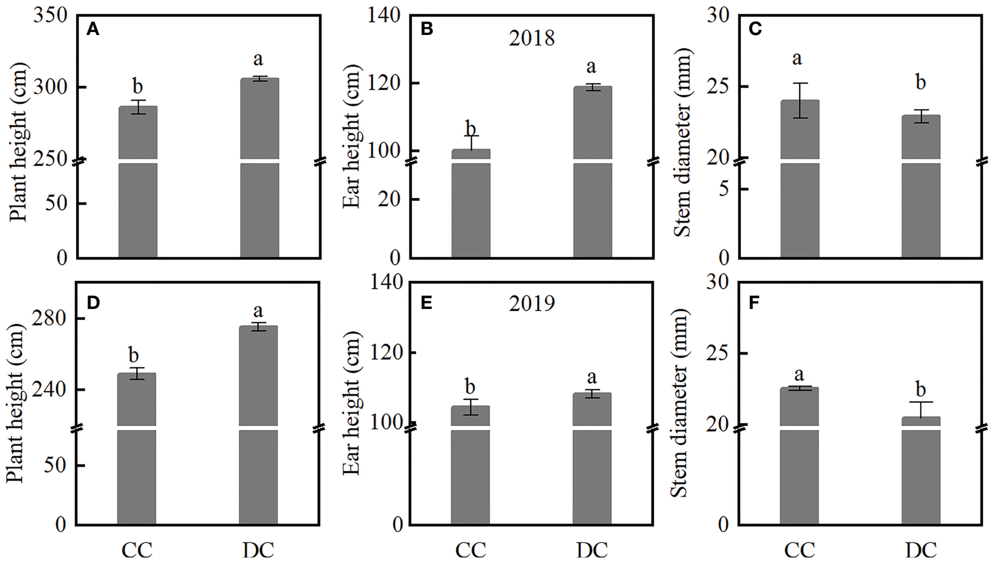

FIGURE 4
The morphology of the maize plants at the R1 stage. **(A–C)**: plant height, ear height, and stem diameter of CC and DC in 2018. **(D–F)**: plant height, ear height, and stem diameter of CC and DC in 2019. DC, Dense cultivation. CC, Common cultivation. Different letters denote significant differences (*P* < 0.05), error bars show standard error of mean.

TABLE 2 Leaf angle and leaf orientation value of different canopies at the R1 stage.

Year	Treatment	Upper canopy		Middle canopy		Lower canopy	
		LA (°)	LOV	LA (°)	LOV	LA (°)	LOV
2018	CC	26.22a	55.31b	29.44a	51.93b	32.22a	46.57a
	DC	23.26b	57.76a	26.11b	53.17a	32.93a	48.40a
2019	CC	25.67a	52.77b	28.11a	45.60b	29.55a	42.84b
	DC	21.70b	70.64a	26.90b	60.80a	29.31a	56.06a

Values are the average of three replicates. DC, Dense cultivation. CC, Common cultivation. LA, leaf angle (the angle between leaf and stem). LOV, leaf orientation value. Upper canopy, above the three-ear leaves. Middle canopy, three-ear leaves. Lower canopy, below the three-ear leaves. Statistical analysis was carried out using the one-way ANOVA test in 2018 and 2019, respectively. Different letters denote significant differences (P < 0.05).

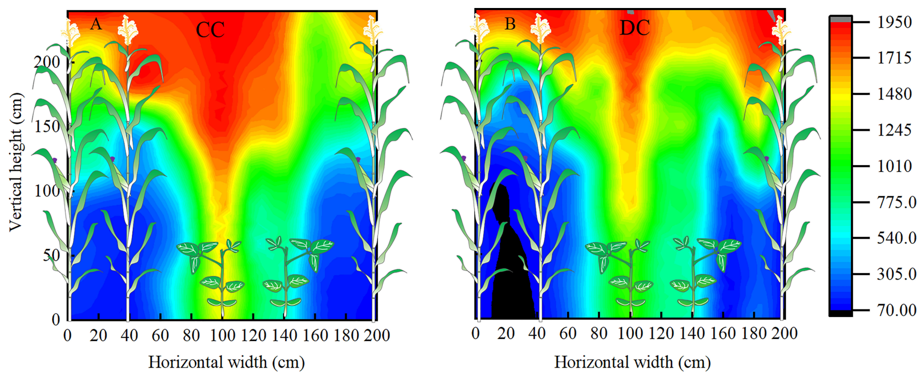

FIGURE 5
Light distribution in maize canopy. **(A, B)**: the light distribution of maize canopy in CC and DC at the R1 stage, respectively. DC, Dense cultivation. CC, Common cultivation. Plot values are photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}).

TABLE 3 Transmittance and light interception rate in different canopy.

Treatment	Transmittance (%)			Light interception rate (%)		
	Upper canopy	Middle canopy	Lower canopy	Upper canopy	Middle canopy	Lower canopy
CC	91.37a	40.84a	23.31a	8.63a	50.52b	17.53a
DC	93.34a	27.83b	18.27b	6.66a	65.52a	9.56b

Values are the average of three replicates. DC, Dense cultivation. CC, Common cultivation. Upper canopy: above the three-ear leaves. Middle canopy: three-ear leaves. Lower canopy: below the three-ear leaves. Different letters denote significant differences (P < 0.05).

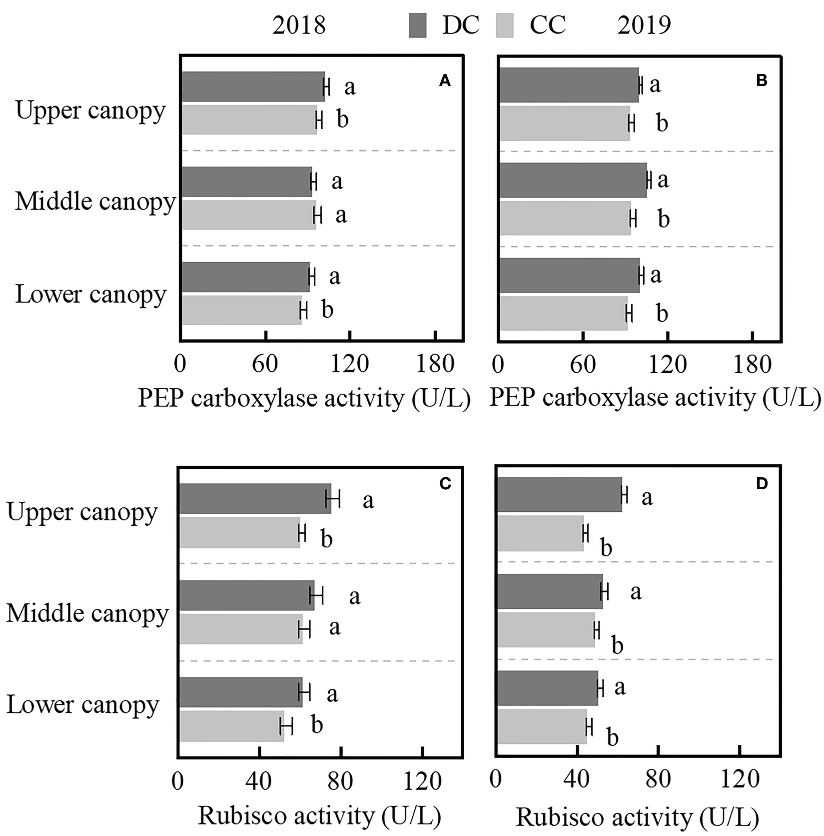

FIGURE 6
Activities of PEP carboxylase and Rubisco in the different canopy leaves at the R1 stage. **(A, B)**: PEP carboxylase activity of upper, middle, and lower canopy leaves in 2018 and 2019. **(C, D)**: Rubisco activity of differences canopy leaves in 2018 and 2019. DC, Dense cultivation. CC, Common cultivation. Different letters denote significant differences (*P* < 0.05), error bars show standard error of the mean.

TABLE 4 Net photosynthetic rate and photosynthetic productivity in different canopy.

Year	Treatment	Pn (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)			Photosynthetic productivity (Pn×LAI) (mg CO_2 m^{-2} s^{-1})			
		Upper canopy	Middle canopy	Lower canopy	Upper canopy	Middle canopy	Lower canopy	Total
2018	CC	24.35b	19.45a	16.54a	21.67b	24.12b	19.85b	66.97b
	DC	26.32a	18.91a	17.64a	47.90a	29.88a	24.69a	100.59a
2019	CC	27.24b	27.12a	20.59a	20.31b	31.71b	39.25b	91.27b
	DC	31.62a	31.30a	21.76a	44.31a	53.75a	58.73a	156.79a

Values are the average of three replicates. DC, Dense cultivation. CC, Common cultivation. Upper canopy: above the three-ear leaves. Middle canopy: three-ear leaves. Lower canopy: below the three-ear leaves. Different letters denote significant differences (P < 0.05). Pn, net photosynthetic rate. LAI, leaf area index.

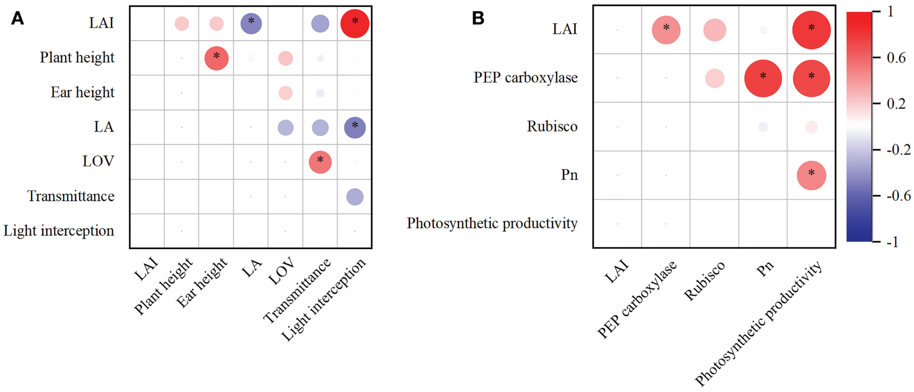

FIGURE 7
Correlation analyzed with light interception **(A)** and photosynthetic productivity **(B)**.

Correlation analysis

Results from correlation analyses are shown in Figures 7A, B. Summarily, light interception was significantly positively correlated ($P < 0.05$) with LAI, which showed a negatively correlated ($P < 0.05$) with LA. In addition, the Pn correlated significantly positively ($P < 0.05$) with PEP carboxylase activity. Similarly, a significant positive correlation ($P < 0.05$) was observed between photosynthetic productivity with Pn and LAI (Figure 7B).

Discussion

Farmer yield potential still has space for further improvement

The outcome of the survey showed that most farmer yields reached 4-6 Mg ha^{-1}, with only four farmers achieving 10-12 Mg ha^{-1}. This suggests that yield more than 10-12 Mg ha^{-1} is theoretically feasible (Figures 2A, B). Subsequently, we investigated the effect of field management and found that most of the farmers maintain planting density of 4.5-5 plant m^{-2}. Moreover, rotary tillage was the local primary tillage modality, while the annual N fertilizer usage range from 200-300 kg ha^{-1} (average 246.4 kg ha^{-1}). Numerous studies have shown that effective field management improves yield. Particularly, a high population density has excellent effect in maize by increasing radiation utilization efficiency and significantly improving grain yield potential (Liu et al., 2017; Gonzalez et al., 2018). Plough tillage increased grain yield, due to the deeper tillage depth reduced nutrition loss by surface runoff (Du et al., 2019). In Southwestern China, annual N fertilizer application in intercropping maize was found to be about 200-240 kg N ha^{-1} (Wang et al., 2017; Yang et al., 2017; Raza et al., 2019a). Accordingly, we designed DC comprising higher population density (6.75 plants m^{-2}), plough tillage, and rational use of N fertilizer (270 kg N ha^{-1}). This system resulted in an average yield increase of 28.8% compared to the CC. Although, it is common that high density and increased fertilization result in higher yield, but our study is more systemic as it is based on the results from an extensive survey that makes our DC more authentic and practical.

Solar radiation is vital for photosynthesis, while radiation intensity has a key role in determining the maize planting density in the local area (Zhang et al., 2006). Previous studies have suggested that CC in Southwestern China usually adopt the low density (4.8 plants m^{-2}) system due to abundance of rainfall and low solar radiation (Ming et al., 2017). Other evidences have also shown that excessive rainfall is unfavorable to increase planting density, while high humidity is not conducive for seeding formation and also leads to vigorous growth as well as lodging (Ming et al., 2017). However, the results in present study clearly indicated that adjusting the field management significantly improves farm yields, and does not cause vigorous growth and lodging. In addition, DC yield was lower than the record for maximum yield from survey data. Four farmers have achieved highest yield; the most probable reason for this difference was different planting region. Another possible reason is application of farmyard manure.

To date, the yield potential of relay intercropped maize under low solar radiation area remains unclear. Some scholars applied model simulations to obtain maximum yield potential in Northwest China (where solar radiation is abundant), as evidenced by 12.0 Mg ha^{-1}, farmer yields was 51% lower than maximum yields potential (Gou et al., 2017). In the present study, we obtained an average yield 9.8 Mg ha^{-1} under DC system, which was 18.3% lower than the potential yield in

Northwest China. Remarkably, Southwest China has lower solar radiation compared to suitable global areas for crop planting, and annual precipitation is 1009.4 mm, which is 3.9-fold in Northwest China (Gou et al., 2017; Liu et al., 2017). Low solar radiation and high precipitation led to a decrease in yield potential under DC. Additionally, we obtained more yield potential under the DC system than what has been reported in many previous studies on maize intercropping in Southwestern China (Wang et al., 2017; Chen et al., 2019; Feng et al., 2019; Raza et al., 2019a; Raza et al., 2019b; Feng et al., 2020). Although we did not achieve the maximum yield potential of maize intercropping, the DC system mediated a marked increase in yield as compared to the CC and what has been reported in previous studies. Based on these findings, it is evident that increasing planting density and fertilization as well as adopting plough tillage can improve yield potential in Southwest China.

Canopy structure under DC improved the light interception

Capture of a crop's light energy is determined by canopy light interception (Liu et al., 2021b). Analysis of canopy structure is an effective way to evaluate light interception ability (Subedi and Ma, 2005). Light interception and LA are closely related, with optimal LA observed to improve light interception of the rice canopy. (Hammer et al., 2009; Sher et al., 2018; Xu et al., 2018). Additionally, higher LAI and LOV mean higher light interception, which is also the case for plant height (Ma et al., 2014; Hu et al., 2016; Senapati et al., 2019). Results of the present study indicated that the DC system resulted in higher plant height than CC, as well as higher LAI and LOV across all the canopies. On the other hand, transmittance of middle and lower canopy declined in DC while light interception rate increased (Tables 2 and Table 3). To find out whether canopy structure plays a role increasing light interception, we further correlated canopy structure and light interception. Results showed that light interception had a significant negative correlation with LA, but a significant positive correlation with LAI (Figure 7). These results indicate that both LA and LAI play a key role in determining light interception in the canopy. The low value of LA in the upper and middle canopies, higher value of LAI in the upper, middle, and lower canopies ensured high light interception in DC. Interestingly, why does decrease of LA under DC? Previous studies have shown that LA increased (leaf inclination angles decrease) with leaf weight and area (Hernández, 2010). Modification in leaf orientation suggest shade avoidance reactions by a reduction in the red:far-red ratio of light in the canopy (Maddonni et al., 2001). As a result, a decrease in leaf weight and area per plant, as well as shade avoidance behaviors, may be major factors contributing to decreased LA in DC.

Canopy structure of DC improved photosynthetic productivity

The photosynthetic productivity under the DC system improved due to the Pn of upper canopy and an increase in the LAI of all canopies (Table 4 and Figure 3). Similarly, activities of two major enzymes, namely PEP carboxylase and Rubisco (Paulus et al., 2013; Atkinson et al., 2020), were high in the upper and lower canopies (Figure 6). Additionally, photosynthetic productivity exhibited a significant positive correlation with Pn and LAI, with Pn also showing a significant positive correlation with PEP carboxylase activity (Figure 7). The increase in PEP carboxylase activity in upper and lower canopies, coupled with Pn in the upper canopy, as well as elevated LAI across all canopies, generated a corresponding increase in photosynthetic productivity under DC.

The several layers in a maize canopy each serve a distinct functions. For instance, leaves around and above the ear commonly provide energy for grain development. Previous studies have shown that enhanced light interception in the middle canopy (100-150 cm) positively affects grain yield (Liu et al., 2011). In the present study, photosynthetic productivity increased under DC in the middle canopy, suggesting a possibility for increased yield. Additionally, we found that leaves at a height of 0-100 cm had improved light interception in wide-narrow row planting patterns in maize (Figure 5). These leaves provide photosynthates that aid in root development and growth (Liu et al., 2011), which subsequently have far-reaching implications for grain yield improvement. Under DC, lower canopy leaves (0-100 cm) exhibited higher Pn and photosynthetic productivity, which consequently enhanced grain yield.

Notably, the previous studies suggested that increase of intercropping grain yield was benefited by the complementarity effect (Gou et al., 2017; Li et al., 2020). The component crops in intercropping have a longer coexistence period than that in relay intercropping. The competition for nutrients is more important than aboveground competition for light in maize-soybean intercropping (Lv et al., 2014). In present relay intercropping of maize and soybean, coexistence period was relatively short (about 48 days), and with greater distance (60 cm) between strip of maize and soybean, which means the increased yield of DC was mainly due to an increase in light interception rate of maize middle canopy, rather than complementarity effect.

Conclusions

In the current study, we surveyed 300 farmers and subsequently designed our experiment on the basis of survey data, to provide a realistic insight into the farmer yield and possible ways to increase the maize productivity in maize-soybean relay intercropping. Our findings indicate that increasing planting density and fertilization, as well as using a plough tillage system, can boost the yield potential of farmers' existing farming practices. Moreover, our findings clearly indicate that optimizing canopy structure improved the light interception and photosynthetic productivity, which subsequently mediated a marked increase in grain yield. Improved LAI and compact LA effectively increases light interception and utilization. Taken together, this study presented a systemic experiment based on extensive survey of farmer fields to provide a practical solution for improving maize yields under the intercropping system, particularly in areas of low solar radiation. This study had some limitations, despite the substantial yield increases by improved field management observed in this study, it is still not enough to explore the potential yield completely. Future research, using new hybrids, irrigation systems, among others, are needed to validate the observed improvement in yield potential of crops under intercropping systems.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

Writing-original draft: GC. Data curation: YR, BL, and HC. Writing, revision and methodology: AM and HG. Formal analysis: TY and JL. Project administration: XS and TP. Software: YW. Supervision: WL, WY. Resources: JD and FY. Funding acquisition: XW. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer [GA] declared a shared affiliation with one of the authors [AM] to the handling editor at the time of review.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1031024/full#supplementary-material

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EDITED BY
Feng Yang,
Sichuan Agricultural University, China

REVIEWED BY
Suleyman I. Allakhverdiev,
Russian Academy of Sciences (RAS), Russia
Aimal Khan,
University of Chinese Academy of Sciences, China

*CORRESPONDENCE
Xun Bo Zhou
xunbozhou@gxu.edu.cn

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Maize-soybean intercropping at optimal N fertilization increases the N uptake, N yield and N use efficiency of maize crop by regulating the N assimilatory enzymes

Jamal Nasar[1], Chang Jiang Zhao[1], Rayyan Khan[1], Hina Gul[2], Harun Gitari[3], Zeqiang Shao[4], Ghulam Abbas[5], Imran Haider[5], Zafar Iqbal[5], Waqas Ahmed[5], Raheela Rehman[6], Qing Ping Liang[7], Xun Bo Zhou[1*] and Juan Yang[1]

[1]Guangxi Key Laboratory of Agro-environment and Agro-products Safety, National Demonstration Center for Experimental Plant Science Education, Agricultural College of Guangxi University, Nanning, China, [2]National Center of Industrial Biotechnology, Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi, Pakistan, [3]Department of Agricultural Science and Technology, School of Agriculture and Environmental Sciences, Kenyatta University, Nairobi, Kenya, [4]College of Resources and Environmental Engineering, Jilin Institute of Chemical Technology, Jilin, China, [5]National Research Center of Intercropping, The Islamia University of Bahawalpur, Punjab, Bahawalpur, Pakistan, [6]Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Faisalabad, Pakistan, [7]Guangxi Agricultural Vocational University, Nanning, China

Introduction: Surplus use of chemical nitrogen (N) fertilizers to increase agricultural Q9 production causes severe problems to the agricultural ecosystem and environment. This is contrary to N use efficiency and sustainable agricultural production.

Methods: Hence, this study was designed to investigate the effect of maizesoybean intercropping on N uptake, N yield, N utilization use efficiency, and the associated nitrogen assimilatory enzymes of maize crops under different N fertilization for two consecutive years 2021-2022.

Results: The findings of the study showed that intercropping at the optimal N rate (N1) (250 kg N ha-1) increased significantly maize grain yield by 30 and 34%, residue yield by 30 and 37%, and 100-grain weight by 33 and 39% in the year 2021 and 2022, respectively. As compared with mono-cropping, at this optimal N rate, the respective increase (of maize's crop N yield indices) for 2021 and 2022 were 53 and 64% for grain N yield, and 53 and 68% for residue N yield. Moreover, intercropping at N1 resulted in higher grain N content by 28 and 31%, residue N content by 18 and 22%, and total N uptake by 65 and 75% in 2021 and 2022, respectively. The values for the land equivalent ratio for nitrogen yield (LERN) were greater than 1 in intercropping, indicating better utilization of N under the intercropping over mono-cropping. Similarly, intercropping increased the N assimilatory enzymes of maize crops such as nitrate reductase (NR) activity by 19 and 25%, nitrite reductase (NiR) activity by 20 and 23%, and glutamate synthase activity (GOGAT) by 23 and 27% in 2021 and 2022, respectively. Consequently, such increases resulted in improved nitrogen use efficiency indices such as N use efficiency (NUE), partial factor nitrogen use efficiency (PFNUE), nitrogen uptake efficiency (NUpE), and nitrogen agronomic efficiency (NAE) under intercropping than mono-cropping.

Conclusion: Thus, this suggests that maize-soybean intercropping under optimal N fertilization can improve the nitrogen status and nitrogen use efficiency of maize crops by regulating the nitrogen assimilatory enzymes, thereby enhancing its growth and yield. Therefore, prioritizing intercropping over an intensive mono-cropping system could be a better option for sustainable agricultural production.

KEYWORDS

maize-soybean intercropping, nitrogen yield, nitrogen use efficiency, nitrogen assimilatory enzymes, agricultural sustainability

1 Introduction

In China, there is increased use of chemical nitrogen (N) fertilizers for agricultural production, which results in wasted resources and environmental pollution (Malunga et al., 2018; Wang et al., 2018; Ahmad et al., 2022). For example, N leaching to subsoil increases soil acidification and groundwater pollution whereas its emission into the atmosphere directly stimulates air pollution (Galanopoulou et al., 2019). Such processes pose serious threats to the agricultural ecosystem and environment, contrary to efficient N use efficiency (NUE) (Galanopoulou et al., 2019; Nasar et al., 2020a; Nasar et al., 2020b). Also, intensive farming and long-term sole cropping system have severely harmed the agricultural ecology and reduced biodiversity (Nasar et al., 2020a). Thus, it is imperative to establish a sustainable agricultural production system that requires zero to little inputs. Hence, opting for intercropping over an intensive mono-cropping system could be a better option in such a scenario.

Intercropping is the simultaneous cultivation of two or more different crop species on the same field (Gitari et al., 2020; Maitra et al., 2020). It is an ancient agronomic practice and is still widespread globally. As opposed to mono-cropping, intercropping shows better growth and yield advantages due to the efficient utilization of the available natural resources (i.e., water, light, land, and nutrients) (Fung et al., 2019; Gao and Meng, 2020; Nasar et al., 2020b; Raza et al., 2021). Intercropping also helps in minimizing negative environmental impacts that threaten the agroecosystems (i.e., climate change, soil acidification, terrestrial eco-toxicity, or cumulative energy demand) (Yang et al., 2017; Nyawade et al., 2020b; Faridvand et al., 2021). In a cereal–legume intercropping, the companion crops efficiently utilize the atmospheric and soil available N. The major source of N under the such intercropping system is its fixation by the legumes, which helps save the soil N pool, increases the amount of soil N, enhances the N uptake in cereals and eventually crop yield (Xiang et al., 2018; Sousa et al., 2022). These improvements can occur through facilitative root interactions, nutrient sharing, and rhizosphere modification (e.g., enzymatic activities, root exudation, and soil pH) in an intercropping system (Li et al., 2019; Liu et al., 2019; Nasar et al., 2021). Such underlying mechanism under the intercropping systems contributes efficiently to soil nutrient cycling and plant nutrition (Nyawade et al., 2019; Nasar et al., 2020a). Additionally, the improved nitrogen assimilatory enzymes (i.e., NR, NiR and GOGAT activity) in the intercropping system equally contributes to the plant N content and its uptake (Nasar et al., 2022a). Previously, many studies have shown that cereal-legume cropping systems can significantly increase the plant N status due to the underlying rhizosphere modification (Sun et al., 2018; Nasar et al., 2020b; Raza et al., 2021), facilitative nutrient sharing through interspecific root interaction between intercrops (Shao et al., 2020) and improved N assimilatory enzymes (i.e., NR, NiR and GOGAT) (Nasar et al., 2022b).

Maize (*Zea mays* L) is grown globally due to its high-yielding food and forage crop production and is also known as the "Queen of Cereals" (Sun et al., 2014). In China, maize production increased by 1633% between 1949 and 2013, with average maize yields from 1 to 6 t ha^{-1} (Muhammad et al., 2022). More than 36 million hectares of maize were planted in the country in 2013, producing more than any other crop, especially on the North China Plain (Zhong et al., 2017). On the other hand, soybean (*Glycine max* L) is an annual grain legume known for its high protein content, vitamins, and minerals (Raza et al., 2020; Mirriam et al., 2022). It is a restorative plant that improves the quality and health of the soil by enriching it with nutrients (Zaeem et al., 2019). Thus, intercropping maize with soybean not only secures the regional food demand and nutritional quality of the forage industry but also improves the nutrient status of the maize crop besides providing an environmentally friendly and promising agricultural system for the future development. It is worth noting that, maize-soybean intercropping has been widely practiced to improve crop and forage yield, utilization of the natural resources, nutrient improvement of the cereal crop and soil health (Du et al., 2020; Raza et al., 2020; Nasar et al., 2022b). Nonetheless, relatively less data is available on the N yield, N use and utilization efficiency *via* regulation of N assimilatory enzymes in the maize-soybean intercropping. Therefore, this study was initiated to investigate the effect of maize-soybean intercropping on the N uptake, N yield, and N use efficiency, and the associated N assimilatory enzymes of maize with different N fertilization. The main objective of the study was to investigate whether maize-soybean intercropping under different N treatments improve the N yield, uptake and its use efficiency by regulating the N assimilatory enzymes of maize crop.

2 Material and methods

2.1 Site description, experimental design and layout

A two-year pot experiment was conducted at the experimental farm of Guangxi University, Nanning, China, in the year 2021-2022. This area is characterized by a subtropical monsoon climate with an annual rainfall of 1080 mm. The experimental site had soil with a loamy texture having an organic matter of 23.7 g kg^{-1}, total N of 0.118%, alkaline N of 109.9 mg kg^{-1}, available P of 73.6 mg kg^{-1}, available K of 79.0 mg kg^{-1}, soil pH of 7.4 and available iron of 97.7 mg kg^{-1}.

Maize (Qing Qing 700 variety) was planted as a mono-crop (MM) and an intercrop (MI) with soybean (Gui Chun 15 variety) in large-sized pots (i.e., 88 cm height, 53 cm width, and 43 cm length) filled with 120 kg of soil. The pots, in four replicates, were randomly placed in a ventilated net house under natural light. Initially, five maize seeds and ten soybean seeds were planted in mono-cropping and intercropping at a plant density of 60,000 maize plants ha^{-1} and soybean seed rate of 20 kg seeds ha^{-1}, respectively. However, later at the V3 growth stage, the maize and soybean plants were reduced to 3 and 5 (3:5) plants per pot, respectively, by uprooting the extra plants to better adapt to the pot environment (Figure 1). For the intercropping, maize and soybean plants were planted in the same pot such that the plant-to-plant and pot-to-pot distances were 5 and 10 cm, respectively. Additionally, the bottom of each pot was covered with small marble pebbles to minimize nutrient

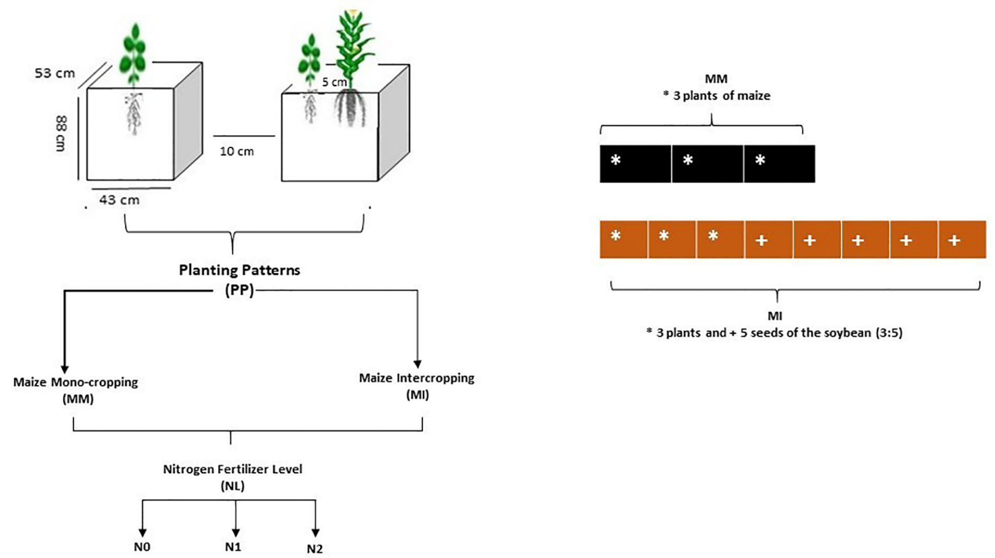

FIGURE 1
Schematic diagram of the experiment. N_0; 0 kg N ha^{-1}, N_1; 250 N kg ha^{-1}, N_2; 300 kg N ha^{-1}. * maize crop; + soybean crop.

leaching. Planting and harvesting were done in mid-September 2021 and mid-February 2022, respectively for the first crop growing cycle, whereas the respective timings for the second cycle were mid-May 2022, and mid-October 2022.

Nitrogen fertilizer was applied as soil dressing before sowing at the rate of 0 kg N ha^{-1} (N_0) for control, 250 kg N ha^{-1} (N_1) for optimal and 300 kg N ha^{-1} (N_2) for conventional practice. In addition, basal doses of phosphorus and potassium fertilizers were applied uniformly to all experimental pots (i.e., P at 100 kg ha^{-1} and K at 100 kg ha^{-1}). The sources of fertilizers used were urea (46% N), diammonium phosphate (P_2O_5 46% P), and potassium chloride (K_2O 60% K). All the plants were watered normally, with weeds and insect pests being controlled with herbicides and pesticides, respectively, when needed. The environmental factors such as temperature (°C) and rainfall (mm) were carefully monitored and recorded (Figures 2A, B).

2.2 Data collection

2.2.1 Grain and residue yield

The grain and residue yield of maize crops were obtained at full maturity when harvesting was done (Raza et al., 2020). The corn from maize crops was removed from the plant and threshed to determine 100-grain weight and grain yield by weighing them on an electric scale. After threshing, the remaining plant straw materials were sun-dried and oven-dried at 65°C for 72 h to obtain residue dry yield.

2.2.2 Grain and residue N content and total N uptake

For the determination of grain and residue N content, the air and oven-dried plant samples were minced and passed through a 1 mm sieve. Nitrogen concentrations were determined as an average of duplicate samples of about 50 mg each by the Dumas

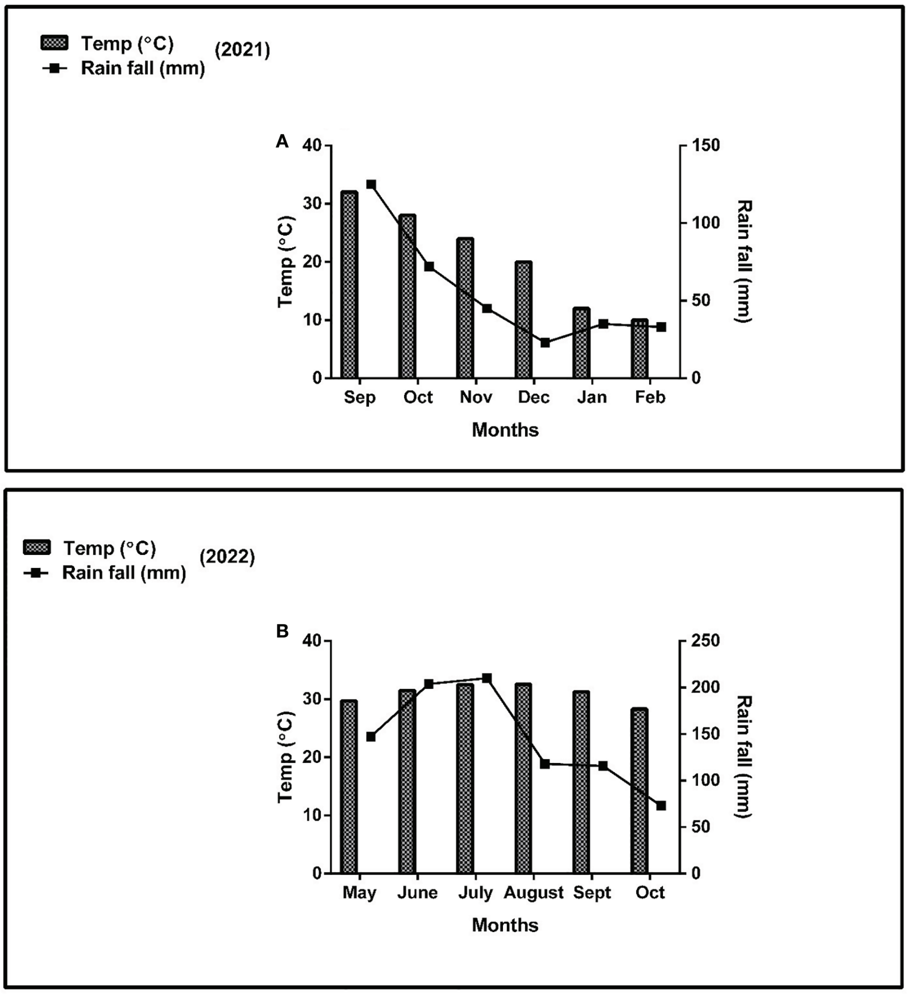

FIGURE 2
Weather forecast (temperature and rainfall) report of the experimental site during the experiment period **(A)**; year 2021 and **(B)**; year 2022.

combustion method (Neugschwandtner and Kaul, 2015) using an elemental analyzer (Vario MACRO cube CNS; Elementar Analysen-Systeme GmbH, Germany). The total N uptake was calculated as indicated in Equation 1 (Nasar and Shah, 2017).

$$\text{Total N uptake (g/pot)} = (\text{GNC} \times \text{Grain yield}) + (\text{RNC} \times \text{Residue yield}) \quad (1)$$

Where GNC and RNC denote grain N content and residual N content, respectively.

2.2.3 Nitrogen yield and nitrogen harvest index

The grain and residue N yield of maize crops were calculated as indicated in Equations 2 and 3 whereas, the N harvest index was computed according to Equation 4.

$$\text{Grain N yield (g/pot)} = \text{Grain yield} \times \text{Grain N content} \quad (2)$$

$$\text{Residue N yield (g/pot)} = \text{Residue yield} \times \text{Residue N content} \quad (3)$$

$$\text{N harvest Index (\%)} = \frac{\text{Grain N yield}}{\text{Residue N yield}} \times 100 \quad (4)$$

2.2.4 Nitrogen use efficiency indices

The nitrogen use efficiency (NUE), partial factor nitrogen use efficiency (PFNUE), nitrogen uptake efficiency (NUpE) and nitrogen agronomic efficiency (NAE) were calculated as indicated in Equations 5, 6, 7 and 8 (Sinebo et al., 2004; Anbessa and Juskiw, 2012).

$$\text{NUE}\left(\text{g pot}^{-1}\right) = \frac{\text{YLD}}{\text{N}_{\text{MIN}} + \text{N}_{\text{f}}} \quad (5)$$

$$\text{PFNUE}\left(\text{g pot}^{-1}\right) = \frac{\text{YLD}_{\text{f}}}{\text{N}_{\text{f}}} \quad (6)$$

$$\text{NUpE}\left(\text{g pot}^{-1}\right) = \frac{\text{total N uptake}}{\text{N}_{\text{MIN}} + \text{N}_{\text{f}}} \quad (7)$$

$$\text{NAE}\left(\text{g pot}^{-1}\right) = \frac{\text{grain N YLD} - \text{grain YLD}}{\text{N}_{\text{MIN}} + \text{N}_{\text{f}}} \quad (8)$$

Where the YLD is the grain yield and NY_r is the residue nitrogen yield of maize crops; N_{MIN} represents soil mineral N at sowing and N_f the fertilizer level; the subscript f stands for fertilizer N.

2.2.5 Nitrogen assimilatory enzymes

The nitrogen assimilatory enzyme activity, such as nitrate reductase (NR), nitrite reductase (NiR), and glutamate synthase (GOGAT) activity in maize leaf samples were determined according to the following protocol.

2.2.5.1 NR activity

To determine NR activity in maize leaves, the frozen plant leaf samples were crushed in 4 mL of 25 mM sodium phosphate (buffered at pH 8.7) containing 1.3 mM EDTA and 10 mM cysteine before being centrifuged at 4000 rpm for 15 minutes at 4°C. In this case, the reaction mixture was made up of 0.1 M KNO_3, and 2.82 mM NADH. Following addition of NADH was a 30-minute incubation period. After 15 minutes, the reaction was stopped followed by addition of 1% sulfanilamide and 0.02% N-phenyl-2-naphthylamine. The absorbance was the calculated at 540 nm following centrifugation at 4000 rpm for 5 min (Imran et al., 2019).

2.2.5.2 NiR activity

NiR activity (NiR, EC 1.7.2.1) in the fresh maize leaves was determined according to the proposed method of Rao et al. (1981). Briefly, a cold 0.1 M potassium phosphate (buffered at a pH of 7.5) was used to homogenize the frozen leaf tissues. The reaction mixture included enzyme extract, 10 mM KNO_2, 1.5% methylviologen, and 5% sodium dithionite ($Na_2S_2O_4$) dissolved in 100 mM $NaHCO_3$, which was added to start the reaction. The 30-minute incubation period of the reaction mixture at room temperature was followed by methylviologen's decolorization. Nitrite concentrations were determined by measuring the absorbance at 540 nm in a solution made up of supernatant, distilled water, 1% (w/v) N (1-naphty1)-ethylenediamine dihydrochloride, and 10% (w/v) sulfanilamide produced in HCl.

2.2.5.3 GOGAT activity

The NADH-glutamate synthase (NADH-GOGAT; EC 1.4.1.14) activity in maize leaves was measured according to Lin and Kao (1996). In this case, frozen leaves were homogenized in a mortar and pestle with an extraction buffer that was pre-cooled and containing 100 mM Tris-HCl (pH 7.6), 1.0 mM $MgCl_2$-$6H_2O$, 10 mM 2-mercaptoethanol, and 1.0 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged for 15 minutes at 4°C at 13,000 rpm. To evaluate the GOGAT enzymes in leaf tissues, the supernatants were used as crude extracts. 25 mM Tris-base, 100 mM -Ketoglutaric acid, 10 mM KCl, 20 mM L-glutamine, and 3 mM NADH were used to treat the crude enzyme extract. Thereafter, NADH oxidation caused the absorbance which was measured at 340 nm.

2.2.6 Land equivalent ratio for nitrogen yield (LER_N)

The land equivalent ratio for nitrogen yield (LER_N) as an indicator used to determine the N yield advantage of intercrops (Mead and Willey, 1980) was calculated as shown in Equation 9:

$$\mathrm{LER_N} = \frac{\mathrm{NY_{MI}}}{\mathrm{NY_{MM}}} \quad (9)$$

Where NY_{MI} and NY_{MM} are the crop N yields for maize under intercropping and mono-cropping, respectively. A $LER_N > 1$, indicates a higher N yield whereas when its<1 then it represents a lower N yield.

2.3 Statistical analysis

The collected data were entered and tabulated in Ms excel 2016. For statistical analysis, two factors factorial analysis was done using the SPSS and Ms statistix 6.1 statistical analysis software, respectively. Means among the treatments were compared by Least Significant Difference (LSD) Test at $p \leq 0.05$ level of probability (Mirzapour et al., 2022) by keeping the nitrogen fertilization as the main effect and planting pattern sub-effect. Graphs were constructed using the graphical software Graph Pad prism 6.1.

3 Results

3.1 Grain and residue yield

Intercropping and N fertilization significantly ($p< 0.05$) affected the grain yield, residue yield and 100-grain weight of maize (Table 1). However, these indices were more evident in intercropping under N_1 treatment than in N_0 and N_2 treatments. For instance, in 2021, intercropping increased the grain yield of maize crops by 16, 30 and 20% in N_0, N1 and N_2, respectively compared with mono-cropping, whereas in 2022, the respective increases of 18, 34 and 19% in 2022 were noted. Moreover, intercropping increased the residue yield of maize crops by 15, 30, and 24% in 2021 and by 19, 37 and 23% compared with mono-cropping. Similarly, intercropping increased the 100-grain weight of maize crops by 3% under N_1 treatment than by 23 and 26% and by 26 and 39 and 29% under N_0 and N_2 treatments in 2021 and 2022, respectively when compared with mono-cropping.

3.2 N yield indices and N harvest index

The collected data showed that intercropping under different N fertilization significantly ($p< 0.05$) increased the N yield indices of the maize crop as compared with mono-cropping (Table 2). However, these indices were more pronounced under N_1 than in N_0 and N_2 treatments. In 2021, intercropping increased the grain N yield of maize by 53% under N_1 treatment than by 27 and 39% under N_0 and N_2 treatments, respectively when compared with mono-cropping. There was a further increase in the grain N yield of maize crop under intercropping of 64% in N_1 vis a vis 32 and 40% under N_0 and N_2, respectively in 2022 as compared with mono-cropping. Similarly, when compared with mono-cropping, intercropping significantly ($p< 0.05$) increased the residue N yield of maize crops by 53% under N_1 treatment than by 25 and 43% under N_0 and N_2 treatments, respectively in 2021, and further increased by 68% under N_1 treatment than by 33 and 44% under N_0 and N_2 treatments, respectively in 2022. However, the N harvest index of maize crops did not show any significant differences in the

TABLE 1 Grain yield, residue yield and 100-grain weight of maize crop as influenced by different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons.

Treatment		Grain yield (g pot^{-1})		Residue yield (g pot^{-1})		100-grain weight (g)	
N fertilizer rate	Plantingpattern	Year 2021	Year 2022	Year 2021	Year 2022	Year 2021	Year 2022
N_0	MM	91.50 ± 8.1 d	93.00 ± 786.0 d	201.36 ± 9.5 d	205.61 ± 854.2 c	22.52 ± 1.6 c	22.13 ± 2.0 d
	MI	106.18 ± 11.6 c	109.94 ± 810.1 c	230.64 ± 7.4 c	244.89 ± 781.8 b	27.66 ± 2.1 b	27.90 ± 3.1 bc
N_1	MM	105.15 ± 4.5 c	105.90 ± 621.4 c	210.40 ± 10.1 d	213.15 ± 750.8 c	27.81 ± 2.1 b	27.83 ± 2.3 c
	MI	136.54 ± 2.9 a	142.29 ± 707.0 a	272.80 ± 10.6 a	291.30 ± 855.2 a	37.09 ± 1.7 a	38.71 ± 3.2 a
N_2	MM	102.05 ± 8.1 cd	103.80 ± 621.4 cd	204.15 ± 8.5 d	207.15 ± 750.8 c	23.81 ± 1.1 c	25.08 ± 3.1 cd
	MI	122.62 ± 3.2 b	123.37 ± 707.0 b	253.55 ± 17.7 b	254.80 ± 855.2 b	30.10 ± 0.6 b	32.46 ± 1.9 b
Significance							
NL		0.000***	0.000***	0.003**	0.001**	0.001**	0.000***
PP		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
NL*PP		0.150^{ns}	0.061^{ns}	0.065^{ns}	0.020*	0.106 ^{ns}	0.269 ^{ns}

The mean values with different lowercase letters (± standard deviation) are significantly different from each other at LSD Test (P ≤ 0.05). *p< 0.05, **p< 0.01, ***p< 0.001, ^{ns} p > 0.05.

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TABLE 2 Grain N yield, residue N yield, N harvest index and LER_N of maize crop as influenced by different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons.

Treatment		Grain N yield (g pot^{-1})		Residue N yield (g pot^{-1})		N harvest index (%)		LER_N	
N fertilizer rate	Plantingpattern	Year 2021	Year 2022	Year 2021	Year 2022	Year 2021	Year 2022	Year 2021	Year 2022
N_0	MM	203.70 ± 19.4 d	208.17 ± 19.6 d	448.66 ± 28.0 e	460.75 ± 28.9 d	45.63 ± 6.1	45.36 ± 5.5		
	MI	259.05 ± 35.8 c	275.43 ± 29.8 c	562.08 ± 24.1 c	613.49 ± 31.4 c	45.98 ± 5.1	44.85 ± 3.7	1.3	1.3
N_1	MM	263.76 ± 16.7 c	262.60 ± 21.5 c	527.42 ± 28.9 cd	527.76 ± 14.3 d	50.06 ± 3.2	49.73 ± 3.5		
	MI	403.88 ± 32.9 a	431.69 ± 34.3 a	807.54 ± 80.2 a	884.07 ± 65.8 a	50.08 ± 1.1	48.87 ± 2.7	1.5	1.6
N_2	MM	241.54 ± 18.8 cd	241.50 ± 21.7 cd	484.35 ± 37.4 de	482.68 ± 38.1 d	50.15 ± 6.1	50.26 ± 5.9		
	MI	336.24 ± 28.6 b	338.45 ± 29.1 b	694.58 ± 72.1 b	697.15 ± 66.7 b	48.55 ± 3.5	48.81 ± 5.7	1.4	1.4
Significance									
NL		0.000***	0.000***	0.000***	0.000***	0.164^{ns}	0.133^{ns}		
PP		0.000***	0.000***	0.000***	0.000***	0.830^{ns}	0.631^{ns}		
NL*PP		0.017*	0.003**	0.013*	0.000***	0.901^{ns}	0.979^{ns}		

The mean values with different lower case letters (± standard deviation) are significantly different from each other at LSD Test ($P \leq 0.05$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ^{ns} $p > 0.05$.

intercropping under N fertilization treatments. In addition, the LER_N was always greater than 1 in all N treatments, indicating a yield advantage of intercropping with N treatments.

3.3 N concentration and total N uptake

When compared with mono-cropping, intercropping significantly ($p < 0.05$) enhanced the N concentration and total N uptake of maize crops under different N fertilization treatments (Figure 3). In 2021, intercropping significantly ($p < 0.05$) increased the N concentration of maize grain and residues by 28 and 18% under N_1 treatment than by 18 and 9% in N_0, and by 21 and 16% in N_2 respectively as compared with mono-cropping (Figures 3A, B). In 2022, intercropping further increased the N concentrations of maize grain and residue by 31 and 22% under N_1 than by 20 and 12% in N_0, and by 19 and 18% in N_2, respectively as compared with mono-cropping (Figures 3D, E). Moreover, intercropping increased the total N uptake of maize crop by 65% under N_1 vis a vis 37 and 45% in N_0 and N_2, respectively in 2021. Nonetheless, higher increases were noted in 2022. For instance, there was 75% increase in N uptake in the intercropping under N_1 than by 41 and 32% in N_0 and N_2, respectively when compared with mono-cropping (Figures 3C, F).

3.4 Nitrogen use and utilization

Intercropping and N fertilization significantly affected the nitrogen use efficiency indices such as NUE, PFNUE, NUpE and NAE of maize crops when compared with mono-cropping (Figures 4, 5). However, these indices were more promising in intercropping under N_1 treatment than in N_0 and N_2 treatments. In 2021, intercropping significantly ($p < 0.05$) increased the NUE by 14% under N_1 treatment than by 7 and 9% under N_0 and N_2, respectively when compared with mono-cropping (Figures 4A, C). In 2022, there was an increase of 16% for this index under intercropping for N_1 vis a vis 8 and 9% for N_0 and N_2, respectively. Moreover, intercropping increased the PFNUE of maize crops by 30 and 34% under N_1 treatments than by 20% and 19% under N_2 treatments in 2021 and 2022, respectively as compared with mono-cropping (Figures 4B, D). Similarly, intercropping increased the NUpE of maize crop by 35% under N_1 treatment than by 16 and 23% under N_0 and N_2 treatments respectively in 2021, and further increased by 40% under N_1 treatment than by 19 and 22% under N_0 and N_2 treatments, respectively in 2022 as compared with mono-cropping (Figures 5A, C). Furthermore, intercropping increased the NAE by 38% under N_1 treatment than by 17 and 28% under N_0 and N_2 treatments in 2021, and further increased by 47% under N_1 treatment than by 21 and 29% under

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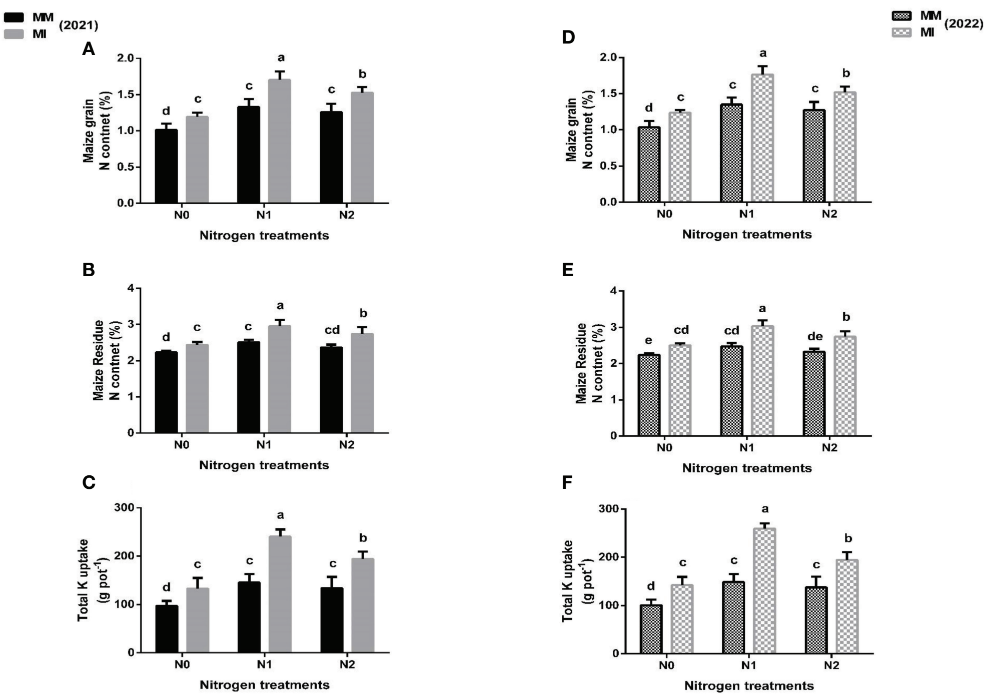

FIGURE 3
Maize grain N content **(A, D)**, residue N content **(B, E)**; total N uptake **(C, F)** under different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons. The column bars with dissimilar lowercase letters are significantly different from each other as per the LSD test (p< 0.05).

N_0 and N_2 treatments respectively in 2022 as compared with mono-cropping (Figures 5B, D).

3.5 Nitrogen assimilatory enzymes

Intercropping and nitrogen fertilization significantly (p< 0.05) affected the nitrogen assimilatory enzymes of maize as compared with mono-cropping. For instance, compared with mono-cropping, intercropping increased the NR, NiR and GOGAT activity of maize crop under different N treatments (Figure 6). However, these activities were more enhanced under N_1 treatment than in N_0 and N_2 treatments. In 2021, the NR activity of maize crops increased by 19% under N_1 than by 10 and 16% under N_0 and N_2, respectively, but this activity was further increased by 25% in intercropping system under N_1 treatment than by 12 and 14% under N_0 and $N_{2,}$ respectively in 2022 as compared with mono-cropping (Figures 6A, D). Similarly, intercropping increased the NiR activity of maize crops by 20% under N_1 treatment than by12 and 15% under N_0 and N_2, respectively in 2021, but it was further increased by 23% in the intercropping system under N_1 treatment than by 14 and 13% under N_0 and N_2, respectively in 2022 as compared with mono-cropping (Figures 6B, E). Moreover, intercropping increased the GOGAT activity of maize crop by 23% under N1 treatment than by 13 and 17% under N_0 and N_2 treatments respectively in 2021, and further increased by 27% under N_1 treatment than by 15 and 13% under N_0 and N_2 treatments respectively in 2022 when compared with mono-cropping (Figures 6C, F).

3.6 Liner regression

The linear regression analysis was used to determine the relationship of the total N uptake and NUE with the N assimilatory enzymes (i.e., NR, NiR and GOGAT activity) of maize crop. The result showed that the total N uptake had significant strong correlations with NR, NiR and GOGAT activity (Figures 7A–F). Equally, NUE had significant positive and strong correlations with NR, NiR and GOGAT activity (Figures 8A–F). Thus, such relationships suggested that changes in the N assimilatory enzymes could significantly bring changes in the total N uptake and NUE of the maize crop under intercropping.

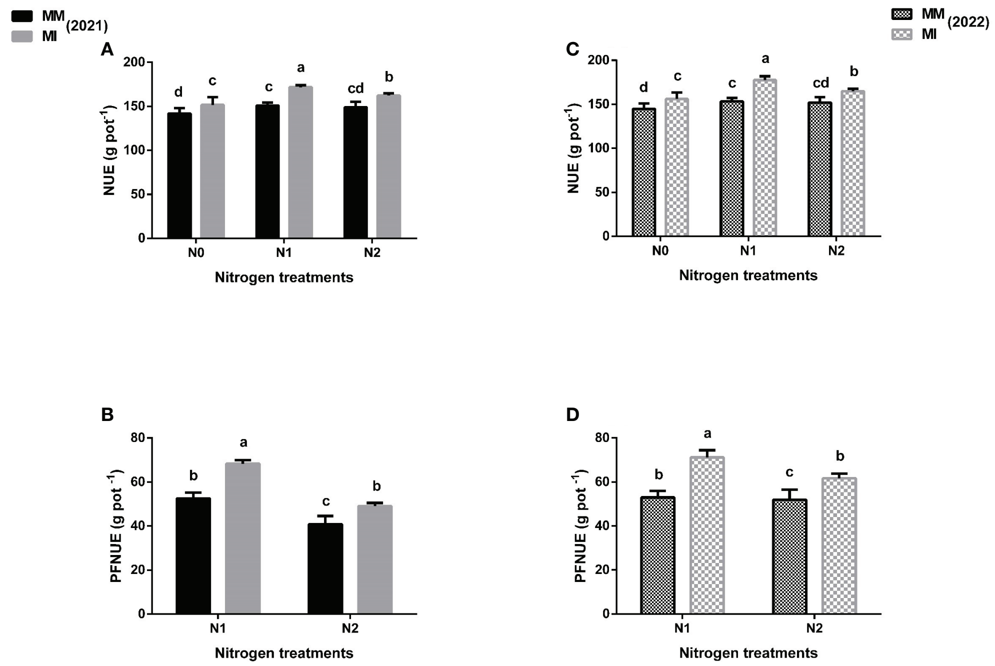

FIGURE 4
Maize nitrogen use efficiency (NUE) **(A, C)**, partial factor nitrogen use efficiency (PFNUE) **(B, D)** under different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons. The column bars with dissimilar lowercase letters are significantly different from each other as per the LSD test (p< 0.05).

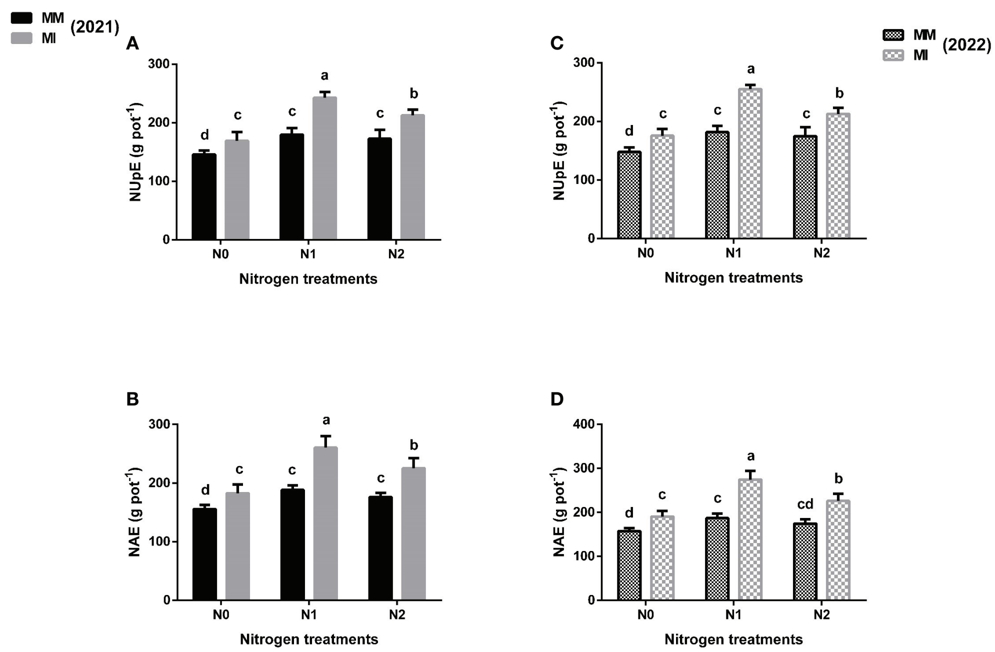

FIGURE 5
Maize nitrogen uptake efficiency (NUpE) **(A, C)**, nitrogen agronomic efficiency (NAE) **(B, D)** under different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons. The column bars with dissimilar lowercase letters are significantly different from each other as per the LSD test (p< 0.05).

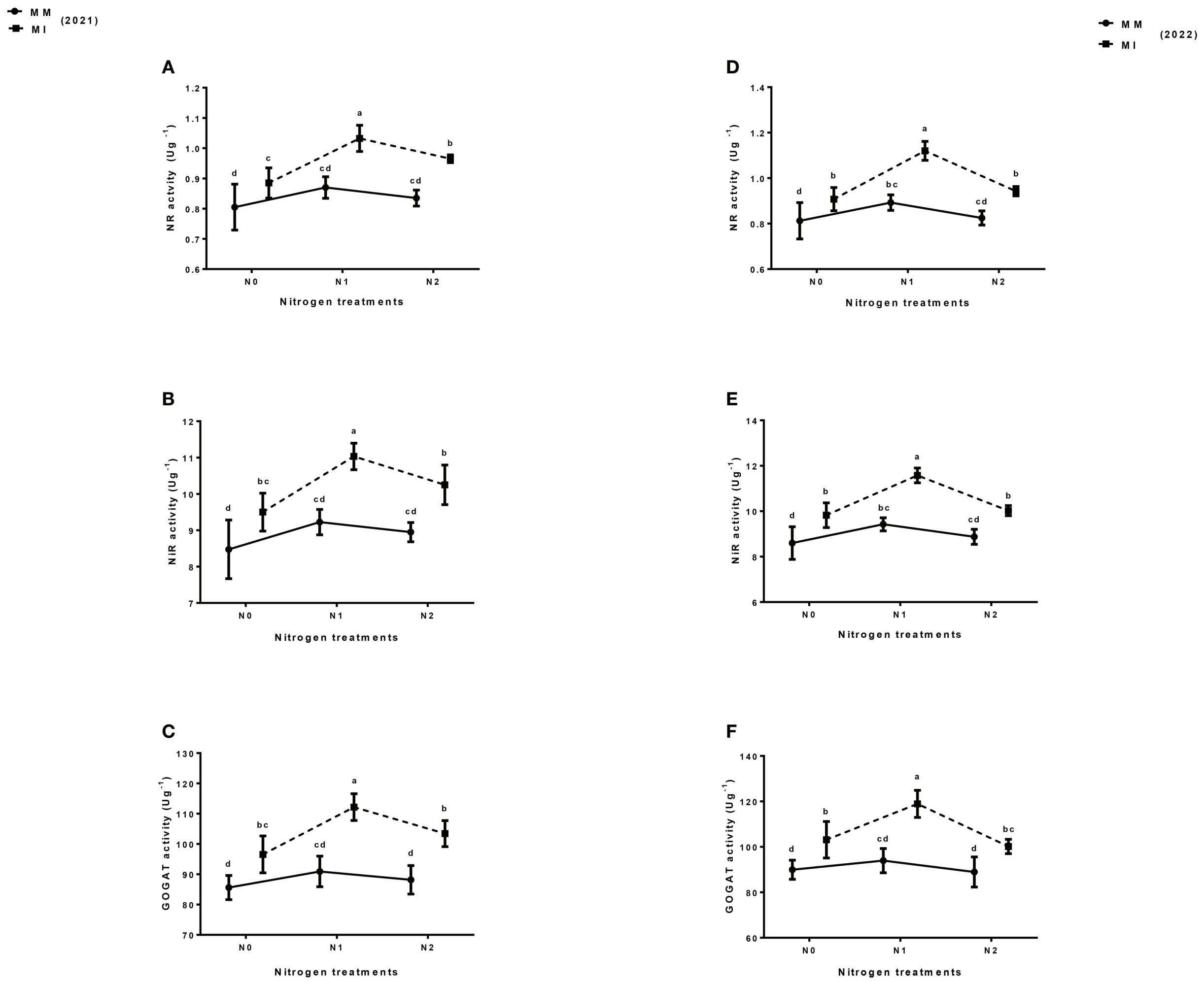

FIGURE 6
Maize nitrogen assimilatory enzymes: nitrate reductase (NR) **(A, D)**, nitrite reductase (NiR) **(B, E)** and glutamate synthase (GOGAT) **(C, F)** activity under different planting patterns (MM, maize mono-cropping and MI, maize intercropping) and N fertilizer application rates (N_0; 0 $_{kg}$ N ha^{-1}, N_1; 250 kg N ha^{-1} and N_2; 300 kg N ha^{-1}) in 2021 and 2022 crop growing seasons. The column bars with dissimilar lowercase letters are significantly different from each other as per the LSD test (p< 0.05).

4 Discussion

Generally, the improved productivity of intercropping is due to the efficient utilization of the available resources (e.g., water, nutrients, land and light) (Neugschwandtner and Kaul, 2015; Rawashdeh, 2016; Gitari et al., 2018a; Gitari et al., 2018b; Gou et al., 2018; Raza et al., 2019). The present study demonstrated that maize-soybean intercropping significantly increased the yield indices, residue yield and 100-grain weight of maize crop. However, these indices were more evident under N_1 treatment than N_0 and N_2. Possibly, this could due to the better utilization of the available natural resources such as land, light, water, and nutrients (Nasar et al., 2020b; Raza et al., 2020; Raza et al., 2022), or could be due to the N fertilization, which is an important element required for plant growth and development (Zhang et al., 2014; Nduwimana et al., 2020). Moreover, legume in intercropping with cereal are also known to improve the N status of cereal crop by facilitative transfer of N to their corresponding cereal crop through the underlying facilitative root interactions, which ultimately leads to an increase yield production of intercropping cereals than mono-cropping (Shao et al., 2020). As previously documented that the efficient use of the available resources (i.e., water, land, light and nutrients) by intercrops have produced more yield than in their mono-cropping system (Latati et al., 2017; Raza et al., 2019; Kisaka et al., 2023). Maize/mungbean intercropping have also shown to increase the grain yield and biomass dry matter of maize crop by 15-29% and 21-34%, respectively than in mono-

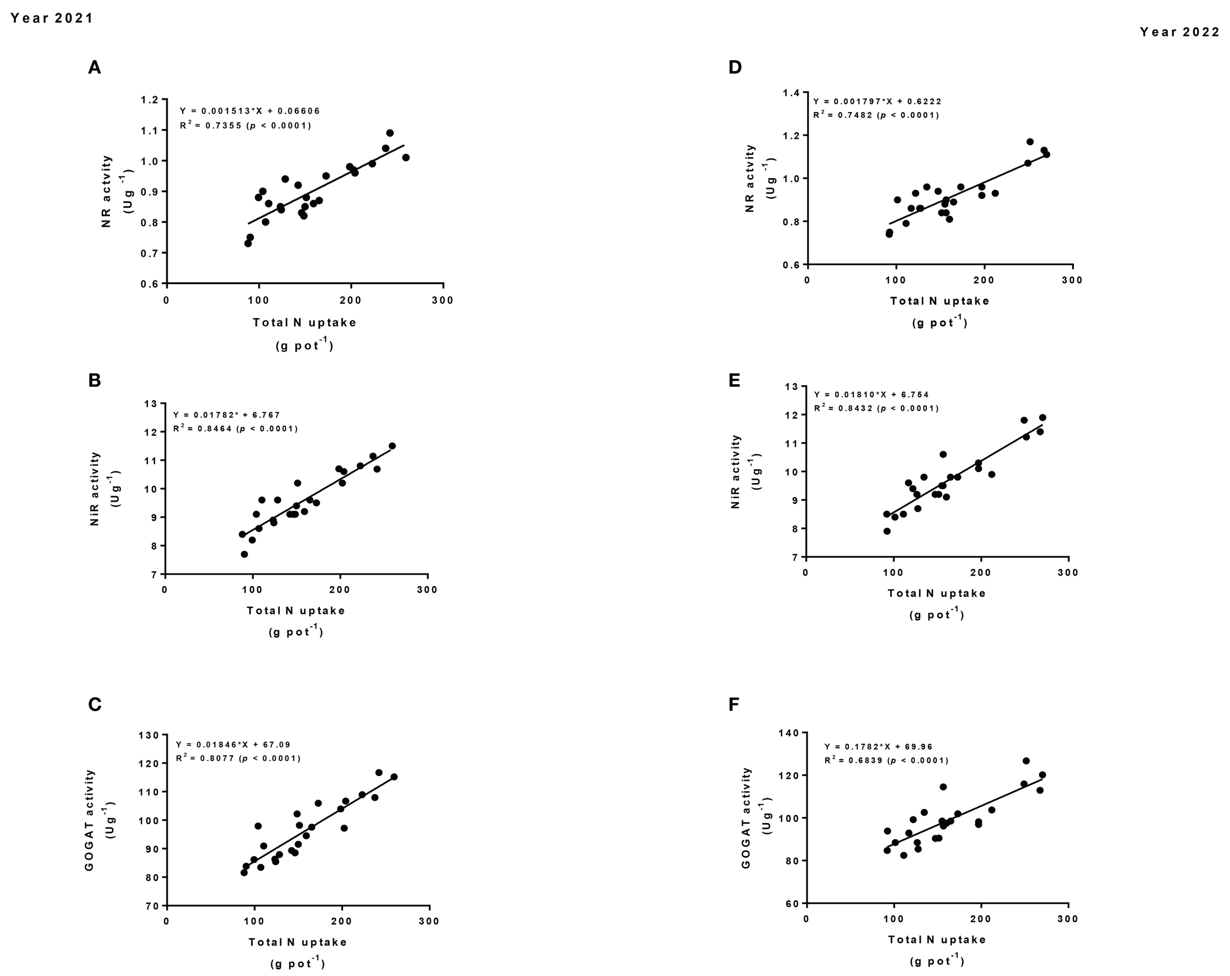

FIGURE 7
Regression analysis of the total N uptake with nitrogen assimilatory enzymes (i.e., NR **(A, D)**, NiR **(B, E)**, and GOGAT activity **(C, F)**) of maize crop in 2021 and 2022.

cropping, which was attributed to better utilization of the available resources and the underlying nutrient sharing of mungbean to its corresponding maize crop during intercropping (Qian et al., 2018). Moreover, maize in intercropping with mungbean or mash bean significantly enhance the yield and biomass dry matter of maize crops particularly under optimal N fertilization, mainly because of the N fixation ability of legumes, which helps improve the N content of maize crop. This helps in reducing the high use of chemical N fertilizers (Saleem et al., 2011), which supports our findings. Intercrops are also known for their better use of the applied fertilizers, which helps in production of more crop yield under intercropping than in mono-cropping systems that are established under the same piece of land with same or different fertilization managements (Shao et al., 2020). For instance, the higher LER_N value (1.33) in oat-pea intercropping than in mono-cropping under optimal N fertilization was mainly because of the better utilization of the applied N fertilizer (Neugschwandtner and Kaul, 2015; Sun et al., 2018). Similarly, in our study we found a higher LER_N values in intercropping than in mono-cropping, indicating better utilization of the N in the intercropping system than in mono-cropping. Previously different intercropping studies have shown higher LER_N value under optimal N fertilization (Neugschwandtner and Kaul, 2015; Sun et al., 2018), which confirmed our results.

Legumes are well known for their ability to fulfill nitrogen requirement through atmospheric N fixation. Thus, legumes in intercropping with cereals can help improve the N content and its uptake by cereals due to the underlying facilitative N transfer through interspecific root interaction (Nasar et al., 2020b; Raza et al., 2020; Shao et al., 2020; Mirriam et al., 2022). In our study we found that soybean when intercropped with maize significantly increased the N content and total N uptake of maize crops than in mono-cropping. However, these indices

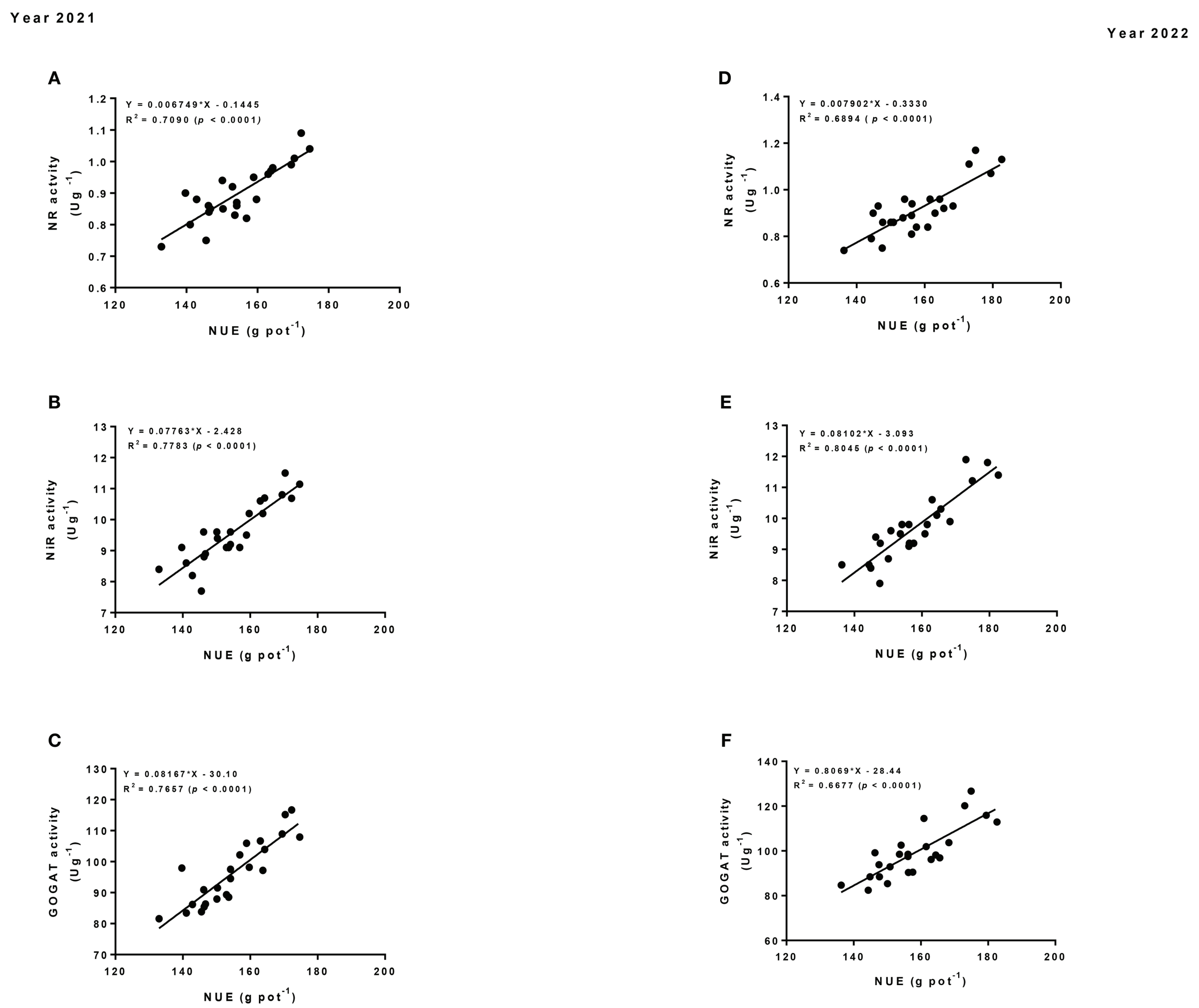

FIGURE 8
Regression analysis of nitrogen use efficiency (NUE) for maize crop with nitrogen assimilatory enzymes (i.e., NR **(A, D)**, NiR **(B, E)**, and GOGAT activity **(C, F)**) in 2021 and 2022.

were more prominent under N_1 treatment than in N_0 and N_2 treatment. There could be several reasons to explain such observation, (i) this could be due the N fixation ability of legume which improved the soil nutrient pool and N availability, thereby enhancing the N content and its uptake of the cereal crop during intercropping (Neugschwandtner and Kaul, 2015; Sousa et al., 2022), (ii) it could also be attributed to the underling nutrient sharing between intercrops or facilitative N transfer from legumes to their corresponding cereal crop (Zhang et al., 2017a; Shao et al., 2020), (iii) it might also be due to the rhizosphere modification, root releasing chemicals and alteration in the soil physio-chemical and enzymatic activities due to mix and different rooting behavior during intercropping (Nasar et al., 2022a). Nitrogen fertilization could also play an important role in improving plant N status, which might improve the soil N availability for plant roots (Yong et al., 2018; Ochieng' et al., 2021). For example, barely in intercropping with fababean was reported to have considerably improved the N content and total N uptake of barely because of the N fixation ability of the companion fababean (Galanopoulou et al., 2019), which confirmed our results. Maize-common bean intercropping has also been shown to have enhanced N contents and its uptake in the maize crop particularly under optimal N fertilizer application (Malunga et al., 2018). Several other cereal-legume intercropping studies have shown to improve the N content and its uptake in cereal crops *via* underlying facilitative N transfer from legume side to their companion cereal crop, rhizosphere modification, soil nutrient availability improvement, root releasing chemicals, changes in the nutrients related soil enzymes and some unknown mechanisms (Zhang et al., 2014; Chen et al., 2017; Hu et al., 2018; Gao and Meng, 2020; Nyawade et al., 2020a; Shao et al., 2020; Nasar et al., 2022a).

The present study also demonstrated that maize-soybean intercropping significantly increased the N yield indices (i.e., grain N yield and residue N yield) and N use efficiency indices (i.e., NUE, PNUE, NUpE and NAE) of maize crop. However, these indices were further increased under N_1 treatments than in N_0 and N_2 treatments. Probably, this might be due to the underlying facilitation, or complementary (Gitari et al., 2018b; Li et al., 2020), sharing of nutrients (Shao et al., 2020), better use of soil available N and the facilitative N transfer from legume to their companion cereals during intercropping (Yong et al., 2018; Nyawade et al., 2020a). Moreover, N fertilization help reduce the belowground interspecific competition and maximize the facilitative interactions for resources between intercrops (Xiao et al., 2013). As earlier reported that legumes in intercropping with cereals modify the rooting system of cereals, enabling them to occupy more space and acquire more nitrogen (Galanopoulou et al., 2019). Moreover, the facilitative N transfer from legumes to cereals can make a reverent contribution to the N nutrition of cereals (Génard et al., 2016; Zhang et al., 2017a). In previous maize-soybean intercropping study it was found that intercropping significantly improves N content, N uptake and N use efficiency of maize crop particularly under optimized N fertilization, mainly because of the underlying rhizosphere modification and nutrient facilitation provided by soybean (Yong et al., 2018). In another maize-soybean intercropping study it was reported that the significant N transfer from soybean to maize improved the NUE of maize when treated with optimal N fertilization (Zhang et al., 2017b; Raza et al., 2022).

Such improved N status and N use efficiency in the cereal-legume intercropping are directly linked to nitrogen assimilatory enzymes such as NR, NiR and GOGAT activities, which are the key enzymes involve in plant nitrogen metabolism (Nasar et al., 2022b). This study showed that maize-soybean intercropping significantly improved the NR, NiR and GOGAT activity of maize crop as compared with mono-cropping. However, these enzymes were more evident when intercropping was practiced under N_1 treatments than N_0 and N_2 treatment. Possibly, this might be attributed to the nitrogen fixation ability of soybean, which helps improve the nitrogen content of maize plant, thereby enhancing the N metabolism and N-related enzymes of maize crop (Nasar et al., 2022a). It might also be due to the underlying rhizosphere alteration, changes in the soil enzymes and the root releasing chemicals, which ultimately triggers the plant nitrogen metabolisms system (Nasar et al., 2022b). Moreover, nitrogen fertilization is also known to improve the nitrogen metabolism of the plant by improving the nitrogen assimilatory enzymes (Ben, 2016). These results are also supported by Nasar et al. (2022a), who found that maize-soybean intercropping under optimal N fertilization significantly improved the N assimilatory enzymes of maize crop, thereby enhancing its nitrogen use efficiency. Dang et al. (2020) also reported that proso millet and mung bean intercropping significantly improved the nitrogen assimilatory enzymes of millet crops, thereby enhancing their N status and yield. This, suggests that maize-soybean intercropping under optimal N fertilization can help improve the N uptake, N yield and N use efficiency *via* regulating N assimilatory enzymes, thereby enhancing its productivity.

5 Conclusion

The findings of this study have clearly shown that maize-soybean intercropping significantly improved the yield and yield attribute of maize compared with a mono-cropping system. However, these indices were more pronounced under optimal nitrogen fertilization. Moreover, intercropping under optimal nitrogen fertilization enhanced the nitrogen assimilatory enzymes such as nitrate reductase, nitrite reductase and glutamate synthase activity. This resulted in an improved nitrogen content and total nitrogen uptake of maize crop, thereby enhancing its nitrogen yield indices and nitrogen utilization efficiency indices such as nitrogen use efficiency, partial factor nitrogen use efficiency, nitrogen uptake efficiency and nitrogen agronomic efficiency as compared with mono-cropping. Hence, our study suggests that maize-soybean intercropping could be a potential cropping system for improving crop productivity, nitrogen uptake, nitrogen yield and nitrogen use efficiency under minimal input, ultimately leading to sustainable agricultural development.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JN: conceptualization, methodology, and writing—original draft. CZ and RK: data curation. HG and ZS: formal analysis. GA and IH: resources. ZI and WA: software. XZ and JY: supervision. HG, QL, and RR: writing— review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Feng Yang,
Sichuan Agricultural University, China

REVIEWED BY
Muhammad Ali Raza,
Islamia University of Bahawalpur, Pakistan
Wen Yin,
Gansu Agricultural University, China

*CORRESPONDENCE
Wei-Ping Zhang
zhangwp@cau.edu.cn

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Maize/peanut intercropping has greater synergistic effects and home-field advantages than maize/soybean on straw decomposition

Surigaoge Surigaoge[1], Hao Yang[1], Ye Su[1], Yu-He Du[1], Su-Xian Ren[1], Dario Fornara[2], Peter Christie[3], Wei-Ping Zhang[1]* and Long Li[1]

[1]Beijing Key Laboratory of Biodiversity and Organic Farming, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China, [2]Davines Group-Rodale Institute European Regenerative Organic Center (EROC), Parma, Italy, [3]Chinese Academy of Sciences (CAS) Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China

Introduction: The decomposition of plant litter mass is responsible for substantial carbon fluxes and remains a key process regulating nutrient cycling in natural and managed ecosystems. Litter decomposition has been addressed in agricultural monoculture systems, but not in intercropping systems, which produce species-diverse litter mass mixtures. The aim here is to quantify how straw type, the soil environment and their combined effects may influence straw decomposition in widely practiced maize/legume intercropping systems.

Methods: Three decomposition experiments were conducted over 341 days within a long-term intercropping field experiment which included two nitrogen (N) addition levels (i.e. no-N and N-addition) and five cropping systems (maize, soybean and peanut monocultures and maize/soybean and maize/peanut intercropping). Experiment I was used to quantify litter quality effects on decomposition; five types of straw (maize, soybean, peanut, maize-soybean and maize-peanut) from two N treatments decomposed in the same maize plot. Experiment II addressed soil environment effects on root decomposition; soybean straw decomposed in different plots (five cropping systems and two N levels). Experiment III addressed 'home' decomposition effects whereby litter mass (straw) was remained to decompose in the plot of origin. The contribution of litter and soil effects to the home-field advantages was compared between experiment III ('home' plot) and I-II ('away' plot).

Results and discussions: Straw type affected litter mass loss in the same soil environment (experiment I) and the mass loss values of maize, soybean, peanut, maize-soybean, and maize-peanut straw were 59, 77, 87, 76, and 78%, respectively. Straw type also affected decomposition in the 'home' plot environment (experiment III), with mass loss values of maize, soybean, peanut, maize-soybean and maize-peanut straw of 66, 74, 80, 72, and 76%, respectively. Cropping system did not affect the mass loss of soybean straw (experiment II). Nitrogen-addition significantly increased straw mass loss in experiment III.

Decomposition of maize-peanut straw mixtures was enhanced more by 'home-field advantage' effects than that of maize-soybean straw mixtures. There was a synergistic mixing effect of maize-peanut and maize-soybean straw mixture decomposition in both 'home' (experiment III) and 'away' plots (experiment I). Maize-peanut showed greater synergistic effects than maize-soybean in straw mixture decomposition in their 'home' plot (experiment III). These findings are discussed in terms of their important implications for the management of species-diverse straw in food-production intercropping systems.

KEYWORDS

C:N ratio, home-field advantage, litter quality, maize/legume intercropping, mixed litter decomposition, N addition, non-additive effects, plant diversity

1 Introduction

Terrestrial plants are estimated to produce 120 Pg of organic carbon (C) annually, and about 60 Pg of this C enters the dead organic matter pool (Datry et al., 2018). Similarly, the amount of crop residue yielded worldwide is estimated at 3758×10^6 Mg/year for 27 food crops (Lal, 2005). The return of litter mass (i.e. straw) to the soil has been commonly practiced to increase crop yield and manage carbon (C) sequestration in agricultural ecosystems (Liu et al., 2014). Plant litter mass decomposition not only accounts for a substantial carbon (C) flux but is a key process regulating nutrient cycling in terrestrial ecosystems (Barbe et al., 2017; Bichel et al., 2017; Chen et al., 2017a). Plant decomposition has been extensively studied in natural ecosystems and monoculture agricultural ecosystems (Handa et al., 2014; Xu et al., 2017). Intercropping is widely practiced worldwide and is considered a good example of sustainable agriculture (Vandermeer, 1992; Bedoussac et al., 2015; Yang et al., 2017), because it maintains crop yields without increasing inputs (Li et al., 2007; Tamburini et al., 2020), and is also associated with greater yield stability (Li et al., 2021b; Wu et al., 2022). Intercropping also increases the diversity of crop residues compared with monocultures (Zhang et al., 2023a), and a key question remains whether and how intercropping may influence the decomposition of crop residues.

The main drivers of litter decomposition are litter type and the soil environment in which the litter decomposes (Powers et al., 2009; Guo et al., 2021). Differences in the chemical composition of plant litter may affect decomposition processes (Berg, 2014). For example, legume species have higher decomposition rates than non-legume species (Xu et al., 2017; da Silva et al., 2021). In addition to the importance of litter type, soil environmental conditions also play a key role in straw decomposition (Hättenschwiler et al., 2005; Chen et al., 2017a). For example, high soil water contents stimulate decomposition (Chen et al., 2017a). Intercropping influences soil water content (Yin et al., 2020), light transmittance (Li et al., 2021a), N dynamics (Chen et al., 2019) and soil enzyme activities (Curtright and Tiemann, 2021), which together influence the rate of decomposition of species-diverse straw mass. For example, intercropping enhances soil total N content, especially in low-fertility soils (Li et al., 2021b). However, it remains largely unknown how straw type and the soil environment in intercropping interact to influence straw decomposition.

Nitrogen addition to soils may also affect plant litter quality and thus influence decomposition processes, with N addition having positive (Vivanco and Austin, 2011; Li et al., 2017), negative (Song et al., 2019), or no effects on litter decomposition rates (Wang et al., 2019). Net N effects on litter decomposition depend on N fertilization rates and litter quality. For example, litter decomposition is inhibited by N additions when fertilizer N rates are high or when litter quality is low (e.g. high lignin content), whereas decomposition is stimulated when ambient N deposition is low and litter quality high (e.g. low lignin content) (Knorr et al., 2005). Here we focus on the effects of nitrogen fertilization on straw decomposition in intercropping systems.

There is considerable variation in the quality of plant litter returned to the soil (Cornwell et al., 2008) and many soil microbial communities are adapted to decompose local litter (Ayres et al., 2009). A growing number of studies show that litter decomposes faster in its habitat of origin (i.e. 'home') relative to some other location (i.e. 'away') (Veen et al., 2015; Li et al., 2017) and this is termed the 'home-field advantage' (HFA) effect (Ayres et al., 2009). The HFA effect occurs when the quality of a given litter type is well recognized by the decomposer community in the environment of the 'home' plot. The "substrate quality–matrix quality interaction" (SMI) hypothesis suggests that the strength of the HFA will be greater as the quality of specific plant litter and the decomposition environment become more and more divergent (Freschet et al., 2012). Using litter mass loss data from 125 reciprocal litter transplants across 35 studies, a meta-analysis found that there was considerable variation in the strength and direction (sometimes opposite to expectations) of the HFA effect (Veen et al., 2015). For example, some studies show accelerated decomposition in the home environmental conditions relative to away conditions (Ayres et al., 2009; Li et al., 2017), whereas other studies show similar or even reduced decomposition at home compared to away (Ayres et al., 2006; Gießelmann et al., 2011). However, our knowledge of the relative roles of litter quality and the soil environment on HFA effects of litter decomposition in intercropping remains limited.

Most studies consider the litter decomposition of single crop species but the litter layer usually consists of a mixture of litter materials from different plant species (Chen et al., 2018). Litter mixtures influence decomposition in two alternative ways, through additive or non-additive effects (Hättenschwiler et al., 2005; Chen et al., 2018). Additive effects do not involve interactions among straw materials from different species during decomposition, with no differences between observed and expected litter decomposition rates in mixtures based on species composition (Chen et al., 2017b). Non-additive effects include an antagonistic effect (slower decomposition in the mixture than expected) or a synergistic effect (faster decomposition in the mixture than expected) (Liu et al., 2020). Numerous studies show that non-additive effects (synergism or antagonism) seem to be more common than additive effects in the decomposition of litter mixtures (Hättenschwiler et al., 2005; Chen et al., 2018). The release of secondary metabolites from specific litter species leads to antagonistic non-additive effects, whereas synergistic mixing effects may occur due to nutrient transfer among litter species and suitable microenvironmental conditions can stimulate the decomposition of poor litter quality (Hättenschwiler et al., 2005). We hypothesize that mixtures of straw from legumes and maize have synergistic mixing effects during decomposition in maize/legume intercropping systems.

The current study aims to quantify how straw type, the soil environment and their combined effects may influence straw decomposition in widely practiced maize/legume intercropping systems. We hypothesize that 1) home-field advantages of straw decomposition depend on species identity and species combinations; and 2) there are synergistic mixing effects on straw decomposition in intercropping systems.

2 Materials and methods

2.1 Study site

The study was conducted at the China Agricultural University Lishu Experimental Station (43.3° N, 124.4° E) in Lishu county, Jilin province, northeast China from May 2020 to May 2021. The mean daily air temperature during the experiment was 8.2°C. The maximum air temperature was 28.5°C observed on 8 June 2020 and the minimum was -23.6°C on 7 January 2021 (Figure S1). The soil type is Vertisol and the texture of the soil is clay loam (23.9% sand, 45.2% silt, and 30.9% clay) at 0–20 cm depth. The surface soil (0–20 cm depth) organic matter content is 16.6 g kg^{-1}, with a pH of 5.45, a total soil N content of 0.96 g kg^{-1}, 18.9 mg of Olsen-P kg^{-1}, and 137 mg of available K kg^{-1} at the start of the long-term experiment (2017).

2.2 Field experimental design

2.2.1 Sources of straw materials

The decomposition study was carried out within a long-term intercropping field experiment (see Zhang et al., 2021 for more details) including two N level treatments and five cropping systems (Figures S2, S3). The study was therefore a two-factor complete randomized block design with three blocks. The first factor was two N levels (no N addition (N0) and N addition (N1) and the second factor was five cropping systems, namely monoculture maize (*Zea mays* L. cv. Xianyu No. 335), monoculture soybean (*Glycinemax* L. Merrill. Jiyu No. 47), monoculture peanut (*Arachis hypogaea* L. cv. Baisha No. 1016), maize/soybean intercropping, and maize/peanut intercropping (Figure S3). A total of 30 plots (2 N levels × 5 cropping systems × 3 blocks) were used in the decomposition study.

In the N-addition treatments, 80 kg N ha^{-1} were applied to the soybean and peanut monocultures as urea, 240 kg N ha^{-1} to the maize monoculture, and 160 kg N ha^{-1} to the two intercropping systems (Table S1). In addition, 52 kg P ha^{-1} (as superphosphate) were applied and 83 kg K ha^{-1} (as potassium sulphate) were also applied to each experimental plot. Three decomposition experiments were conducted to quantify litter and soil effects on straw decomposition and potential home field advantages (HFA). In October 2019 the stems and leaves of maize, soybean, and peanut from the monocultures and intercropping systems were collected randomly from the long-term experiment after harvest, and the straw materials were used in experiments I-III.

2.2.2 Experiment I (different straw types decomposing in the same plot)

The litter quality effect on straw decomposition was quantified by decomposing different straw types in the same plot in experiment I (Figures S3, S4). Ten straw treatments were used from five cropping systems (maize, soybean, peanut, maize-soybean mixture, and maize-peanut mixture) and 2 N levels (5 × 2 = 10 straw treatments) in the long-term experiment. All straw materials were decomposed in three newly established monoculture maize plots adjacent to the long-term experiment (Figures S3, S4). Monoculture maize plots were selected to represent standard soil conditions because three-fifths of the plots contained maize. Each maize monoculture plot received 240 kg N ha^{-1}, 52 kg P ha^{-1} (as superphosphate), and 83 kg K ha^{-1} (as potassium sulphate) fertilizers as in the long-term experiment (Table S1).

2.2.3 Experiment II (same straw type decomposing in different plots)

The potential effects of the soil environment on straw decomposition were assessed by decomposing the same straw types in different plots in the long-term intercropping experiment (Experiment II, Figures S2–S4). The same straw (monoculture soybean straw from N1 fertilizer) was decomposed in 30 plots (5 cropping systems × 2 N levels × 3 blocks). The initial chemical quality trait (i.e. C/N ratio) of soybean was intermediate between maize and peanut, and soybean straw materials were therefore selected as the standard straw.

2.2.4 Experiment III (straw types decomposing in their 'home' plots)

Potential straw type and soil environmental effects on straw decomposition were determined by decomposing each straw type in its 'home' plot in the long-term intercropping experiment (Experiment III, Figures S2-S4). The straw materials from 30 plots (5 cropping systems × 2 N levels × 3 blocks) were decomposed in their corresponding plots ('home' plots), i.e., the straw materials were returned to their original plots. The straw materials decomposed *in-situ* in experiment III ('home' plot), in contrast to experiments I and II which are considered as two control experiments (or 'away' plots) for decomposition.

2.3 Straw and soil sampling

After overwinter air-drying, all stem and leaf sample were clipped into 2-3 cm-long fragments in early May 2020 and oven-dried at 40°C for 72 h to constant mass. All straw samples were placed in 15 × 10 cm polyethylene litterbags (mesh size 180 μm) with 4 g dried straw. The actual situation of straw remaining in the field was simulated by mixing percentages of stems and leaves of the three monoculture crops calculated according to the biomass of the corresponding monocultures estimated from 2017 to 2019 at harvest. In this way the following combinations were obtained: monoculture maize straw (stem 53% + leaf 47%), monoculture soybean straw (stem 57% + leaf 43%) and monoculture peanut straw (stem 55% + leaf 45%). The percentage of mixed straw of both crops in the intercropping systems (maize/soybean and maize/peanut) was calculated by the biomass in their respective intercropping systems from 2017 to 2019 at harvest. We thus prepared: (a) maize-soybean mixture straw (maize 75% + soybean 25%), which was composed of stem 40% and leaf 35% of intercropping maize and stem 15% and leaf 11% of soybean, and (b) maize-peanut mixture straw (maize 81% + peanut 19%), which comprised stem 43% and leaf 38% of maize and stem 10% and leaf 9% of peanut.

The number of litterbags in experiment I was 150 (i.e. 5 straw types × 2 N levels × 1 soil condition × 5 retrievals × 3 blocks), in experiment II 150 (i.e. 1 straw type × 5 cropping systems × 2 N levels × 5 retrievals × 3 blocks), and in experiment III 150 (5 straw and soil combinations × 2 N levels × 5 retrievals × 3 blocks) (Figure S4). Thus, a total of 450 litterbags were buried in the soil at 10 cm depth in the field experiment on 27 May 2020. The litterbags were placed in the center of two crop rows in the monoculture and intercropping systems, respectively (Figures S4, S5). Litterbags from each plot were sampled after decomposition for 44, 74, 109, 136, and 341 days and oven-dried (60°C, 48 h). Soil samples (0-10 cm depth) were taken from each plot using a soil auger (5-cm-diameter) and soil water content was determined by oven-drying (105°C, 48 h) to constant mass. Straw materials were removed from the litterbags and each sample was washed and gently sieved through a 0.25-mm mesh to remove any adhering soil particles. The straw samples were then transferred to labeled paper envelopes and oven-dried (60°C, 48 h) to constant mass. The dry straw samples were then weighed and the straw mass loss was determined from the initial mass and the samples were retained for further analysis. Oven-dried straw samples prior to decomposition were ground with a ball mill for chemical analysis. The initial straw C and N concentrations were determined using a C/N analyzer (Vario Micro cube, Elementar, Lagenselbold, Germany). The concentrations of cellulose, hemicellulose and lignin were determined using an Ankom A200 fiber analyzer (Ankom Technology, Macedon, NY) based on a modified Van Soest method.

2.4 Calculations

Straw mass loss was calculated using the oven-dried weights at each retrieval (Chen et al., 2017a):

$$\text{Mass loss} = (1 - \frac{M_t}{M_0}) \times 100 \qquad \text{eqn 1}$$

where M_t is the dry mass of straw remaining in litter bags at each sampling time and M_0 is the initial dry mass of straw.

Decay constants (*k*) were obtained assuming simple negative exponential decay (Olson, 1963):

$$\ln(\frac{M_t}{M_0}) = -kt \qquad \text{eqn 2}$$

where M_t is the straw mass at time t and M_0 is the initial straw mass. The *k* value can also indicate decomposition rate.

The contribution of litter effects and soil effects to the home-field advantage (HFA) was compared between experiments I-II ('away' plots) and III ('home' plots). Both experiments I and III used the same straw materials from two intercropping systems and two N levels of the long-term experiment, but a different plot environment was used for decomposition ('home' soil in experiment III vs. maize plots in experiment I), thus the soil effect on home-field advantage was assessed from the difference between experiments I and III. Both experiments II and III used the same plots with two intercropping systems and two N levels for decomposition in the long-term experiment, but used different straw types (4 straw treatments in experiment III *vs.* soybean straw in experiment II), thus the litter effect on home-field advantage was assessed from the difference between experiments II and III. The litter effect and soil effect on home-field advantage were calculated as follows (Li et al., 2017):

$$\text{Soil effect on HFA} = \ln(\frac{k\text{III}_{\text{Tx}}}{k\text{I}_{\text{Tx}}}) \qquad \text{eqn 3}$$

$$\text{Litter effect on HFA} = \ln(\frac{k\text{III}_{\text{Tx}}}{k\text{II}_{\text{Tx}}}) \qquad \text{eqn 4}$$

where Tx indicates the five types of straw in N0 and N1 treatments and *k*III, *k*II, and *k*I are the *k*-values of Tx treatment in experiments III, II, and I, respectively. Positive soil effects on home-field advantage (HFA) indicate that a specific straw decomposed faster in the 'home' soil (experiment III) than in the given soil (monoculture maize plot, experiment I), and positive litter effects on home-field advantage indicate that one straw type (experiment III) decomposed faster than the standard straw (soybean straw, experiment II) in the same plot (Li et al., 2017).

The relative mixture effect (RME) on straw mass loss at the end of decomposition was calculated as:

$$\text{RME} = \frac{(\text{O} - \text{E})}{\text{E}} \times 100\,\% \qquad \text{eqn 5}$$

where O was the observed mass loss of straw mixture and E was the expected value based on the case of each component straw type decomposed separately (Wardle et al., 1997).

2.5 Statistical analysis

Generalized linear mixed-effects models (GLMMs) were used to test for potential effects of fertilizer N (N0 *vs*. N1), straw type or/and cropping system (maize, soybean, peanut, maize-soybean, and maize-peanut) and their interactions on straw mass loss and soil water content at 5 retrievals (Table S2). Nitrogen and straw type were included as fixed factors, and block and day were included as random effects (Bolker et al., 2009). Generalized linear mixed-effects models (GLMMs) were also used to test for potential effects of fertilizer N (N0 *vs*. N1), straw type or/and cropping system (maize, soybean, peanut, maize-soybean, and maize-peanut) and their interactions on initial straw quality (the concentrations of C, N, cellulose, hemicellulose and lignin, C/N ratio and lignin/N ratio), liter effect on HFA, soil effect on HFA, and relative mixture effect; N and straw type were included as fixed factors and the block was included as a random effect (Table S2). The R package 'nlme' was used for the linear-mixed effect models. Moreover, Pearson correlation analysis was used to test for potential relationships among initial straw quality and decomposition rate (k). Significant differences among treatments were determined by the *post-hoc* Tukey HSD test at $P< 0.05$. Furthermore, Student's *t*-test was used to test the difference of HFA and RME from zero. We used R version 4.1.2 (R Core Team, 2022) for all statistical analysis.

3 Results

3.1 Effects of litter quality on straw mass loss (Experiment I)

Nitrogen did not significantly affect straw mass loss but straw type significantly affected straw mass loss in the same plot (maize plots) environment ($P< 0.01$). The mass loss values of maize, soybean, peanut, maize-soybean, and maize-peanut straw materials were 59, 77, 87, 76, and 78%, respectively, of the initial mass after 341 days of decomposition (Figures 1A, B). Peanut straw had the highest straw mass loss, which was significantly higher than mass loss of maize/peanut straw mixtures and maize/soybean straw mixtures and soybean, and maize had the lowest straw mass loss ($P< 0.05$) (Figures 1A, B). Nitrogen application significantly decreased the C/N ratios of straw while straw type significantly affected straw C/N ratios. The C/N ratio of maize was the highest (63.45 ± 5.60), followed by maize-soybean (58.77 ± 2.81), soybean (49.26 ± 2.53), and maize-peanut (47.71 ± 1.05). Peanut C/N ratio (21.69 ± 1.59) was significantly lower than those of other straw types (Table 1). The concentrations of cellulose and hemicellulose were highest in maize straw, but the lignin concentration was lower than in soybean and or peanut straw (Table 1). The initial C/N ratios as well as C and hemicellulose

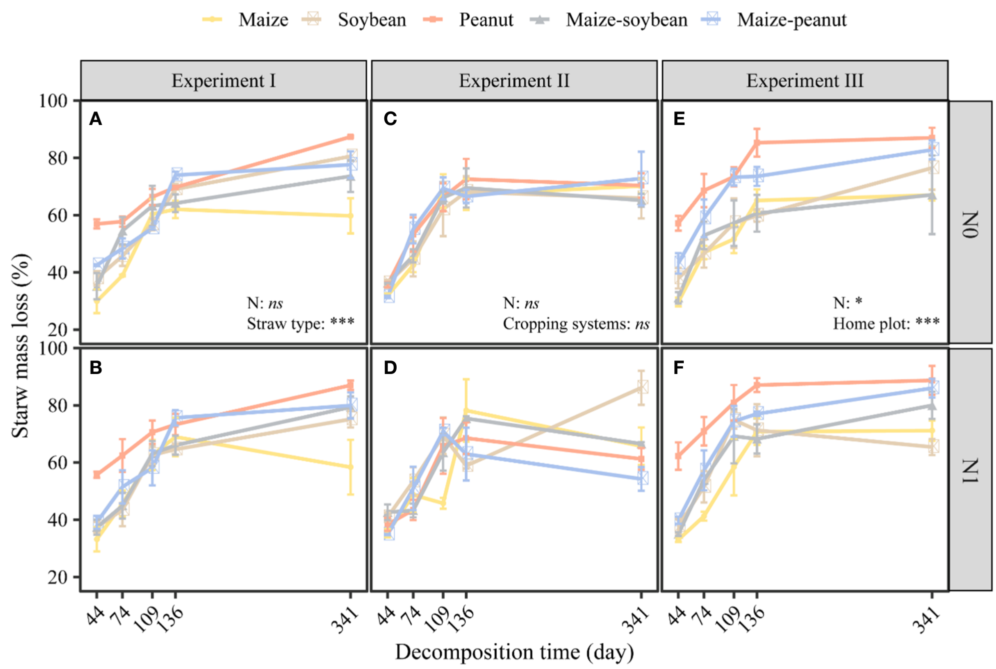

FIGURE 1
Effects of nitrogen addition and straw type on mass loss of different straws in the new monoculture maize plots **(A, B)**; effects of nitrogen addition and cropping system on straw mass loss of soybean straw **(C, D)**; and effects of nitrogen and cropping system on straw mass loss in the home plots **(E, F)**. In experiment I, different straw types decomposed in the same plot (new maize plots). In experiment II the same soybean straw decomposed in different plots. In experiment III, straw types from each plot decomposed in their home plot. *$P < 0.05$, *** $P < 0.001$, *ns*: not significant. Data are mean ± SE (n=3).

contents had a negative effect on decomposition but lignin and lignin/N ratio had no significant effects on the decomposition rate (*k*) under the same environmental conditions (Figure 2A).

3.2 Effects of cropping system on straw mass loss (Experiment II)

Nitrogen addition and cropping system did not significantly affect the mass loss of the same soybean straw materials (Figures 1C, D). Nitrogen significantly decreased soil water content but cropping system did not affect soil water content (Figure S6).

3.3 Effects of the 'home' plot on straw mass loss (Experiment III)

The mass loss of maize, soybean, peanut, maize-soybean and maize-peanut straw types was 66, 74, 80, 72, and 76% respectively, of the initial mass after 341 days of decomposition (Figures 1E, F). In general, peanut straw had significantly ($P< 0.001$) higher mass loss than maize, soybean, maize-soybean, or maize-peanut straw types during the experimental period (Table S3).

Nitrogen and cropping systems significantly affected the mass loss of straw in the 'home' plot ($P< 0.05$). Nitrogen fertilizer significantly increased the straw mass loss in 'home' plots. Peanut straw left to decompose in peanut monoculture plots had higher mass loss than other straw types including maize-peanut straw mixtures in maize/peanut intercropping systems. Maize-soybean straw mixtures, maize, and soybean had the lowest mass loss in their 'home' plots among the five straw types (Figures 1E, F). The initial C/N ratios, C and hemicellulose had a negative effect, but lignin and lignin/N ratio had no significant effects on straw mass loss in their 'home' plot (Figure 2B).

3.4 Effects of N addition and straw type on the home-field advantage effect

A significant litter type effect ('litter effect') was found in the home-field advantage (HFA) decomposition for maize-peanut straw mixture under nitrogen additions (0.59 ± 0.13; Figure 3A). This indicates that maize-peanut straw mixture decomposed faster than the standard straw (soybean straw) under higher N additions in the 'home' plot conditions. A positive 'soil effect' in the HFA decomposition for maize-peanut straw mixtures was found under zero N addition (0.17 ± 0.03), indicating that maize-peanut straw mixture decomposed faster in 'home' plots than in a given plot (maize plot condition) under zero N addition. The 'soil effect' in the HFA of maize-peanut straw mixtures was significantly higher than the HFA in maize-soybean straw mixtures ($P< 0.05$; Figure 3B).

3.5 Effects of N addition and straw type of relative mixing effects on litter decomposition

After the five retrieval times of the litter bags were taken into consideration, the RME values of both maize-peanut (13.54 ± 5.02%)

TABLE 1 Initial quality traits of straw types (monoculture and mixture) from the different N addition treatments in experiments I – III.

N rate	Straw type	C (mg g^{-1})	N (mg g^{-1})	Cellulose (mg g^{-1})	Hemicellulose (mg g^{-1})	Lignin (mg g^{-1})	C/N	Lignin/N
N0	Maize	423.58 ± 2.57 aB	6.31 ± 0.65 bB	347.39 ± 3.22 aA	337.19 ± 3.32 aA	30.42 ± 3.3 cA	68.56± 6.82 aA	4.99± 0.86 bA
	Soybean	415.43 ± 1.54 aB	8.45 ± 0.29 bB	247.57 ± 5.59 bA	158.32 ± 9.55 cA	109.61 ± 7.5 aA	49.29± 1.87 bA	12.97± 0.75 aA
	Peanut	394.16 ± 11.28 bB	16.67 ± 0.43 aB	260.11 ± 13.59 bA	146.66 ± 15.69 dA	101.61 ± 8.68 bA	23.64± 0.26 cA	6.12± 0.65 bA
	Maize-soybean	420.78 ± 1.41 aB	6.98 ± 0.29 bB	325.01 ± 0.69 aA	305.4 ± 2.39 bA	51.14 ± 1.67 cA	60.52± 2.6 aA	7.33± 0.07 bA
	Maize-peanut	416.93 ± 4.82 aB	8.27 ± 0.2 bB	336.75 ± 1.64 aA	304.85 ± 2.03 bA	43.81 ± 2.24 cA	50.47± 1.46 bA	5.29± 0.15 bA
N1	Maize	436.5 ± 1.88 aA	7.56 ± 0.55 bA	343.69 ± 7 aA	338.04 ± 7.37 aA	40.02 ± 1.13 cA	58.34± 4.36 aB	5.37± 0.56 bA
	Soybean *	416.44 ± 5.79 aA	8.51 ± 0.42 bA	250.41 ± 7.78 bA	166.17 ± 7.22 cA	121.67 ± 6.8 aA	49.24± 3.2 bB	14.44± 1.5 aA
	Peanut	397.6 ± 6.78 bA	21.07 ± 3.18 aA	244.12 ± 25.18 bA	121.59 ± 10.18 dA	88.75 ± 13.79 bA	19.75± 2.91 cB	4.28± 0.65 bA
	Maize-soybean	428.99 ± 1.74 aA	7.57 ± 0.43 bA	313.98 ± 4.24 aA	300.47 ± 4.56 bA	52.22 ± 2.6 cA	57.01± 3.04 aB	6.97± 0.68 bA
	Maize-peanut	424.62 ± 0.7 aA	9.45 ± 0.14 bA	314.99 ± 4.61 aA	290.99 ± 4.36 bA	46.5 ± 4.8 cA	44.94± 0.65 bB	4.91± 0.43 bA
P-value								
N		0.049	0.040	0.136	0.171	0.546	0.021	0.750
Straw type		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
N × Straw type		0.787	0.322	0.757	0.288	0.371	0.531	0.258

Means with different letters are significantly different (Tukey's post hoc test; $P< 0.05$). Uppercase letters indicate differences between zero-N addition and N addition, and lowercase letters indicate differences among straw types. In experiment II the same straw (soybean from N1 treatment) decomposed in different plots, marked with an asterisk. Data are mean ± SE (n = 3).

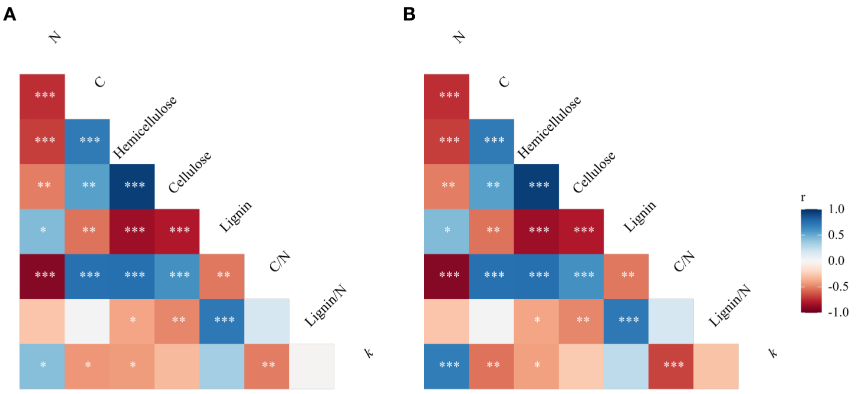

FIGURE 2
Pearson correlation matrix of potential relationships between decomposition rate (*k*) and initial straw quality (concentrations of C, N, cellulose, hemicellulose and lignin, C/N ratio and lignin/N ratio) in **(A)** experiment I and **(B)** experiment III. Associations are colored by direction of effect (blue, positive; red, negative), with associations significant (* $P< 0.05$, ** $P< 0.01$, *** $P< 0.001$).

and maize-soybean mixture (14.05± 5.30%) decomposition were positive in the zero N-addition control in experiment I ($P< 0.05$; Figure 4). The magnitude of the RME of the maize-peanut mixture decomposition was significantly greater than that of maize-soybean mixtures in experiment III ($P< 0.05$; Figure 4). In addition, the RME of maize-peanut mixtures was positive in non N-addition (19.60± 3.96%) and N-addition treatments (14.28± 5.37%), whereas the RME of maize-soybean mixtures (9.84± 4.25%) was significantly positive only with nitrogen application in experiment III ($P< 0.05$; Figure 4).

4 Discussion

The results indicate that straw type had significant effects on the mass loss of straw in the same soil (i.e. maize plots in experiment I) and in the 'home' plot environment (experiment III), and this is mainly attributable to differences in straw quality (i.e. the C/N ratios). The mass loss of the same soybean straw materials was unaffected by the soil environment in the different cropping systems (experiment II). Nitrogen addition significantly increased straw mass loss in the 'home' plots (experiment III). Maize-peanut straw mixture decomposition showed higher occurrence and magnitude of 'home-field advantage' effects than mixed maize-soybean straw. Both maize/soybean and maize/peanut straw mixtures showed synergistic mixing effects (RME) on decomposition in both 'home' (experiment III) and 'away' plots (experiment I), while maize-peanut straw mixture decomposition had greater synergistic effects than maize-soybean straw mixture decomposition in the 'home' plots (experiment III).

4.1 The home-field advantage effects *via* straw quality and soil properties

The results here support our first hypothesis that home-field advantages (HFAs) of straw decomposition depend on species identity and on litter mass species combinations. A 'litter identity

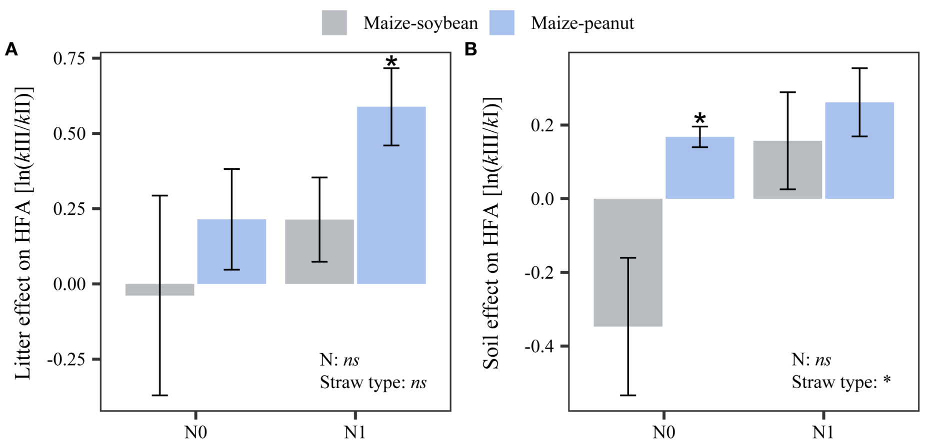

FIGURE 3
Effects of nitrogen addition and straw type on the home-field advantage effect *via* **(A)** litter type ('litter effect') and **(B)** soil environment ('soil effect'). Asterisks above bars indicate significant differences between the home-field advantage effect and zero ($P< 0.05$). Statistical significance of the main effects of nitrogen application and straw type on HFA was also shown (*$P< 0.05$, *ns*: not significant).. Data are mean ± SE (n=3).

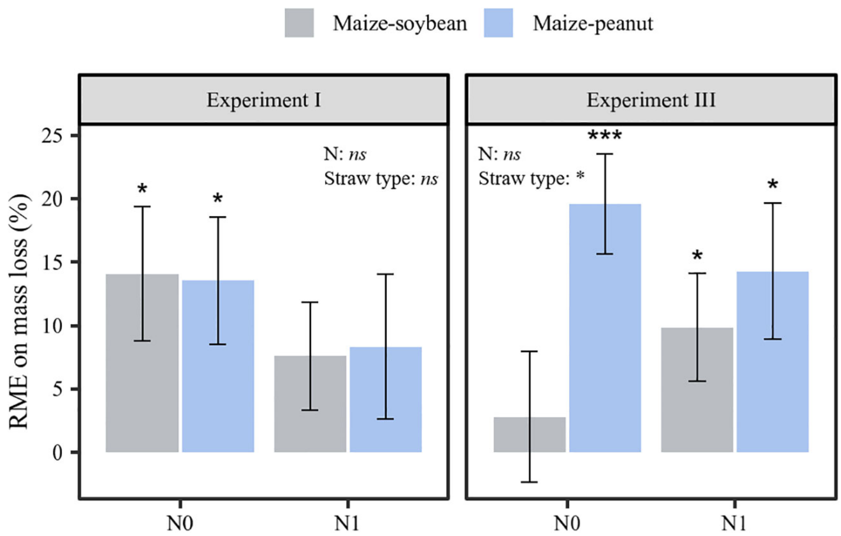

FIGURE 4
The relative mixing effects (RME) on straw mass loss in experiments I and III for maize-soybean and maize-peanut mixtures at N0 and N1 conditions. Data are mean ± SE (n=3). Asterisks above bars show significant differences between mean value and 0 (*P < 0.05, *** P < 0.001). Statistical significance of the main effects of nitrogen application and straw type on RME was also shown (*P < 0.05, *ns*: not significant).

effect' of maize-peanut mixture straw on decomposition was found which was driven by increased nitrogen additions (Figure 3A). These findings suggest that the quality of maize-peanut mixture straw was significantly different from soybean straw, and the HFA effect occurred *via* litter quality. A 'soil environment effect' on the decomposition of maize/peanut mixture straw was also found in the unfertilized plots (Figure 3B). This suggests that the soil environment in maize/peanut intercropping is significantly different from that in the maize monoculture and that the HFA effect occurred because of 'home' soil properties.

There is increasing recognition that plants are associated with species-specific decomposer communities (McGuire and Treseder, 2010; Freschet et al., 2012). The HFA would likely occur when these decomposer communities are adapted to decompose local plant litter materials (Madritch and Lindroth, 2011; Osburn et al., 2022). There were no litter or soil effects associated with HFA in many other treatments. For example, we did not detect any HFA in maize and soybean straw materials decomposing in their 'home' plots (Figure 3), and these results support those from a worldwide meta-analysis in which HFA effects showed considerable variation in their strength and direction (Veen et al., 2015).

The results here indicate that straw type significantly affected straw mass loss during decomposition in the maize plot environment (experiment I) and in the 'home' plot conditions (experiment III). Different decomposition rates were also found in maize, soybean, and wheat straw materials (Xu et al., 2017). Peanut had the highest straw mass loss which was higher than maize/peanut straw mixtures, maize/soybean straw mixtures and soybean, and maize had the lowest straw mass loss among the five straw types (Figures 1A, B). Generally, residues with higher C/N ratios decompose more slowly than those with lower C/N ratios (Guo et al., 2021). Maize had the highest C/N ratio (63.4), followed by maize/soybean (58.8), soybean (49.3), and maize/peanut (47.7), and peanut (21.7) was significantly lower than other straw types (Table 1). The peanut straw had the highest mass loss during the period of decomposition whereas maize straw, soybean straw, and their mixture were more recalcitrant to decomposition. In addition, the current study indicates that the C/N ratio was a more important factor than lignin concentration in explaining the effects of straw quality on decomposition (Figure 2; Table 1). This result is consistent with those of a recent meta-analysis in which C/N ratio was a controlling factor in leaf litter decomposition across different biomes (Guo et al., 2021).

Nitrogen application did not affect straw mass loss in the 'away' plots (experiments I and II) but significantly increased soil water content and thus the straw mass loss in the 'home' plots (experiment III). Nitrogen and cropping systems did not significantly affect the mass loss of soybean straw materials (experiment II) (Figures 1C, D). These findings, together with those from experiments I-III, suggest that straw type was more important than the soil environment in affecting decomposition. The differences among litters were generally much larger than the effects of fertilization on litter decomposition (Hobbie, 2008). A recent meta-analysis also indicates that litter quality plays a more important role in driving leaf and fine root decomposition than soil and decomposers across biomes (Guo et al., 2021).

4.2 Maize-peanut, not maize-soybean, had home-field advantage in decomposition of straw mixtures in intercropping systems

The decomposition of maize-peanut straw mixtures was associated with a higher occurrence and magnitude of HFA effects than the decomposition of maize-soybean straw mixtures (Figure 2). The current results indicate that maize/peanut intercropping differed from maize and soybean monocultures in terms of straw quality and soil environment. This supports the "substrate quality-matrix quality interaction (SMI) hypothesis" in which HFA effects become larger when the quality of 'home' and 'away' litters become more dissimilar (Ayres et al., 2009; Freschet et al., 2012; Li et al., 2017). The C/N ratio of peanut straw was significantly lower than those of maize and soybean straw materials (Table 1) thus promoting greater HFA effects in maize-peanut straw decomposition than in maize-soybean straw decomposition through enhanced litter quality. Two meta-analysis studies also found that HFA effects increased with the initial litter quality (N content and C/N ratio) (Zhang et al., 2013; Veen et al., 2015). In addition, HFA can be affected by dissimilarity in the decomposer community and tends to become stronger when plant communities are more dissimilar (Veen et al., 2015). The maize/peanut intercropping system can significantly modify soil microbial community composition and the dominant microbial species relative to maize and peanut monocultures (Li et al., 2016), thus promoting HFAs in maize-peanut straw decomposition *via* changes in the soil environment. The current results indicate that maize-peanut residue retention in their home field may be an alternative method of straw return practice in maize/peanut intercropping.

4.3 The relative mixing effects on maize-legume mixtures

The present study partly supports the second hypothesis that synergistic mixing effects of straw decomposition occur in intercropping systems. The RME associated with maize-peanut and maize-soybean mixture decomposition was positive under zero N-addition in new maize monoculture plots (experiment I). Moreover, the RME of maize-peanut mixture decomposition was significantly positive under two nitrogen levels; and the RME of maize-soybean mixtures was significantly positive under N addition in their 'home' plots (experiment III) (Figure 4). These findings suggest a synergistic effect of maize-soybean and maize-peanut litter mixtures on decomposition. Previous studies indicate that litter mixtures may show more non-additivity (synergism or antagonism) rather than additivity in their decomposition rates (Hättenschwiler et al., 2005; Hou and Lü, 2020). The synergistic effects of decomposition of maize-soybean and maize-peanut litter mixtures may be due to nutrient transfer (e.g. nitrogen, phosphorus) from high-quality legumes to low-quality maize straw or the complementarity effects of soil fauna and decomposers (Schimel and Hättenschwiler, 2007; Luan et al., 2022).

The RME of maize-peanut straw mixture decomposition was significantly higher than that of maize-soybean straw in their 'home' plots ($P< 0.05$, Figure 4). Across agroforestry systems it has been shown that non-additive effects were most pronounced when a high-N-concentration litter was mixed with a low-N-concentration litter (Wang et al., 2014). RME values increased in magnitude with increasing dissimilarity in the traits of litter mixtures, with positive effects related to trait dissimilarity in the case of nutrient traits and negative effects related to trait dissimilarity in recalcitrance traits (Canessa et al., 2022). The lower C/N ratio of peanut straw than of soybean straw materials (Table 1) thus promoted higher RME values in maize-peanut straw decomposition than in maize-soybean straw decomposition through enhanced litter quality. The current results partly support the higher yield of maize intercropped with peanut than with soybean in the same long-term intercropping experiment due to increased nutrient release and transfer (Zhang et al., 2021; Yang et al., 2022; Zhang et al., 2023b).

The overall straw mass loss in the current experiments was about 70% of the initial mass by day 136, and there was relatively little further straw decomposition subsequently (from days 136 to 341). This latter effect may be explained by the recalcitrant components left in this stage and the dry and cold winters in northeast China. The average temperature was 20.7°C and the cumulative precipitation was 514 mm at the early stages of decomposition (days 1 to 136). In contrast, the average temperature was -2.8°C and the cumulative precipitation was 36.6 mm at the later stages of decomposition (Figure S1).

Furthermore, the proportion of different litter species has been 1:1 in most previous studies. Here, to simulate the actual situation of straw remaining in the field, the percentage of the legume was nearly 20% in mixed straw while the RME effect of the maize-legume was also observed, and the higher percentage of legume may lead to greater synergistic mixing effects. Maize residue retention is a recommended practice in China and globally, and the current results indicate that mixing maize and legume residues can improve straw management practices by promoting straw decomposition and nutrient release.

5 Conclusion

Straw type significantly affected the straw mass loss in the same plots (i.e. maize plots) and in the 'home' plot environment, and this is largely explained by the different straw C/N ratios (litter quality). Neither N addition nor cropping system significantly affected the mass loss of soybean straw (experiment II). Straw type explained more the variation in straw decomposition than did the soil environment. Nitrogen addition did not affect the same straw mass loss in other locations but increased the straw mass loss in the 'home' plots. In addition, maize-peanut straw mixtures had a higher occurrence and magnitude of HFA effects than maize-soybean straw mixture materials. There was a synergistic mixing effect of maize-peanut straw mixture and maize-soybean straw mixture decomposition in both 'home' and 'away' plots. Maize-peanut straw mixtures showed a significantly higher relative mixing effect than maize-soybean straw mixture decomposition in their 'home' plots (Figure S7). The results suggest that *in situ* maize/peanut residue retention is to be recommended due to its higher straw decomposition and nutrient release rates in intercropping systems in temperate regions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

SS and W-PZ designed the research. SS, W-PZ, YS, Y-HD, HY and S-XR collected the data. SS and W-PZ analyzed the data. SS drafted the paper, and LL, DF, PC, W-PZ and SS contributed substantially to the revisions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1100842/full#supplementary-material

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EDITED BY
Muhammad Ali Raza,
Islamia University of Bahawalpur, Pakistan

REVIEWED BY
Shakeel Ahmad,
Bahauddin Zakariya University, Pakistan
Muhammad Nawaz,
Ayub Agriculture Research Institute,
Pakistan

*CORRESPONDENCE
Xiaoli Chen
cxlrxl@aliyun.com
Xiaolong Ren
rxlcxl@aliyun.com

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Nitrogen application enhances yield, yield-attributes, and physiological characteristics of dryland wheat/maize under strip intercropping

Sadam Hussain[1,2,3], Muhammad Asad Naseer[1,2,3], Ru Guo[1,2,3], Fei Han[1,2,3], Basharat Ali[4,5], Xiaoli Chen[1,2,3*], Xiaolong Ren[1,2,3*] and Saud Alamri[6]

[1]College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China, [2]Institute of Water Saving Agriculture in Arid Areas of China, Northwest A&F University, Yangling, Shaanxi, China, [3]Key Laboratory of Crop Physic-ecology and Tillage Science in Northwestern Loess Plateau, Ministry of Agriculture, Northwest A&F University, Yangling, Shaanxi, China, [4]Institute of Crop Science, University of Bonn, Bonn, Germany, [5]Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan, [6]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

Intercropping has been acknowledged as a sustainable practice for enhancing crop productivity and water use efficiency under rainfed conditions. However, the contribution of different planting rows towards crop physiology and yield is elusive. In addition, the influence of nitrogen (N) fertilization on the physiology, yield, and soil water storage of rainfed intercropping systems is poorly understood; therefore, the objective of this experiment was to study the contribution of different crop rows on the physiological, yield, and related traits of wheat/maize relay-strip intercropping (RSI) with and without N application. The treatments comprised of two factors viz. intercropping with three levels (sole wheat, sole maize, and RSI) and two N application rates, with and without N application. Results showed that RSI significantly improved the land use efficiency and grain yield of both crops under rainfed conditions. Intercropping with N application (+N treatment) resulted in the highest wheat grain yield with 70.37 and 52.78% increase as compared with monoculture and without N application in 2019 and 2020, respectively, where border rows contributed the maximum followed by second rows. The increase in grain yield was attributed to higher values of the number of ears per square meter (10-25.33% more in comparison to sole crop without N application) during both study years. The sole wheat crop without any N application recorded the least values for all yield-related parameters. Despite the absence of significant differences, the relative decrease in intercropped maize under both N treatments was over 9% compared to the sole maize crop, which was mainly ascribed to the border rows (24.65% decrease compared to the sole crop) that recorded 12 and 13% decrease in kernel number and thousand-grain weight, respectively than the sole crop. This might be attributed to the reduced photosynthesis and chlorophyll pigmentation in RSI maize crop during the blended growth period. In a nutshell, it can be concluded that wheat/maize RSI significantly improved the land use efficiency and the total yield compared to the sole crops' yield in arid areas in which yield advantages were mainly ascribed to the improvement in wheat yield.

KEYWORDS

co-growth period, crop rows, nitrogen, physiology, productivity, SPAD

1 Introduction

The world's burgeoning population (FAO et al., 2019), consequences of climate change (Challinor et al., 2014), and water shortage (Varis et al., 2017) are acknowledged to be the major challenges for global food security. Cereal grains are consumed as staple foods in many parts of the world. Therefore, their production is to be increased to meet the food needs of the increasing population (Grote et al., 2021). Furthermore, improved food security will also depend on the judicious use of available resources including land, water, and nutrients (Vågsholm et al., 2020). Rainfed agriculture is one of the major water-saving practices which occupy more than 80% of farmland area in the world and 60% in East- and South- Asia (Wani et al., 2009; David et al., 2011). According to an estimate, about 60% of world grains are produced from rainfed areas (UNESCO, 2009). However, the yield of crops in rainfed areas is affected severely under changing climatic scenarios (Bakhsh and Kamran, 2019). Several approaches, including mixed cropping practices like intercropping, have been reported as widespread land management practice to enhance resource use efficiency. These can be used for achieving higher yield and productivity under rainfed conditions (Li et al., 2020; Waha et al., 2020).

Intercropping, growing two or more crops simultaneously in the same field, has been reported as a sustainable agronomic approach that not only promotes crop growth but also boosts grain yield (Maitra et al., 2021). Many advantages have been demonstrated with intercropping including better soil quality (Roohi et al., 2022; Wolińska et al., 2022), enhanced microbial populations (Obi et al., 2022; Zhao et al., 2022), reduced pest populations (Yang et al., 2022), high nutrient acquisition efficiency (Zhu et al., 2022), improved agronomic and physiological parameters (Jo et al., 2022) and overall greater crop yield (Brahimi et al., 2022). During the last few years, this practice has gained considerable attention in irrigated agriculture (Cuartero et al., 2022); various studies have reported a greater increase in grain yield under irrigated conditions (Cuartero et al., 2022; de Sá Souza et al., 2022). However, published reports demonstrated that this practice requires a huge amount of irrigation water (Jannoura et al., 2014). On the other hand, the unavailability of fresh water is one of the major yield-limiting factors in rainfed areas (Dotaniya et al., 2022). With the exception of recent studies (Erythrina et al., 2022; Jo et al., 2022), there are no published reports on intercropping under rainfed agriculture, particularly intercropping with dual cereal crops. In addition, previous studies mainly focused on strip intercropping (Alarcón-Segura et al., 2022; Jo et al., 2022), however, limited information exists on relay-strip-intercropping (Raza et al., 2022).

Wheat and maize are among the widely cultivated cereal crops, both in irrigated and rainfed agriculture. In the Loess Plateau of China, where rainfall is a major source of water for crop cultivation, strip intercropping of these crops is a common practice (Li et al., 2020). In intercropping, the "border effect" i.e., the ability of border rows to capture more inputs than others and yield more, is a common phenomenon (Li et al., 2020; Wang et al., 2021; Zhang et al., 2021). However, there is a lack of information on the contribution of inner and border rows towards crop yield, particularly, in wheat-maize intercropping systems. Intercropping significantly changes the canopy structure, which in turn can affect the ventilation, light interception, and leaf area (Li et al., 2021). Precious studies clearly demonstrated that inner rows intercept less sunlight when compared with border rows. There is a direct relationship between sunlight inception with crop photosynthesis rates. It is also well demonstrated that leaf area greatly influences the photoassimilates production and its supply to other organs which in turn can affect the yield of crops (Raza et al., 2022). Similarly, previously published reports have stated that changing the crop geometry alters the leaf area and, in this way, indirectly influences the production of photoassimilates for better growth and yield (Raza et al., 2022).

Nitrogen is an essential element for plant growth and development. Its application directly influences plant growth, development processes, plant nutrient cycling, and photosynthetic carbon (Zhang et al., 2007). Plants' response to N application is highly dose-dependent (Liang et al., 2019). For example, in a recent study, Wang et al. (2022) demonstrated that increasing N rates decreased N fertilizer utilization by crop plants. During the last few years, most of the studies have discussed N's influence on field-grown crops under a monocropping system. However, less attention has been devoted to the influence of N fertilization on the growth, physiological traits, and productivity of crops, particularly when grown in strip-intercropping. For this work, it was hypothesized that N application would improve crop physiology, yield, and land use efficiency in the wheat-maize intercropping system under rainfed conditions. The objectives of this study were to i) assess the effect of N fertilization on the physiology and yield performance of the rainfed intercropping system, ii) and evaluate the performance of border- and inner-rows in terms of physiological traits and yield of intercrops under N fertilization.

2 Materials and methods

2.1 Site description

The experimental site was established at Northwest Agriculture & Forestry University in 2019 and 2020 to explore the influence of wheat/maize intercropping and N treatments on photosynthetic and yield traits. The site at the experimental area is a loam soil with 26% field capacity and had been in under spring maize cultivation during the last three years. The meteorology data of the site location are from the nearest meteorological station and are given in Figure 1. The regional climate had the following properties: the yearly average temperature of 14.5°C and 575 mm of annual precipitation between 1970 and 2019, of which >65% of rainfall is concentrated from July to September. Pre-trial soil at the 0-30 cm layer had the following properties: 0.92 and 0.052, 0.015 and 0.096 g kg-1 of total N, available N, phosphorus (P), and potassium (K), respectively; 11.82 ± 0.5 of organic matter and 8.14 of soil pH.

2.2 Experimental design

The experimental field was divided following the randomized complete block design in which three intercropping viz. sole wheat, sole maize, and relay-strip-intercropping of both crops and two nitrogen levels viz. without- and with-N (basal application at 150 and 235 kg ha^{-1} for wheat and maize, respectively) application were maintained with three replicates. Each treatment plot was 10.5 m in length and 9 m in width comprising one meter follow area between the experimental plots which have north-to-south orientation for crop rows. Relay-strip-intercropping plots, with three strips in total, have eight and four rows of wheat and maize with 1.6 and 1.9 m widths, respectively in each strip. The whole planting geometry is given in Figure 2. The commonly cultivated wheat and maize cultivars viz. Yongliang 4 and Xianyu 335 were used with seedling rates of 180 kg ha^{-1} and 66,670 plants ha^{-1}, respectively. A row spacing of 50, 30, and 20 cm was maintained for inter- and intra-maize and wheat crops, respectively for sole-crops and RSI treatments. Planting geometry was the same as the local practices (Ma et al., 2020). For RSI experimental unit, 30 cm distance was maintained between the adjacent crop rows. Wheat was sown on October 21 and October 13, and maize was sown on April 06 and March 30 during the first and second experimental years, respectively. The competitive growth phase between the two crops was about 2 months during both years. According to standard grower practice, phosphorus and potassium were applied at 176 and 40 kg ha^{-1} by using tricalcium phosphate {$Ca_3(PO_4)_2$} and sulphate of potash, respectively. All fertilizers, including treated N, were applied as the basal dose of both crops under both sole- and RSI treatments. During both trial years, no irrigation was supplemented for sole crops and TSI treatments.

2.3 Data collection and analysis

2.3.1 Yield and related traits

During both experimental years, at the maturity stage, plants from sole- and intercropped- rows were harvested from each experimental unit, containing eight and four rows (R) of wheat and maize, respectively, to obtain the yield and related traits. After

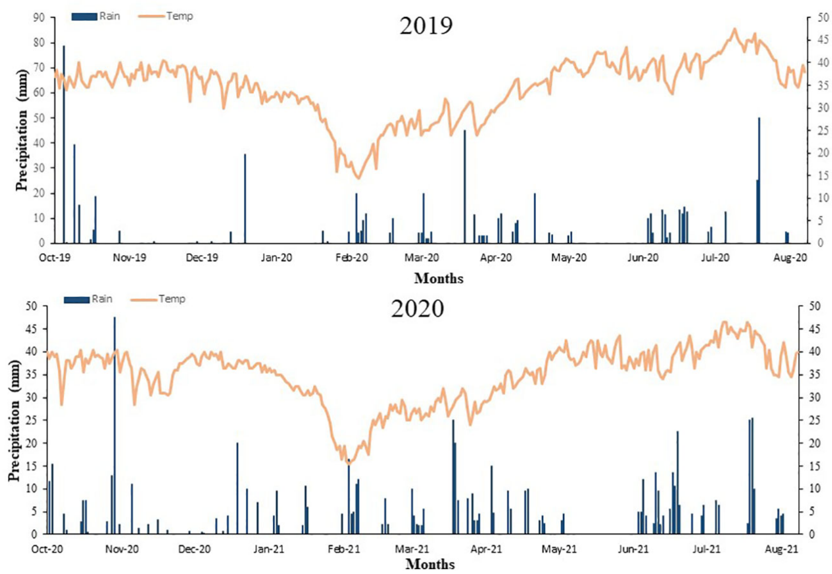

FIGURE 1
Daily weather data including the precipitation and average temperature during both experimental years.

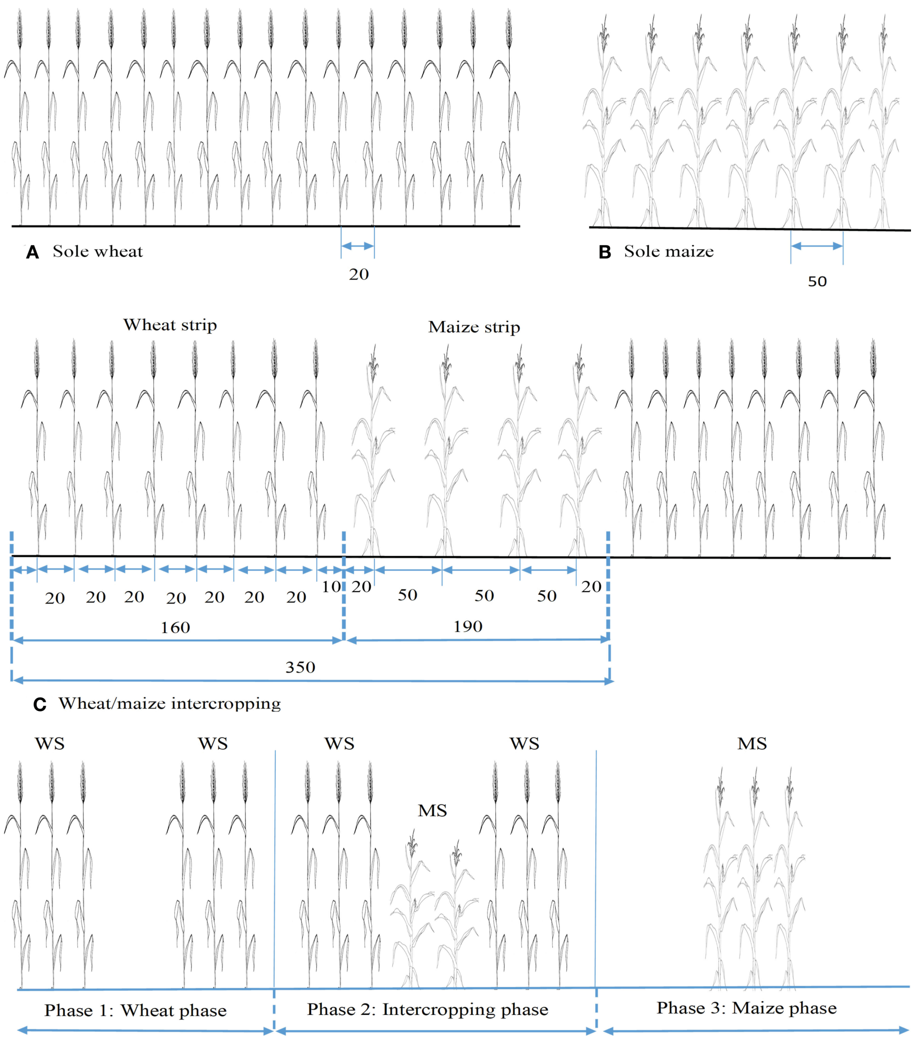

FIGURE 2
Layout of sole- **(A, B)** and strip- **(C)** intercropping of both crops.

manually threshing, the sun-dried grains of both crops were weighed. The ear number per meter (EN) in each row was counted. To calculate the kernels on each ear, approximately 20-40 ears/cobs per row were counted and then averaged. Furthermore, approximately 10-12 replications were harvested in each row to determine the thousand grains' weight. In the current work, for RSI-wheat, R1 and R8 were described as border rows while adjusting rows viz. R2 and R7 were specified as 2nd border rows. While R3 and R6, and R4 and R5 were characterized as 3rd and center crop rows. Similarly, for the maize crop, under RSI system, R1 and R4 were described as the border while the remaining crop rows R2 and R3 were identified as the center crop rows. As opposed to RSI, we ignored the border effect for sole cropping treatments. For yield calculation, plants from the middle of the experimental area were harvested to reduce the experimental error.

2.3.2 Photosynthetic attributes and SPAD values

A high efficient photosynthesis system LI-Cor, LI-6400XT was employed for Pn, Tr, and Gs measurement. For SPAD values, we used a dual-wavelength chlorophyll meter (SPAD 502, Minolta Camera Co., Ltd., Japan). For photosynthetic indices, an LED leaf chamber was used for the measurements. Furthermore, leaf water-use-efficiency was premeditated as the ratio of Pn and Tr. For that, we took the samples at about 10:00 a.m. on sunny days. For measurements, fully emerged top leaves were considered prior to the flag leaf stage and VT stage in wheat and maize, respectively. After that, flag- and ear leaves were used respectively in wheat and maize. For each experimental unit, the measurements were made by using approximately 10-12 leaves in the border- and center rows. The critical growth stages of both crops, as described in our previous study (Li et al., 2019), were considered for the measurements, including the SPAD value.

2.3.3 Soil water storage

The soil moisture meter Diviner 2000 was used for assessing the soil water contents. For that, sampling was done from each 10 cm soil profile until the soil depth of 160 cm. The measurements were made during the critical crop growth phases, a detailed procedure is described in our previous study (Li et al., 2020). The soil water storage was determined as the multiplication of water content and soil depth, as previously reported by Li et al. (2020).

2.3.4 Land equivalent ratio

We considered the partial land equivalent ratios of both crops (LRpw + LRpm), as previously described (Li et al., 2020), to estimate the total land equivalent ratio (Eq. 1). Measurements were made by considering the grain yield of both crops under the sole (Gyws and Gyms for wheat and maize, respectively) as well as strip-intercropping system (Gywi and Gymi for wheat and maize respectively), and planting ratios (Pw and Pm), using the equation as described by (Yu et al., 2015) with slight modifications.

$$\text{Land equivalent ratio} = \frac{Pw \;\; Gywi}{Gyws} + \frac{Pm \;\; Gymi}{Gyms} \qquad 1$$

2.4 Statistical analyses

Statistical Package for the Social Sciences (SPSS 20.0) software was used to perform the statistical analyses. One-way ANOVA was used for analyzing the mean difference at a 5% probability level. Correlation analyses were performed between grain yield and components for different rows, and between the Pn and yield components at various stages in both crops, and their means were differentiated by performing Duncan's multiple range tests at $p< 0.05$.

3 Results

3.1 Yield and yield-related traits

Data showed that wheat-maize intercropping system non significantly affected the ear number per meter row, kernel number per ear, and thousand kernel weight of both crops during both study years under N treatments (with- and without N application), except for ear number per meter row for wheat in 2019 and 2020 (Table 1). Intercropping and N application resulted in 25.33 and 10.60% more number of wheat ears per meter row during 2019 and 2020, respectively in comparison to sole crop without N application. The sole wheat crop without any N application recorded the least values for all yield related parameters (Table 1).

Wheat-maize intercropping system, N application and years significantly (P<0.001) affected the individual and total yield of both crops (Table 2). However, their interactive effect regarding yield was non-significant (Table 2). Results showed that intercropping with N application (+N treatment) resulted in the highest wheat grain yield with 70.37 and 52.78% increase as compared with monoculture and without N application in 2019 and 2020, respectively. However, sole plantation of maize and N application resulted in more yield. It was followed by intercropping and N application (Table 2). Likewise, intercropping with N application resulted in 33.60 and 29.80% more maize yield in 2019 and 2020, respectively in comparison to sole maize grown under N absence. Leaf equivalent ratios recorded for intercropping and N were significantly more during both years (Table 2).

TABLE 1 Effect of wheat-maize intercropping system on ear number per meter row (EN), kernel number per ear (KN), and Thousand kernel weight (TKW) during both study years under with- (+N) and with-out nitrogen (-N) application.

Year	Cropping system	N levels	EN (g)	KN (g)	TKW (g)
2019	Sole wheat	-N	400 c	30.96 a	32.4 a
		+N	425.16 bc	36.9 a	38.53 a
	Intercropped wheat	-N	485 ab	35.09 a	34.30 a
		+N	533.34 a	40.70 a	39.46 a
2020	Sole wheat	-N	411.67 d	34.66 a	31.03 b
		+N	455.33 c	40.16 a	36.33 ab
	Intercropped wheat	-N	505.66 b	32.8 a	33.94 ab
		+N	543.33 a	38.16 a	37.70 a
2019	Sole maize	-N	6.20 a	487 a	266.64 a
		+N	6.9 a	507.33 a	288.84 a
	Intercropped maize	-N	6.11 a	465 a	257.67 a
		+N	6.86 a	485 a	283.16 a
2020	Sole maize	-N	6.23 b	462 ab	246.34 a
		+N	6.70 a	487.67 a	268.5 a
	Intercropped maize	-N	6.24 ab	439.34 b	237.84 a
		+N	6.68 ab	457 ab	261.6 a

The means with the different lowercase letters are significantly different based on three-way anova analysis.

TABLE 2 Effect of wheat-maize intercropping system on wheat and maize yields (t ha^{-1}) and land equivalent ratios (LER) of averaged yield with- (+N) and with-out nitrogen (-N) application.

Year	Cropping system	N levels	Wheat	Maize	Total yield (g)	LER
2019	Monocropping	-N	4.86 d	8.11b	6.49	–
		+N	5.95 c	9.48a	7.71	–
	Intercropping	-N	7.17 b	7.82b	7.50	1.14
		+N	8.28 a	9.06a	8.67	1.17
2020	Monocropping	-N	5.21 d	6.86 c	6.04	–
		+N	6.02 c	8.29 a	7.16	–
	Intercropping	-N	7.20 b	6.34 d	6.77	1.12
		+N	7.96 a	7.71 b	7.84	1.15
Year			NS	***		
Cropping system			***	***		
Nitrogen			***	***		
Cropping system * Nitrogen			NS	NS		
Year * Cropping system * Nitrogen			NS	NS		

Weighted means of both crops in both systems i.e., intercropping, and sole cropping system, were expressed as the total yield. The yield of wheat and maize crops under intercropping treatment was the equivalence values of covered land area of each crop. The means with the different lowercase letters are significantly different based on three-way anova analysis. * Indicates p<0.05; ***, p<0.001; NS indicates non-significant.

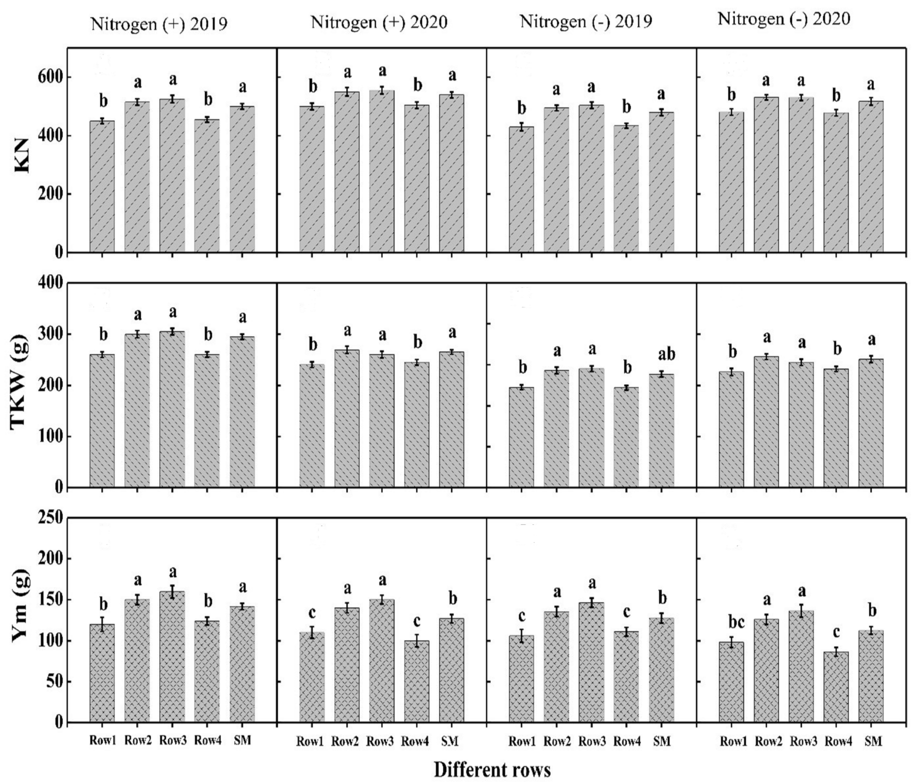

FIGURE 3
The kernel number per ear (KN), thousand kernel weight (TKW) and per meter grain yield (Ym, g) of different maize rows under with- and without N application. Different letters on the top of each bar indicate significant differences among the crop rows, as calculated by Tukey's HSD test at $P \leq 0.05$.

Data regarding yield and related traits in maize showed significant difference among crop rows during both years and N treatments. Higher kernel number, thousand seed weight, and grain yield were recorded for Row-2 followed by Row-3 and the values were statistically at par (P<0.05) with sole maize rows during both years and N addition (Figure 3). The minimum values were depicted for Row-1 followed by Row-4 under both years and N treatments. For wheat, various rows performed differently with regard to ear number, kernel weight, thousand seed weight, and grain yield during both years and N treatments. Among crop rows, Row-1 depicted higher values of these traits, however, showed a statically similar response to Row-8 during both years and under both N rates (Figure 4). Further, Row-2 (R2) and Row-7 (R7) recorded lower values than border rows, however, significantly higher than those of Row-3 (R3), Row-4 (R4), Row-5 (R5), Row-6 (R6) as well as sole wheat rows which showed non-significant difference among each other and recorded lower values than other rows (Figure 4).

3.2 Leaf area index

Leaf area index varied significantly among wheat and maize rows at various growth stages with and without N application. In maize, the leaf area index increased linearly with the passage of time till the VT stage. It started declining thereafter (Figure 5). At V3 and V6 stages, maximum values were recorded for sole maize and center rows in 2019 and 2020, respectively. However, an opposite trend was recorded at lateral stages at which maximum values were recorded for central rows followed by border rows while minimum was recorded for sole maize rows. For wheat crop, there was non-significant difference for leaf area index among the crop rows under both N treatments and during both study years, except for jointing stage during both years and heading stage in 2020. At jointing stage, border rows promoted leaf area index during both years (Figure 6). At heading stage, border rows and center rows recorded the highest values in 2019 and 2020, respectively. Results revealed that N application significantly promoted leaf area index in maize and wheat in comparison to no N application.

3.3 Photosynthesis, stomatal conductance, transpiration, and water use efficiency

There was a significant difference among crop rows, growth stages and N application in maize and wheat for net photosynthesis (Pn), transpiration rates (Tr), stomatal conductance (Gs) and leaf water use efficiency of maize during both years. Among growth

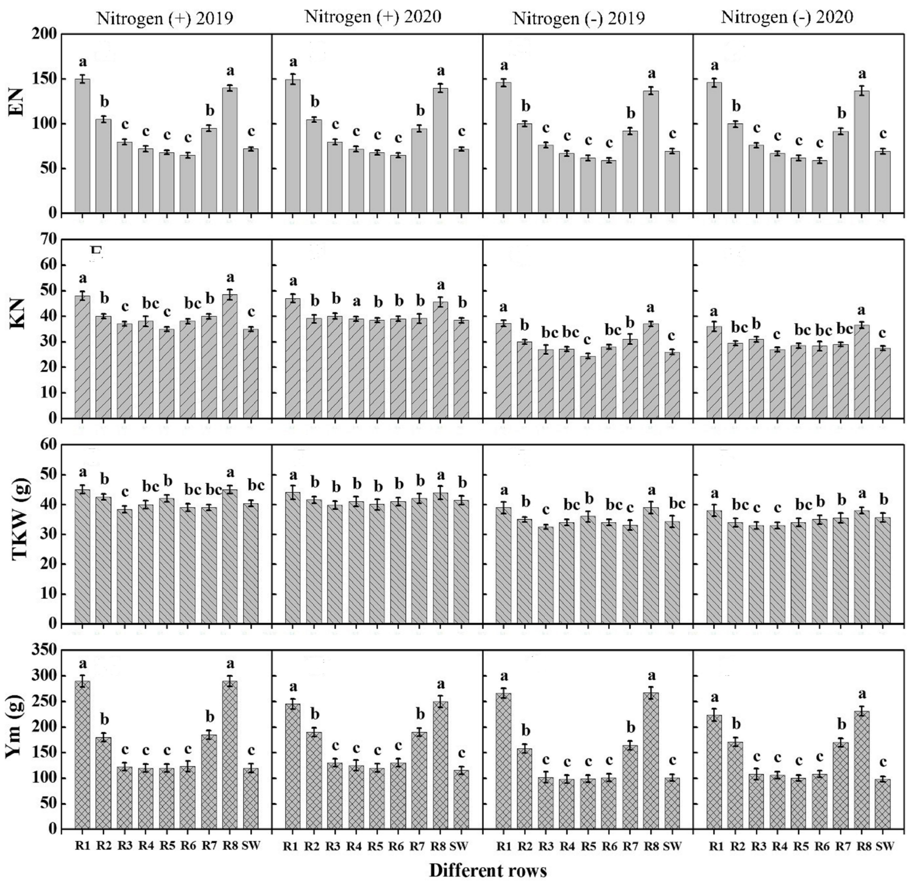

FIGURE 4
The kernel number per ear (KN), ear number per meter row (EN), thousand kernel weight (TKW, g) and per meter grain yield of different wheat rows under with- and without N application. Different letters on the top of each bar indicate significant differences among the crop rows, as calculated by Tukey's HSD test at P≤ 0.05. R, row; SW, sole wheat.

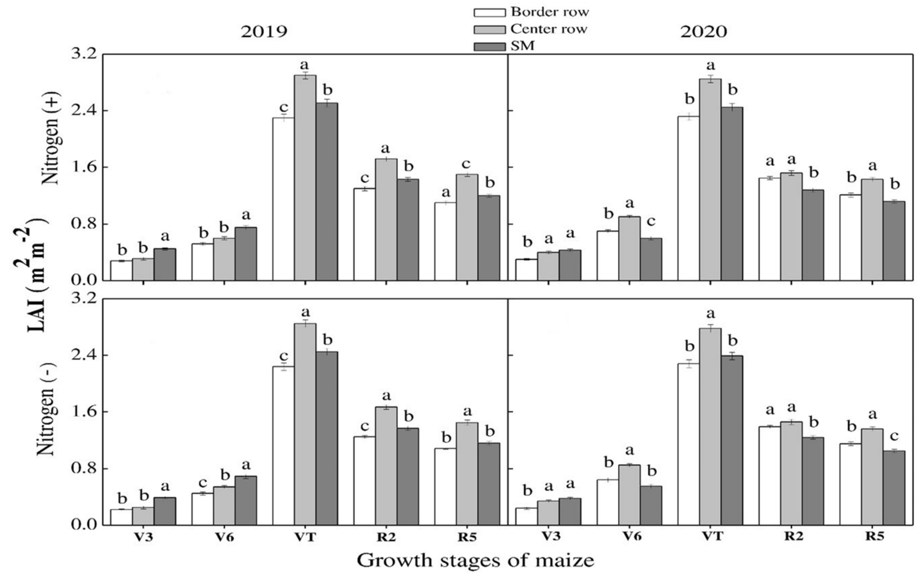

FIGURE 5
Leaf area index (LAI) of the border-, center- and sole maize (SM) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at P≤ 0.05.

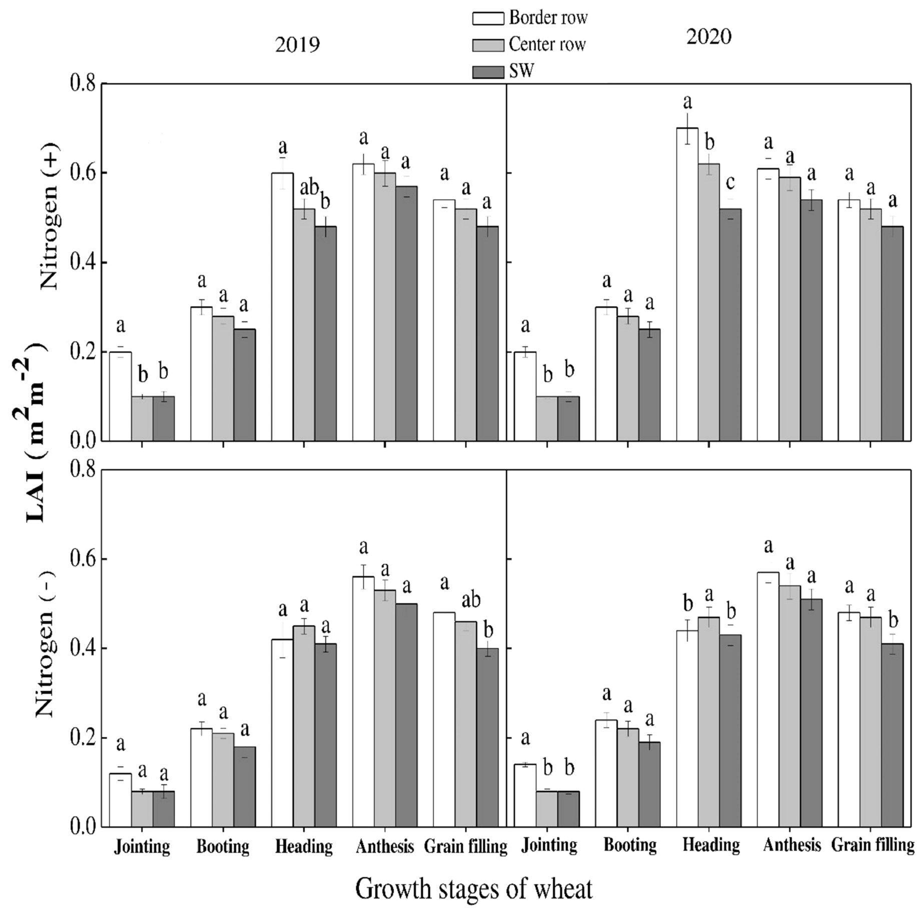

FIGURE 6
Leaf area index (LAI) of the border-, center- and sole wheat (SW) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at P≤ 0.05.

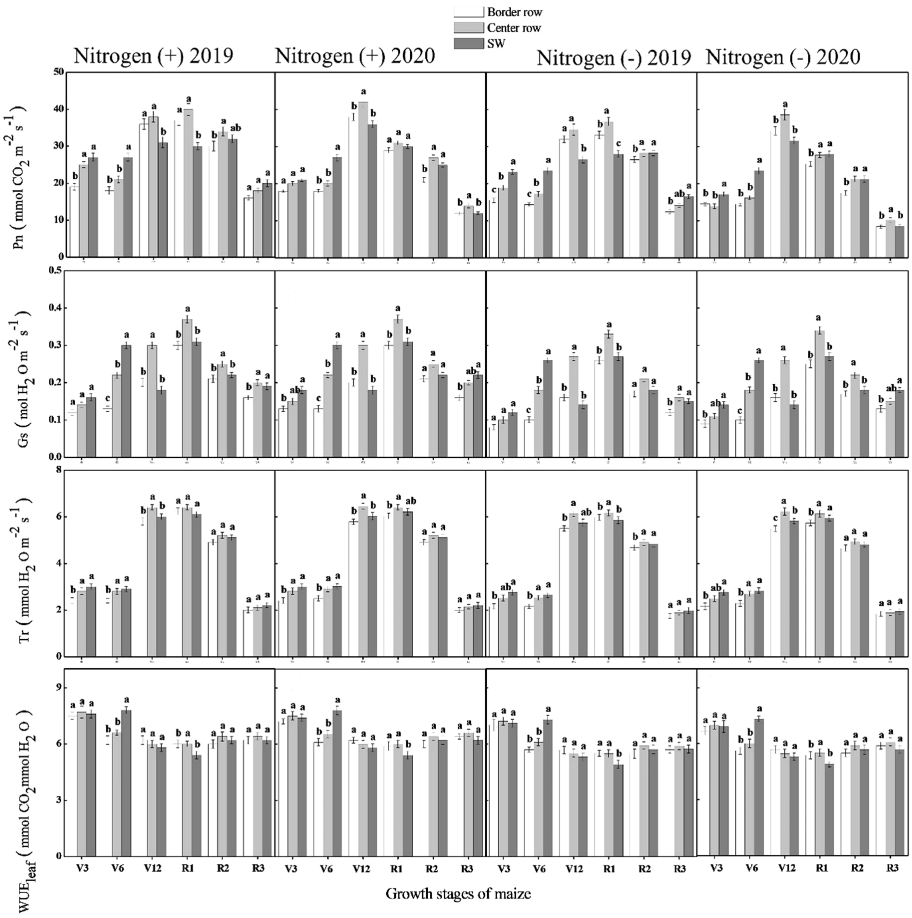

FIGURE 7
Photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and leaf water use efficiency (WUEleaf) in the border-, center- and sole maize (SM) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at $P \leq 0.05$.

stages, maximum Pn, Gs, TR and WUE were recorded for V12, R1, V12-R1, and V3-V6 stages, respectively during both years. For crop rows, at V3 and V6 stages, maximum Pn, Gs, Tr and WUE were recorded for sole maize rows during both years. Whereas, at lateral growth stages, center maize rows depicted significantly higher values of these traits during both years except for WUE for which a non-significant difference was depicted for crop rows (Figure 7). For wheat crop, maximum values of these traits i.e., Pn, Gs, Tr and WUE were recorded at anthesis stage during both years. Furthermore, border rows depicted higher values of these traits at all growth stages during both years. Among N treatments, data showed that significantly higher values of the aforementioned traits were recorded for N application as compared with N absence treatment in both crops during both years of experiment (Figure 8).

3.4 SPAD values

SPAD values in maize varied significantly with varying N rates, crop growth stages and rows during both study years (Figure 9). For growth stages, SPAD values kept on increasing until V12 stage. These were significantly more at this stage and started decreasing thereafter. Minimum SPAD values were recorded at R5 stage. Different maize rows showed significant variation at various stages. At V3 and V6 stages, maximum values were recorded for sole maize rows. However, at V12 and VR stages, crop center rows recorded maximum values under both N treatments during both years except for N absence condition in 2019 in which maximum values were recorded for sole maize rows. At lateral growth stages, there was a non-significant difference among crop rows during both years. Similarly, there was a significant difference for SPAD values among crop rows and growth stages of wheat during both years. The SPAD values decreased with successive growth stages until the grain filling stage at which minimum values were recorded. For crop rows, data showed that maximum values were recorded for border rows at all growth stages during both years followed by center rows and sole wheat crop. Among N treatments, significantly higher values were recorded for N application when compared with no N application in both crops during both years (Figure 10).

3.5 Soil moisture storage

Soil moisture storage varied significantly among crop stages and N rates in maize during both years. Among growth stages,

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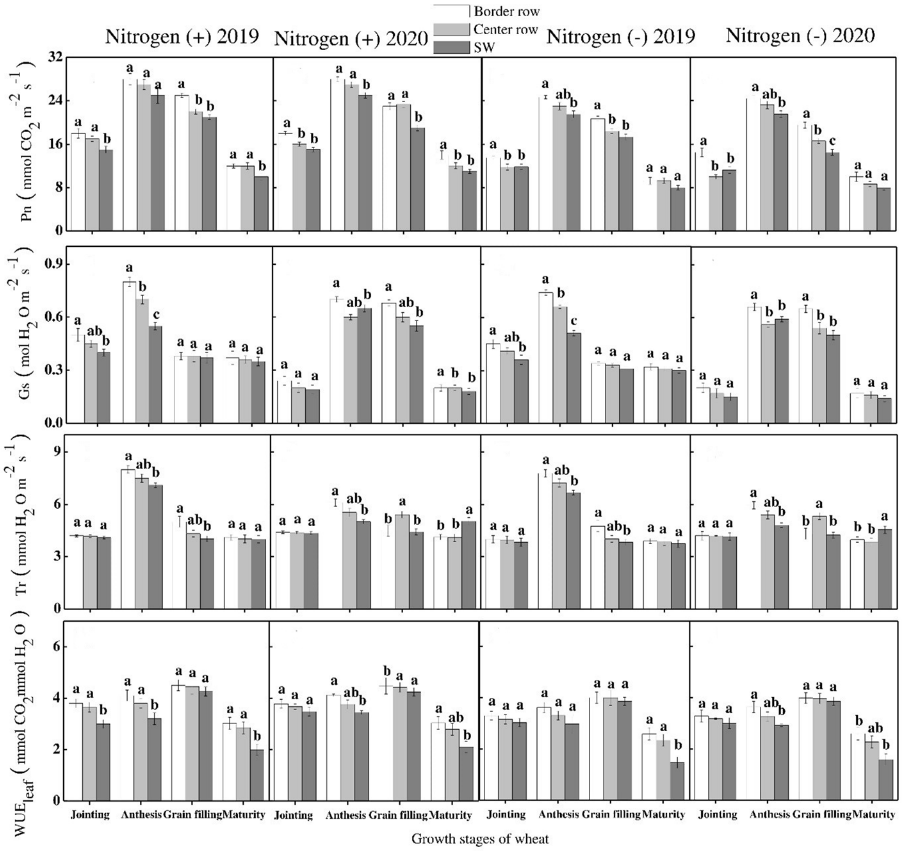

FIGURE 8
Photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and leaf water use efficiency (WUEleaf) in the border-, center- and sole wheat (SW) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at $P \leq 0.05$.

maximum values were recorded at V3 stage during both years. There was a non-significant difference among various rows at different growth stages except for VT stage. At VT stage, maximum values were recorded for border rows followed by center crop rows. Similarly, minimum values were recorded for sole maize crop (Figure 11). For wheat crop, there was a non-significant influence of crop rows on soil moisture storage at various stages with- and without- N application during both study years. Although the results were non-significant, the rows between the strips of both crops recorded higher values than sole and intercropped wheat rows. Among N treatments, N application significantly promoted the soil water storage during both years (Figure 12).

4 Discussion

Intercropping with balanced N application is essential for increasing crop productivity on sustainable basis, through efficient use of inputs (Chen et al., 2017). In this work, intercropping significantly improved the yield of wheat with an opposite trend to maize in which a significant decrease in yield was depicted. Furthermore, the overall grain yield of intercropped rows was greater than the monocropped rows. Previous studies have clearly demonstrated that planting geometry influences light interception and thus affects the crop productivity (Wen et al., 2019; Chapepa et al., 2020). However, further studies are needed to find out the contribution of rows of different crops to grain yield under intercropping system. In this work, under rainfed conditions and during both experimental years, the land equivalent ratio was greater than 1. This established that intercropping increased crops yield besides increasing the cropping intensity. These results are in line with previous published reports in which authors have demonstrated that intercropping system significantly promoted the crop productivity and land use efficiency under irrigated conditions (Brahimi et al., 2022; Wei et al., 2022). Our results thus clearly depict that strip intercropping promoted the overall crop yield under rainfed conditions and semi-arid climate with an average annual rainfall of about 600 mm. When compared with sole wheat crop, the higher yields of intercropped rows were mainly associated with higher numbers of ears per plant and thousand kernel weight. Higher yield related traits were mainly associated with the availability of sufficient resources for intercropped wheat before sowing of corn crop. Also, higher values might be associated

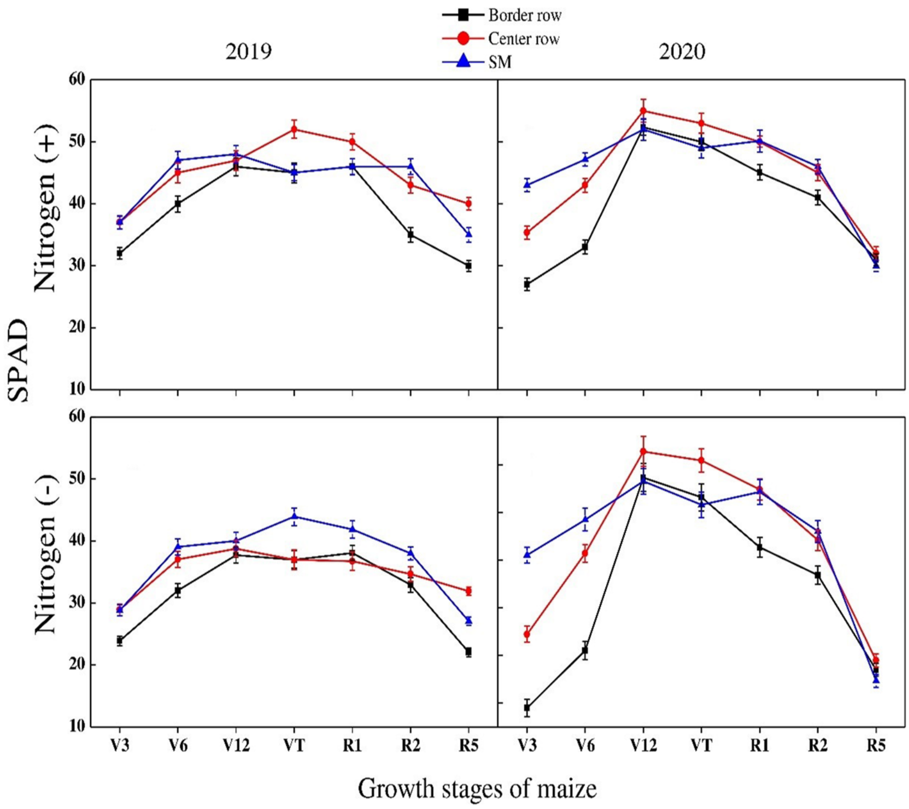

FIGURE 9
SPAD values of the border-, center- and sole maize (SM) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at $P \leq 0.05$.

with higher competitive ability of wheat crop for available resources i.e., moisture and nutrients under intercropping system. Similarly, lower maize yield under intercropping treatments was associated with lower yield-related parameters i.e., kernel number and thousand grain weight under the same treatment than that of sole maize. In line with these results, Li et al. (2001b) demonstrated that maize crop gained significantly higher growth and productivity after harvesting of wheat crop which may be associated with availability of more soil moisture and other resources. Similar results were reported in a recent study of Wang et al. (2015) in which authors reported improved yield in intercropped-maize rows than sole crop rows due to the availability of sufficient soil moisture. More recently, working with the same cropping system, a study of our group demonstrated that under intercropping system, wheat crop showed more horizontal root growth than corn crop, and resulted in less water consumption (Ma et al., 2018). Under arid regions, such type of root growth results in reduced ability of corn strips to attain full growth and reduced grain yield of intercropped strips (Ma et al., 2018). The same has been reported in current study. The results of this work, therefore, established that water scarcity is a vital growth restricting factor for maize crop, particularly under intercropping system. In line with these, working with intercropped maize, Gou et al. (2016) established that low temperature with fewer sunny days limited the growth and developmental processes of the crop. Nitrogen fertilization has been considered a dominant tool for increasing crop productivity (Hirel et al., 2001). And it is well known that higher N rates generally promote grain yield (Fan et al., 2011). Nitrogen is among the essential chlorophyll molecules which help in improving the leaf's enzymatic activity to promote the photosynthetic process (Nasar et al., 2022). A number of previous studies have demonstrated the relationship among N application rates, photosynthetic activity, and grain yield (Sharma et al., 2019; Minhas et al., 2020). Most studies clearly mentioned that N fertilization promoted grain formation mainly by increasing the photosynthetic rates and better assimilates production (reviewed by Fernandes et al., 2022). A similar was reported in this study where N addition promoted the grain yield and related traits in both crops under sole- and combined cultivation. Furthermore, in line with previous studies, higher yield under N application was highly associated with better photosynthesis rates and chlorophyll pigment formation.

Planting geometry in terms of row arrangements influenced the yield and related parameters. For example, Li et al. (2001a) recorded significantly higher grain and biomass yields for border and next to border rows in wheat. Similar was recorded in this study where border and next to border rows recorded higher yield than others. Lower yield in other crop rows might be attributed to more shading effect and less light interception as a result of heighted plants around those rows. Higher yield in border and next-to-border rows might be due to the excessive water and nutrient uptake

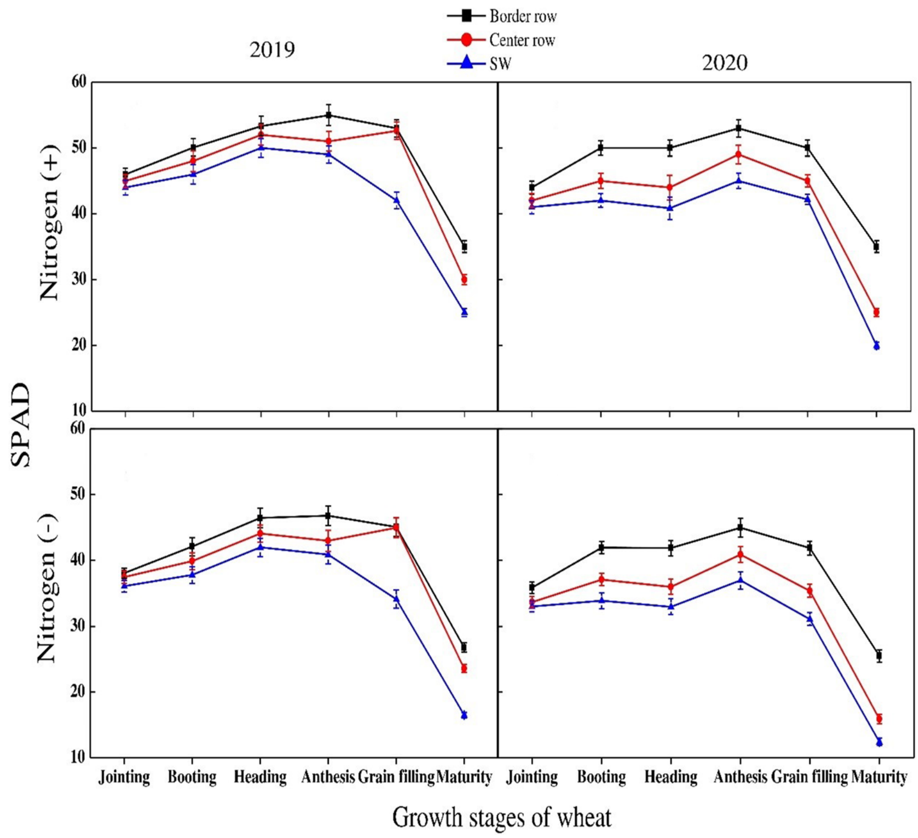

FIGURE 10
SPAD values of the border-, center- and sole wheat (SW) crop at various growth stages under with- and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages, as calculated by Tukey's HSD test at $P \leq 0.05$.

from the contiguous maize strip, as reported in a recent study of (Ma et al., 2018). Improved soil conditions and crop growth rates facilitate grain yield of border rows. Furthermore, our findings revealed that except the border rows and sole wheat strips, there was a non-significant difference for grain yield between the intercropped rows. This showed that increased number of rows and extended distance among crop rows may reduce the endowment of border and next-to-border row. In comparison with sole wheat strip, higher grain yield of border rows was attributed to higher values of kernel number, number of tillers and thousand grain weight. Furthermore, the rows between wheat and maize strips depicted higher values of soil water storage than sole crop strips, which may facilitate the formation of tillers. High values of number of ears in the border rows, due to availability of more moisture, were also reported by Zhu et al. (2016). In comparison with sole crop strips, higher photosynthesis rates in border rows at reproductive stage in wheat also contribute to an increase in number of grains and their weight. The results of this study are in line with Raza et al. (2022) who reported that more light availability at crop's reproductive stages promoted the assimilates formation which in turn promoted the formation of grains. Similarly, in a recent study, Zhang et al. (2018) noted that more light interception in crop border rows increased the number and weight of grains, which ultimately promoted the overall grain yield. On the other hand, Gou et al. (2016) reported a significant decrease in wheat grain weight when grown under intercropping system. They reported this decrease was due to reduced grain filling percentage in intercropped wheat rows. It is well established from the previously published reports that photosynthesis is the foundation for biomass accumulation as well as grain formation (Panfilova et al., 2019; Zhao et al., 2021). In this work, border rows of intercropped depicted significantly higher photosynthesis rates when compared with sole crop rows, which was mainly attributed to higher nutrient uptake and light reception, as reported in previous study of Wang et al. (2017). Furthermore, current results demonstrated that border rows depicted higher values of stomatal conductance and chlorophyll content, same as reported in previous studies of Gaju et al. (2016) where authors have reported higher values of chlorophyll content associated with improved moisture uptake and light interception of border rows.

Contrary to the wheat crop, our results demonstrated lower values of yield and its components of maize border rows when compared with sole maize. However, statistically similar values for grain yield were recorded between the center and border maize rows. These findings indicated that greater number of maize rows reduced the contribution of marginal rows and ultimately reduced the maize yield reduction under intercropping system. A previous study of our group, working with the same cropping system, clearly demonstrated that during co-growth period, depleted soil moisture

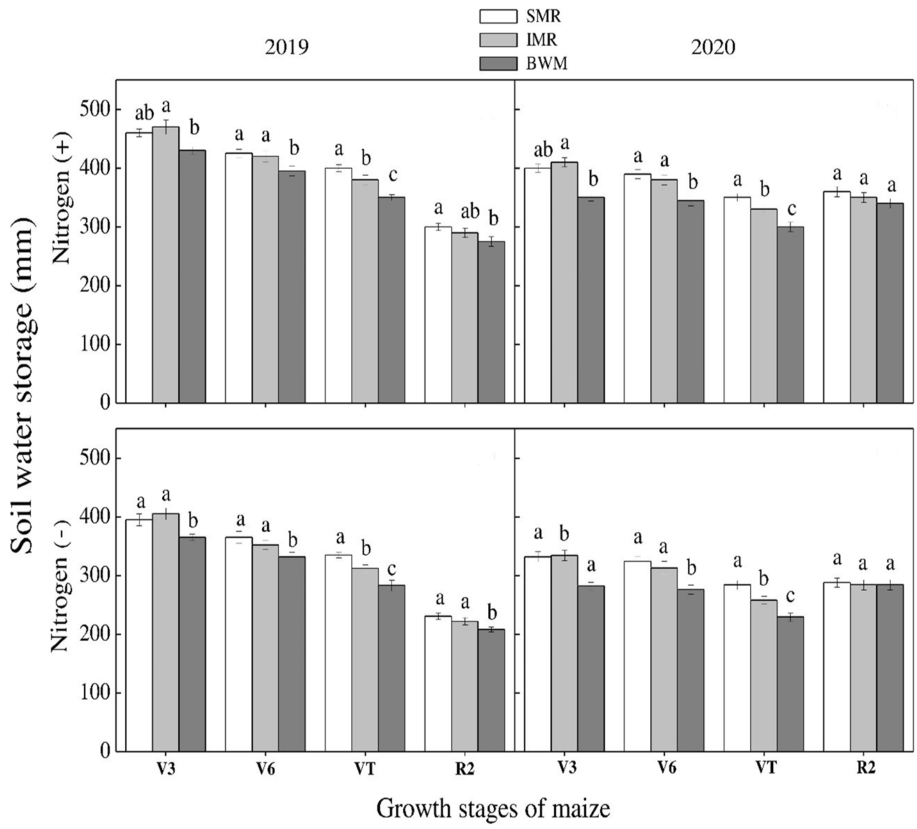

FIGURE 11
Soil water storage under sole maize row (SMR), intercropped maize row (IMR) and the rows between the intercropped strips of both crops at various growth stages under with and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages of maize, as calculated by Tukey's HSD test at P ≤ 0.05.

levels in intercropped wheat crop further created water scarce conditions for marginal corn rows (Ma et al., 2018). Similar results were reported in this work. In addition, during blended growth period, photosynthesis rates in external maize rows were lower than those of sole maize crop which may be the leading cause of decreased number of grains and their weight. In line with these, similar was reported in a previous study of Andrade et al. (1999) where authors have reported reduced number of grains per cob in marginal crop rows associated with less availability of soil moisture and reduced photosynthetic rates. Similarly, during the blended growth period, increased height of wheat crop may have caused the shading effect for the companion crop. A significant reduction in chlorophyll content of maize crop due to shading has been well reported in previous study (Naseer et al., 2022). Similarly, Pang et al. (2018) demonstrated that shading reduced CO_2 absorption and in this way reduced the production of photosynthetic pigments. At the same time, photosynthetic rates in the external crop rows of intercropping were reduced than the sole crop strips. On the other hand, a significant recovery in photosynthetic rates has been observed after harvesting of wheat crop which might be associated with the absence of shading and improved light interception after harvesting of companion crop. Thus, from the above results, it is concluded that reduced assimilates production particularly during the blended-growth period result in lower thousand grain weight and ultimately grain yield in marginal crop rows.

Our results established that intercropping treatments and N application significantly increased the water use efficiency of both crops. A positive relation between the photosynthetic rates and leaf water use efficiency has been reported in a number of previous studies (Lawson and Vialet-Chabrand, 2019; Eyland et al., 2021). According to Ma et al. (2018), crop border rows potentially contributed to increase the leaf water use efficiency associated with high light interception. Sole wheat crop and intercropped maize recorded statistically similar values during the experimental period and border rows recorded lower water use efficiency than center crop rows. Furthermore, after wheat harvest, companion corn rows depicted higher water use efficiency. Thus, during this recovery phase, moisture conservation practices should be adapted to increase the yield of companion crop, particularly under semi-arid conditions without irrigation supplements. Freshwater shortage has emerged a worldwide problem for agricultural crops (Garrido-Cardenas et al., 2020; Yusuf et al., 2020). Intercropping has been reported as a sustainable approach for enhancing crop performance under water scares conditions (Bitew and Abera, 2019). In current work, our results demonstrated that intercropping treatments improved crop yield and overall productivity under rainfed conditions. Similarly, some other

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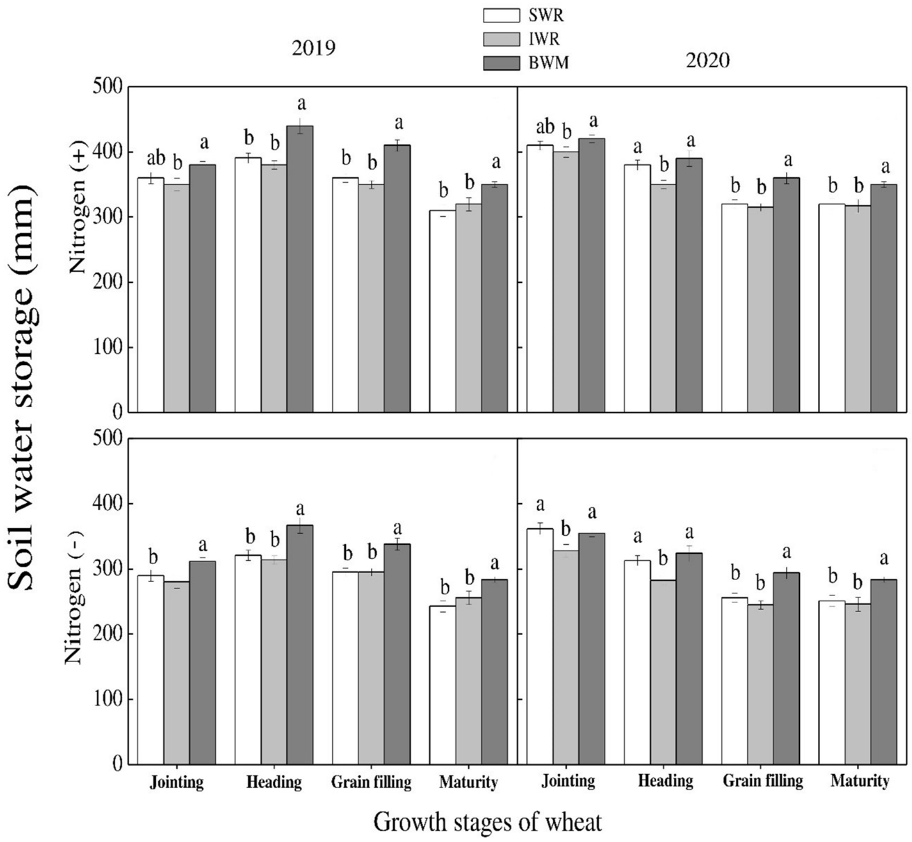

FIGURE 12
Soil water storage under sole wheat row (SWR), intercropped wheat row (IWR), and the rows between the intercropped strips of both crops (BWM) at various growth stages under with and without N application. Different letters on the top of each bar indicate significant differences among the various crop growth stages of wheat, as calculated by Tukey's HSD test at $P \leq 0.05$.

studies also reported that intercropping promoted crop/s yield and land use efficiency more than sole cropping system (Layek et al., 2018; Nasar et al., 2020). Higher yield in intercropping was attributed to improved moisture and nutrient uptake from the soil, and above-ground plant performance (Nwokoro et al., 2022; Zhao et al., 2022). In this work, there was a positive correlation for number of ears and grain yield. Therefore, the planting density can be appropriately increased to better harvest the advantage of the border rows. In conclusion, our results demonstrated that intercropping promoted the yield of wheat, however, there was a decline in intercropped maize which draw our attention to adapt suitable approaches such as cultivation of shade-tolerant cultivars, for increasing the productivity of intercropped maize. The use of organic nutrient sources including straw or biochar also helps in improving the water retaining capacity of the soil (Guo et al., 2022). These practices may contribute towards better growth of maize during recovery phase (Li et al., 2020).

5 Conclusions

Wheat-maize intercropping and N application promptly improved the LUE and overall grain yield in arid regions without irrigation supplements. This increase in grain yield was due to the significant improvement in wheat yield where border rows contributed maximum followed by second rows which were further attributed to the higher values for yield-related traits during both study years. Regardless of the absence of significant differences, intercropped maize, under with and without N application, recorded somewhat decrease in grain yield during both years which was mainly due to the border and second rows in which lower values were attributed to the reduced photosynthesis and chlorophyll pigmentation during the blended growth period. This study identified the greatest possibility for wheat-maize strip intercropping production improvement in rainfed areas in China and provided important information for optimizing the geometry of maize-wheat intercropping, improving regional productivity, and ensuring food security.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

SH, XC and XR planned and designed the research experiment. SH performed the experiments, collected the data and analyzed it. MN, RG, FH, SA and BA monitored the experiments and gave final shape to the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Lingyang Feng,
Institute of Advanced Agricultural Sciences,
Peking University, China

REVIEWED BY
Giuseppe Di Miceli,
University of Palermo, Italy
Xianjiang Chen,
Queen's University Belfast, United Kingdom
Jialin Yu,
Peking University, China

*CORRESPONDENCE
Yanhong Yan
yanyh@sicau.edu.cn

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Mixed legume–grass seeding and nitrogen fertilizer input enhance forage yield and nutritional quality by improving the soil enzyme activities in Sichuan, China

Muhammad Tahir[1,2,3], Xiao Wei[1], Haiping Liu[1], Jiayi Li[1], Jiqiong Zhou[1], Bo Kang[4], Dongmei Jiang[4] and Yanhong Yan[1]*

[1]College of Grassland Science and Technology, Sichuan Agricultural University, Chengdu, China, [2]State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, [3]School of Life Sciences, University of Chinese Academy of Sciences, Beijing, China, [4]Animal Science and Technology College, Sichuan Agricultural University, Chengdu, China

Information regarding relationships between forage yield and soil enzymes of legume–grass mixtures under nitrogen (N) fertilization can guide the decision-making during sustainable forage production. The objective was to evaluate the responses of forage yield, nutritional quality, soil nutrients, and soil enzyme activities of different cropping systems under various N inputs. Alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), orchardgrass (*Dactylis glomerata* L.), and tall fescue (*Festuca arundinacea* Schreb.) were grown in monocultures and mixtures (A1: alfalfa, orchardgrass, and tall fescue; A2: alfalfa, white clover, orchardgrass, and tall fescue) under three N inputs (N1: 150 kg ha^{-1}; N2, 300 kg ha^{-1}; and N3: 450 kg ha^{-1}) in a split plot arrangement. The results highlight that A1 mixture under N2 input had a greater forage yield of 13.88 t ha^{-1} year^{-1} than the other N inputs, whereas A2 mixture under N3 input had a greater forage of 14.39 t ha^{-1} year^{-1} than N1 input, but it was not substantially greater than N2 input (13.80 t ha^{-1} year^{-1}). The crude protein (CP) content of grass monocultures and mixtures significantly ($P < 0.05$) increased with an increase in the rate of N input, and A1 and A2 mixtures under N3 input had a greater CP content of 18.91% and 18.94% dry matter, respectively, than those of grass monocultures under various N inputs. The A1 mixture under N2 and N3 inputs had a substantially greater ($P < 0.05$) ammonium N content of 16.01 and 16.75 mg kg^{-1}, respectively, whereas A2 mixture under N3 had a greater nitrate N content of 4.20 mg kg^{-1} than the other cropping systems under various N inputs. The A1 and A2 mixtures under N2 input had a substantial higher ($P < 0.05$) urease enzyme activity of 0.39 and 0.39 mg g^{-1} 24 h^{-1} and hydroxylamine oxidoreductase enzyme activity of 0.45 and 0.46 mg g^{-1} 5 h^{-1}, respectively, than the other cropping systems under various N inputs. Taken together, growing legume–grass mixtures under N2 input is cost-effective, sustainable, and eco-friendly, which provide greater forage yield and improved nutritional quality by the better utilization of resources.

KEYWORDS

mixed seeding, nitrogen fertilizer, forage yield, nutritional quality, soil nutrients, soil enzymes

1 Introduction

The growing world population has increased the demand for animal products, and the relationship is likely to get even closer in the future. With the instant development of animal husbandry, the demand for forages has emerged frequently (Hisham et al., 2022). A consistent forage supply is critical for grass-based livestock husbandry and food security. Forage grass cultivation, a pivotal ring of the feed production industry, has obvious seasonal and regional characteristics all year around (Garcez Neto et al., 2021). The natural grasslands are the primary source of forage, but cultivating legume–grass mixtures other than their respective monocultures is more beneficial because they provide higher biomass production and balanced feed for livestock (Liu et al., 2022; Tahir et al., 2022). Meanwhile, these legume–grass mixtures could face significant challenges due to lack of soil fertility, fierce competition, and scarcity of suitable species (Liu et al., 2022).

Forage production has been tightly bound up with the environment, as it is a basic industry that requires resources including land, energy, water, and labor. The cultivation of legume–grass mixtures emerged as a practical approach for increasing the forage biomass production and nutritional quality and sustaining the soil nutrient balance with minimal environmental impact (Peeters et al., 2006). Additionally, simple mixtures of two to four species may offer the best means to provide plant diversity and limit seedling competition compared with complex ones (Foster et al., 2014). Moreover, nitrogen (N) fertilization is an essential practice for the maintenance of mixture productivity, considering that a deficiency of this nutrient is a primary factor in triggering forage degradation. Generally, N inputs increase the abundance of beneficial microbes in soil that trigger the activities of microbial enzymes for nutrient mineralization (Fan et al., 2011; Sun et al., 2015). Contrarily, the negative impacts of N inputs on the soil health have also been reported by reducing the organic matter and microbial population (Marschner et al., 2003). Therefore, much attention is needed while applying N fertilizer to soils for greater forage production as N could be lost to the environment which, in turn, will become a bottleneck problem for sustainable agriculture.

The Sichuan province of China is regarded as one of the leading producers of livestock husbandry, but forage deficit, both as quantitative and qualitative, is the main constraint on the advancement of livestock husbandry in this region (Yang et al., 2023). This shortage is usually caused by the lack of soil fertility and environmental stresses that could adversely affect crop growth and development. Therefore, farmers rely heavily on N inputs to obtain a greater forage yield to counter the increased demand of forage for livestock. It is estimated that only 47% of the N added globally to soils is converted to and harvested in product form, whereas more than 50% of N is lost to the environment, which leads to waste of forage resources, threats to biodiversity and bodies of water, and increased emissions of polluting gases (Gurgel et al., 2020). Given these facts, it is of paramount importance that the current livestock systems adopt measures that utilize this nutrient with maximum efficiency. Moreover, the optimal N input rates for growing legume–grass mixtures in Sichuan, China, are not well established. Apparently, there is an urgent need to explore the combined effects of N fertilizer inputs and mixed planting on forage production, which can not only fulfill forage needs but also, more importantly, mitigate the adverse impact on the environment.

Consequently, the current study aimed to investigate the responses of forage yield and quality, soil nutrients, and soil enzyme activities to different planting patterns and N inputs. The results of this study may provide guidance for forage production in Sichuan, China, by mixed planting and optimal N inputs that could mitigate the negative environmental impacts leading towards sustainable agricultural systems.

2 Materials and methods

2.1 Research site and plant materials

A field experiment was conducted on September 15, 2018 at Modern Agriculture Research and Development Base of Sichuan Agricultural University, Chongzhou, China (103°07′ E, 30°30′ N). The legumes alfalfa [*Medicago sativa* L. (cv. Xibuzhixing)] and white clover [*Trifolium repens* L. (cv. Ladino)] and grasses orchardgrass [*Dactylis glomerata* L. (cv. Amba)] and tall fescue [*Festuca arundinacea* Schreb. (cv. Meishijia)] were selected as forage materials.

2.2 Soil characteristics and weather description

The soil in the upper 20 cm of the experimental field is purple clay loam with uniform fertility and has the following properties:

pH, 6.30; organic matter, 37.6 g kg^{-1}; alkali hydrolyzed nitrogen, 135.70 mg kg^{-1}; total nitrogen, 1.81 g kg^{-1}; available phosphorous, 10.20 mg kg^{-1}; and available potassium, 101.10 mg kg^{-1}. The climate of the experimental site is subtropical monsoon humid with an annual average temperature of 15.9°C, rainfall of 1,012.4 mm, and sunlight of 1,161.5 h.

2.3 Experiment design and treatment information

The field experiment was carried out in a split plot arrangement with three biological replications. A total of two legume–grass mixtures (A1: alfalfa, tall fescue, orchardgrass; A2: alfalfa, red clover, tall fescue, orchardgrass) and four monocultures (alfalfa, red clover, tall fescue, and orchardgrass) were planted in a net plot size of 5 m × 3 m under three pure N inputs (N1: 150 kg ha^{-1}, N2, 300 kg ha^{-1}, and N3: 450 kg ha^{-1}). The main plots included three N levels, while the subplots included two legume–grass mixtures and four monocultures. The first N dose was applied at the emergence stage, while the rest of the doses were applied after each mowing. The basal inputs of P_2O_5 and K_2O fertilizers were applied at 96 and 160 kg ha^{-1} $year^{-1}$ to all plots, respectively. The seeds were handed-scattered into the soil and were covered with a thin layer of soil. Weeds that appeared in all plots were removed by hand. The seeding rates used for growing legume–grass mixtures and their corresponding monocultures are presented in Table 1.

2.4 Sampling and measurement

2.4.1 Biomass yield

The first, second, third, and fourth cuttings for forage yield were performed during the initial flowering stage of alfalfa on March 24, May 6, July 23, and September 25 in 2019, respectively. Before harvesting, the side rows of each plot were removed, 50 cm of both sides was removed, and the area of (5–0.5 × 2) × (3–0.6) = 9.6 m^2 was harvested to a stubble height of 5 cm. The fresh weight of the samples was recorded, and then approximately 300 g of the samples was air-dried at 65°C for 72 h up to a constant weight in an oven to estimate the dry matter (DM) content, which was later used to calculate the DM yield.

TABLE 1 Seeding rates used for growing the monocultures and their mixtures (kg ha^{-1}).

Plant materials	Monocultures	Mixtures[a]	
		A1	A2
Alfalfa	22.50	6.75	3.38
White clover	7.50	-	1.13
Tall fescue	37.50	13.13	13.13
Orchardgrass	15.00	5.25	5.25

[a]The mixtures were grown in a legume–grass ratio of 3:7.

2.4.2 Nutritional quality

The dried samples were ground to pass a 1-mm screen for nutritional quality analysis. The water-soluble carbohydrate (WSC) content analysis was referred to the thracenone–sulphuric acid method, while the crude protein (CP) content was measured by the Kjeldahl method (Bremner, 1996). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to a previously described method (Van Soest et al., 1991).

2.4.3 Soil sampling

The soil samples at 15 cm depth were randomly collected from the experimental site prior to seeding and after each mowing. The five sub-samples were taken and then bulked to one sample for each replication. The soil samples were stored in cloth bags and air-dried at room temperature to a constant weight. The roots, stones, and other debris in the soil samples were removed, and the soil samples were passed through a 2 mm sieve and then stored in the laboratory until analysis.

2.4.4 Soil nutrients

The pH was measured in 1/5 (w/v) aqueous extract using a pH meter. The contents of phosphorus and potassium were determined by inductively coupled plasma spectrometry after nitric-perchloric acid digestion. Soil organic matter was determined by the dilution heat method, while that of alkali hydrolyzed nitrogen was determined by the alkaline hydrolysis diffusion method (Bao, 2000). The total nitrogen was measured by the Kjeldahl method (Bremner, 1996). Soil nitrate N and ammonium N were extracted with potassium chloride (KCl, 2 mol/L), and their concentrations were measured by the flow injection method (FIA star 5000 Analyzer, FOSS, DK).

2.4.5 Urease enzyme activity

The phenol-sodium hypochlorite colorimetry method was followed to measure the urease enzyme activity (Qin et al., 2016). Briefly, 10 g of dried soil was placed in a 100 ml Erlenmeyer flask, and then 2 ml of toluene, 10 ml of 10% $CO(NH_2)_2$, and 20 ml of citrate buffer were added. The samples were placed in an incubator for 24 h at 38°C. After incubation, CH_2O was added into each sample to a constant volume of 100 ml and mixed well. The supernatant (1 ml) was taken and mixed with deionized water (9 ml), phenol (4 ml), and sodium hypochlorite solution (3 ml). The samples were placed at room temperate for 20 min. The absorbance of the samples was measured at 578 nm using a spectrophotometer.

2.4.6 Hydroxylamine oxidoreductase enzyme activity

Hydroxylamine oxidoreductase (HAO) enzyme activity was determined by the ammonium ferric sulfate o-phenanthroline method. Briefly, 1 g of dried soil was taken in a test tube, and 20 mg of $CaCO_3$ was added and mixed well. Then, 1 ml of 0.5% hydroxylamine hydrochloride, 1 ml of 1% glucose as hydrogen donor, and 5 ml of H_2O were added. The samples were incubated for 5 h at 30°C in an incubator. The control sample was set without adding hydroxylamine hydrochloride and soil. After the incubation, the mixture was transferred in different test tubes, and 2 ml of alumina potassium alum saturated reagent was added. The samples were vortexed, and 1 ml of supernatant was taken and mixed with 1 ml buffer (1 mol/L CH_3COONa and 1 mol/L CH_3COOH), 1 ml ferric ammonium sulfate solution (0.004 mol/L), and 1 ml o-phenanthroline ethanol solution (0.01 mol/L). The color of the solution was developed for 10 min. A spectrophotometer was used to measure the absorbance at 510 nm.

2.4.7 Nitrate reductase enzyme activity

The α-naphthylamine-p-aminobenzene sulfonic acid colorimetry method was followed to determine the nitrate reductase (Nar) enzyme activity (Li et al., 2014). Briefly, 1 g dried soil was taken in a test tube, and 20 mg of $CaCO_3$ was added and mixed well into the former. Then, 1 ml 0.8 mmol/L 2,4-DNP solution, 1 ml 1% KNO_3 solution, and 1 ml 1% grape poplar solution as hydrogen donor were added, and these were also mixed well. After mixing, 15 ml H_2O was added to form the liquid seal, and the solution was incubated for 24 h at 30°C in the incubator. The control sample was set without adding 1% KNO_3 and soil. After incubation, the solution was transferred to a different test tube, and 1 ml alumina potassium alum saturated reagent was added. Then, 1 ml of supernatant was taken and mixed with deionized water, 4 ml 0.1% α-naphthylamine solution, and 0.5% p-aminobenzenesulfonic acid. The color of the solution was developed for 15 min, and then a spectrophotometer was used to measure the absorbance at 520 nm.

2.4.8 Nitrite reductase enzyme activity

Nitrite reductase (Nir) enzyme activity was also determined using the α-naphthylamine-p-aminobenzene sulfonic acid colorimetry method (Li et al., 2014). Briefly, 1 g dried soil was taken in the test tube, 20 mg $CaCO_3$ was added, and these were mixed well. Then, 2 ml 0.25% $NaNO_2$ solution, 1 ml 1% glucose as hydrogen donor, and 15 ml H_2O were added. The samples were incubated for 24 h at 30°C in the incubator. The control sample was set without adding 0.25% $NaNO_2$ solution and soil. After incubation, the mixture was transferred in a different test tube, and 1 ml alumina potassium alum saturated reagent was added. Then, 1 ml of supernatant was taken and mixed with deionized water, 4 ml of 0.1% α-naphthylamine solution, and 0.5% p-aminobenzenesulfonic acid. Color was developed for 15 min, and then absorbance was measured at 520 nm with a spectrophotometer.

2.5 Statistical analysis

All the results reported are the mean of three replicates, and the relevant data were analyzed using the SPSS software (version 28.0: IBM Corp., Armonk, NY, USA). The forage yield, nutritional quality, soil nutrients, and soil enzyme activities were analyzed using a two-way analysis of variance with Duncan's multiple-range test. The relationships between the variables—forage yield, nutritional quality, soil nutrients, and soil enzyme activities—were determined by calculating the Pearson's correlation coefficients and were plotted by using Origin 2022. The tables and graphics were created using Excel 2019 and GraphPad Prism 8, respectively.

3 Results

3.1 Effect of N input on the forage yield of different cropping systems

The forage yield differed substantially among the cropping systems ($P < 0.001$) and N inputs ($P < 0.001$), and the interaction between the cropping systems and N inputs was significant ($P < 0.001$) (Table 2). With an increase in the rate of N input, the forage yield of the alfalfa, orchardgrass, tall fescue, and A2 mixture significantly increased ($P < 0.05$), while the forage yield of white clover and A1 mixture first substantially increased and then decreased ($P < 0.05$). The alfalfa forage yield of N3 was greater than N2 and N1 by 1.3% and 7.9%, respectively; the white clover forage yield of N2 was greater than N1 and N3 by 15.2% and 15.7%, respectively; the orchardgrass forage yield of N3 was greater than N1 and N2 by 27.5% and 4.4%, respectively; the tall fescue forage

TABLE 2 Forage yield (t ha^{-1} $year^{-1}$) of different cropping systems under various N inputs.

Cropping system	Nitrogen level			SEM	Significance	
	N1	N2	N3			
Alfalfa	12.63aA	13.54aA	13.72aAB	0.2420	Cropping system	***
White clover	8.12bC	9.57aB	8.07bD	0.2695	Nitrogen level	***
Orchardgrass	10.07bB	13.28aA	13.89aAB	0.6140	Interaction	***
Tall fescue	7.925bC	10.05aB	10.36aC	0.4154		
A1	12.92aA	13.88aA	12.85aB	0.2550		
A2	10.66bB	13.80aA	14.39aA	0.6108		
SEM	0.4868	0.4509	0.5657			

A1, mixture of alfalfa, tall fescue, and orchardgrass; A2, mixture of alfalfa, white clover, tall fescue, and orchardgrass; N1, 150 kg ha^{-1}; N2, 300 kg ha^{-1}; N3, 450 kg ha^{-1}; SEM, standard error of the mean.

Lowercase letters represent the significant difference within the same row, while uppercase letters indicate the significant difference within the same column.

***Significance at P < 0.001.

yield of N3 was greater than N1 and N2 by 23.5% and 2.9%, respectively; the A1 forage yield of N2 was greater than N1 and N3 by 6.9% and 7.4%, respectively; and the A2 forage yield of N3 was greater than N1 and N2 by 25.9% and 4.1%, respectively. Taken together, the A1 mixture had a greater forage yield of 13.88 t ha^{-1} $year^{-1}$ than the other N inputs, while that of A2 mixture under N3 had a greater forage yield of 14.39 t ha^{-1} $year^{-1}$ than N1, but it was not substantially greater than the N2 input (13.80 t ha^{-1} $year^{-1}$). These findings highlight that growing legume–grass mixtures under N2 input is sustainable and cost-effective, which provides greater forage yield by efficient resource utilization.

3.2 Effect of N input on the nutritional quality of different cropping systems

The nutritional quality of different cropping systems under various N inputs is presented in Figure 1. The cropping system and N level significantly influenced the CP content ($P < 0.001$), and the interaction among the cropping system and N level was also significant ($P < 0.001$) (Figure 1A). The legume monocultures had a greater CP content than the others; however, the CP content in the grass monocultures and mixtures substantially increased ($P < 0.05$) with an increase in the rate of N input. Additionally, the A1 and A2 mixtures had a greater CP content of 18.91% and 18.94% of DM, respectively, under N3 input than the grass monocultures under various N inputs. The cropping system substantially affected the WSC content ($P < 0.001$), and while the N level did not influence the WSC content, but their interaction was significant on the WSC content ($P < 0.05$) (Figure 1B). The white clover had a greater WSC content (3.5%–3.9% DM) followed by the A2 mixture (2.7%–3.5% DM), while alfalfa (2.4%–2.5% DM) and orchardgrass (2.5%–2.9% DM) had a lower WSC content than the other cropping systems at different N inputs. The cropping system substantially influenced the fiber contents ($P < 0.001$), while the N level had a non-significant effect, but their interaction was significant on the fiber contents ($P < 0.001$) (Figures 1C, D). The legume monocultures had lower NDF and ADF contents than those of grass monocultures and mixtures under various N inputs. Meanwhile, the mixtures had lower NDF (40.44%–43.86% DM) and ADF (26.32%–28.89% DM) contents at different N inputs when compared with the orchardgrass monoculture. Taken together, cultivating grasses in combination

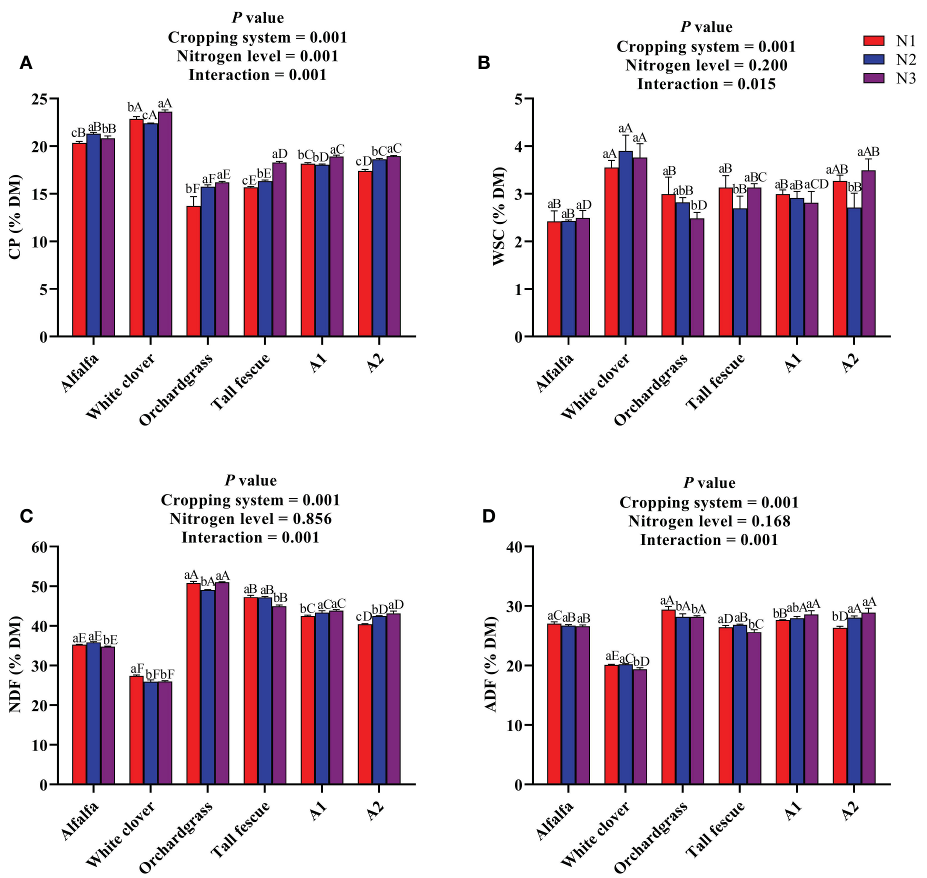

FIGURE 1
Effect of N input on the nutritional quality of different cropping systems. **(A)** Crude protein, **(B)** water-soluble carbohydrates, **(C)** neutral detergent fiber, and **(D)** acid detergent fiber. A1, mixture of alfalfa, tall fescue, and orchardgrass; A2, mixture of alfalfa, white clover, tall fescue, and orchardgrass; N1, 150 kg ha^{-1}; N2, 300 kg ha^{-1}; N3, 450 kg ha^{-1}. The bars show the standard errors. Lowercase letters represent the significant difference within the same cropping system under various N inputs, while uppercase letters indicate the significant difference within different cropping systems under the same N input. Significance was employed at 0.05.

with legumes improved the nutritional quality of forages, while N fertilization did not affect the nutritional quality of forages except the CP content.

3.3 Effect of N input on the soil nutrients of different cropping systems

The effect of N input on the soil nutrients of the different cropping systems is shown in Figure 2. The total N content was neither affected by the cropping system nor the N level, and their interaction was also non-significant on total N (Figure 2A). The total N content of the different cropping systems under various N inputs ranged from 1.21–1.58 g kg^{-1}; the total N content of the A1 mixture under N2 input (1.58 g kg^{-1}) was numerically greater than those of other cropping systems under various N inputs. The ammonium N content was substantially affected by the cropping system ($P < 0.001$) and N level ($P < 0.001$), and their interaction was also significant for ammonium N content ($P < 0.001$) (Figure 2B). With an increase in the rate of N input, the ammonium N content of alfalfa and mixtures significantly increased ($P < 0.05$), while the ammonium N content of white clover and grass monocultures first increased and then decreased ($P < 0.05$). The A1 mixture had a greater ammonium N content of 16.01 and 16.75 mg kg^{-1} under N2 and N3 inputs than the other cropping systems (except white clover under N2 input: 17.02 mg kg^{-1}) under various N inputs. The nitrate N content was significantly influenced by the cropping system ($P < 0.001$) and N level ($P < 0.001$), and their interaction was also significant for nitrate N content ($P < 0.001$) (Figure 2C). With an increase in the rate of N input, the nitrate N content of grass monocultures and mixtures substantially increased ($P < 0.05$), whereas the nitrate content of legume monocultures first increased and then decreased ($P < 0.05$). A1 and A2 had a greater nitrate N content of 3.6 and 4.2 mg kg^{-1} under N3 input compared with the other cropping systems under various N inputs. Taken together, the legume–grass mixed cultivation substantially increases the contents of available N in the soil when the N input rate was ≥300 kg ha^{-1}.

3.4 Effect of N input on the soil enzyme activities of different cropping systems

The influence of N input on the soil enzyme activities of different cropping systems is presented in Figure 3. The urease enzyme activity was substantially affected by the N level ($P < 0.001$),

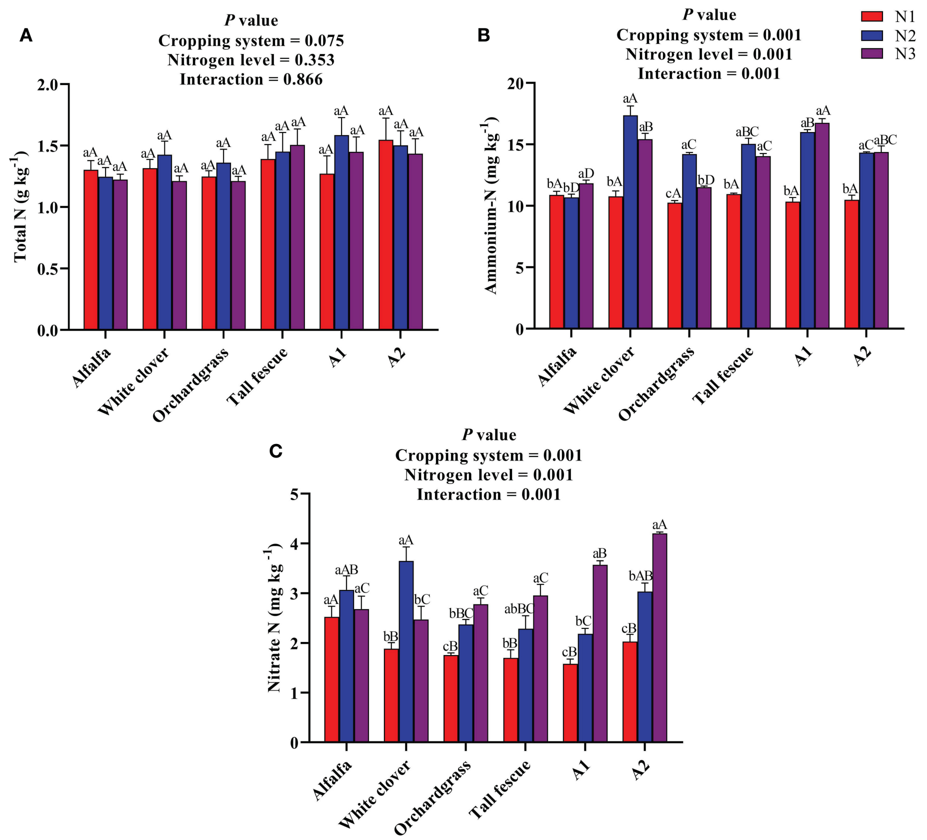

FIGURE 2
Effect of N input on the soil nutrients of different cropping systems. **(A)** Total N, **(B)** ammonium N, and **(C)** nitrate N. A1, mixture of alfalfa, tall fescue, and orchardgrass; A2, mixture of alfalfa, white clover, tall fescue, and orchardgrass; N1, 150 kg ha^{-1}; N2, 300 kg ha^{-1}; N3, 450 kg ha^{-1}. The bars show the standard errors. Lowercase letters represent the significant difference within the same cropping system under different N inputs, while uppercase letters indicate the significant difference within different cropping systems under the same N input. Significance was employed at 0.05.

cropping system (P < 0.001), and their interaction (P < 0.001) (Figure 3A). The urease activity of all cropping systems significantly increased (P < 0.05) with an increase in the rate of N input, whereas no substantial difference was observed among the N2 and N3 inputs of the cropping systems. The A1 and A2 mixture had a greater urease activity of 0.39 and 0.39 mg g^{-1} 24 h^{-1} under N2 input than the other cropping systems under various N inputs. The HAO enzyme activity was significantly influenced by the N level (P < 0.001) and cropping system (P < 0.001), and their interaction was also significant for the HAO enzyme activity (P < 0.001) (Figure 3B). The HAO enzyme activity of all cropping systems, except the white clover monoculture, first increased and then decreased with an increase in the rate of N input, and it was substantially greater under N2 input compared with the other N inputs. The A2 and A1 mixtures had the comparative HAO enzyme activities of 0.46 and 0.45 mg g^{-1} 5 h^{-1} with orchardgrass HAO enzyme activity of 0.46 mg g^{-1} 5 h^{-1} under N2 input, which were greater than those of other cropping systems under various N inputs. The Nar enzyme activity differed among the cropping systems (P < 0.001) and N level (P < 0.001), and the interaction of the cropping system and N level was significant for the Nar enzyme activity (P < 0.001) (Figure 3C). The Nar enzyme activity of white clover, tall fescue, and A2 mixture significantly increased (P < 0.05), whereas the Nar enzyme activity of the A1 mixture substantially decreased (P < 0.05) with an increase in the rate of N input. The A1 mixture had a lower Nar enzyme activity of 0.48 mg g^{-1} 24 h^{-1} under N3 input than the other cropping systems under various N inputs (except tall fescue under N1 input). The Nir enzyme activity was significantly influenced by the cropping systems (P < 0.001) and N level (P < 0.001) and their interaction (P < 0.001) (Figure 3D). With an increase in the rate of N input, the Nir activity of all cropping systems increased first and then decreased. The alfalfa and white clover monoculture had lower Nir activities of 0.40 and 0.44 mg g^{-1} 24 h^{-1} under N3 input than the other cropping systems under various N inputs. Taken together, the N addition and legume–grass mixed cultivation significantly improve the urease and HAO enzyme activities which play a crucial role in releasing more available N in the soil for plant uptake.

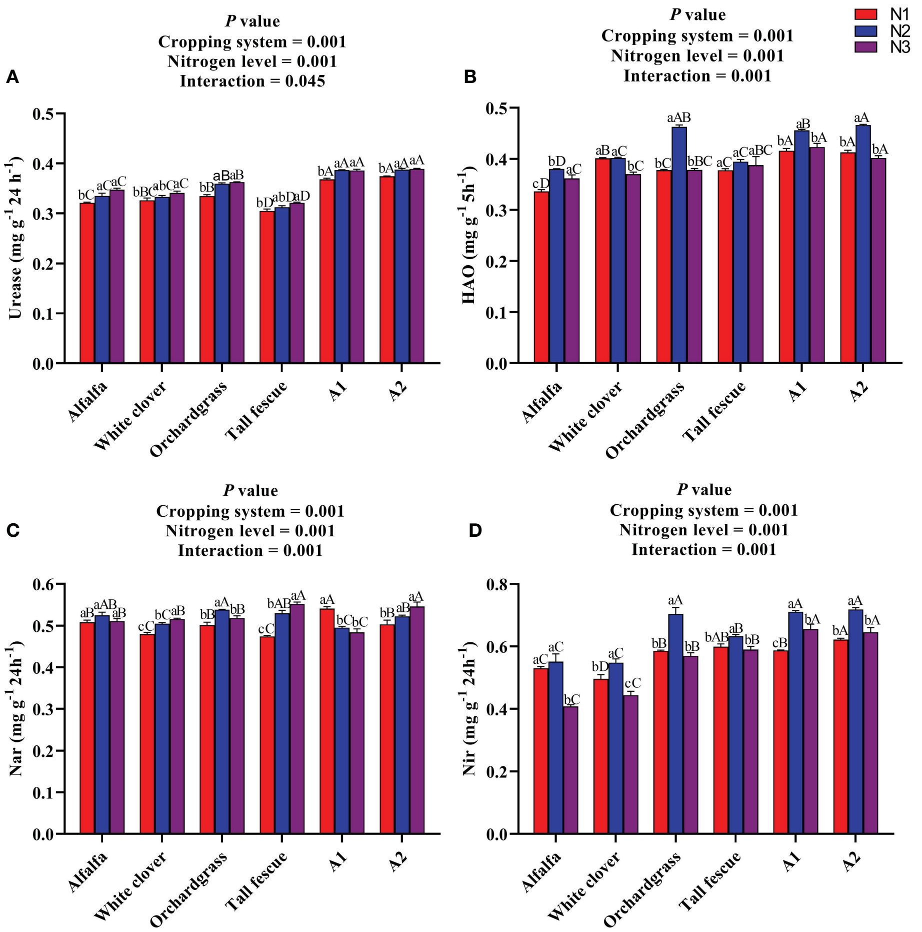

FIGURE 3
Effect of N input on the soil enzyme activities of different cropping systems. **(A)** Urease, **(B)** hydroxylamine oxidoreductase, **(C)** nitrate reductase, and **(D)** nitrite reductase. A1, mixture of alfalfa, tall fescue, and orchardgrass; A2, mixture of alfalfa, white clover, tall fescue, and orchardgrass; N1, 150 kg ha^{-1}; N2, 300 kg ha^{-1}; N3, 450 kg ha^{-1}. The bars show the standard errors. Lowercase letters represent the significant difference within the same cropping system under different N inputs, while uppercase letters indicate the significant difference within different cropping systems under the same N input. Significance was employed at 0.05.

3.5 Relationships between forage yield, soil nutrients, and soil enzyme activities

The relationships among forage yield, nutritional quality, soil nutrients, and soil enzyme activities are presented in Figure 4. The forage yield was substantially positively correlated ($P < 0.05$) with soil enzyme activities, nitrate N, ADF, and ADF, while it was significantly negatively correlated ($P < 0.05$) with WSC. The CP had substantially positive relationships ($P < 0.05$) with nitrate N and WSC, while it had significantly negative correlations ($P < 0.05$) with NDF, ADF, and Nir activity. The Nir enzyme activity was significantly positively correlated ($P < 0.05$) with fiber contents, total N, ammonium N, urease, and HAO activity. The urease activity was substantially positively associated ($P < 0.05$) with soil nutrients, while the HAO activity was significantly positively correlated ($P < 0.05$) with total N, urease activity, and ammonium N.

4 Discussion

4.1 Response of soil enzyme activities of different cropping systems to N input

Soil enzyme activities are important in decomposing organic matter, recycling nutrients, and influencing microbial functions (Sinsabaugh et al., 2008). Urease is an enzyme that degrades urea and is widely regarded as an accurate predictor of N mineralization (Das and Varma, 2011). In this study, the legume–grass mixtures had greater urease enzyme activity than their corresponding monocultures, and its activity increased with an increase in the rate of N input, but there was no substantial difference among the N2 and N3 inputs. This advantageous effect of mixed sowing and N input on urease enzyme activity in soil could be attributed to an increase in microbial population as well as the release of a greater proportion of nitrogenous substances (ammonium N and nitrate N) in root exudates that can induce urease enzyme activity and become available for plant uptake. Improvement in urease enzyme activity is highly dependent on the availability of substrates like urea or ammonium-based fertilizers for nitrogen-cycling enzymes, which results in increased enzyme activity as a positive association has been reported between the substrate and urease activity (Ibrahim et al., 2020). Meanwhile, when the N input increased from N2 to N3, the urease enzyme activity did not increase significantly, which may be due to the absorption of mineral N by soil microorganisms or buildup of NH^{+4} that suppressed the urease activity (Kumari et al., 2020).

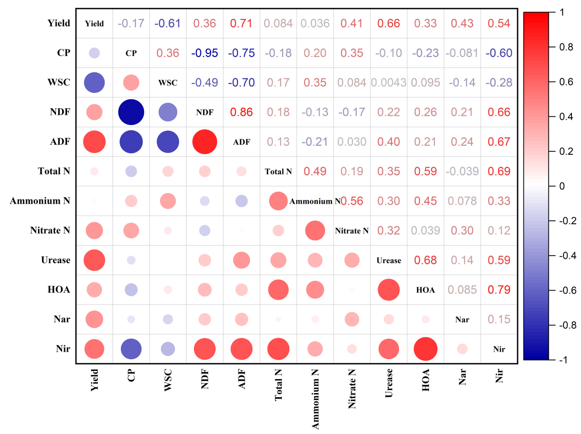

FIGURE 4
Associations among forage yield and nutritional quality, soil nutrients, and soil enzyme activities. CP, crude protein; WSC, water-soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; HAO, hydroxylamine oxidoreductase; Nar, nitrate reductase; and Nir, nitrite reductase.

HAO is a key enzyme in the nitrification pathway, and its activity is usually dependent on the abundance and community structure of ammonia-oxidizing bacteria. In this study, the HAO enzyme activity increased first and then decreased with an increase in the rate of N input, and the legume–grass mixtures and orchardgrass had a greater HAO enzyme activity at N2 input. This could be attributed to the soil environment of legume–grass mixtures and orchardgrass which was more conducive for the growth of ammonia-oxidizing bacteria that ultimately led to an increase in HAO enzyme activity. It has been established that N addition to soil increases the abundance of ammonia-oxidizing bacteria that further improved the HAO enzyme activity, which is beneficial to enhance the available N for plant uptake (Carey et al., 2016). Meanwhile, the decrease of HAO at a higher rate of N input might be related to environmental stresses such as acidification, which influences the substrate availability and abundance of ammonia-oxidizing bacteria, leading to a lower HAO enzyme activity (Liu et al., 2018).

Nar and Nir are the key enzymes for the denitrification process in which nitrate and nitrite are reduced into NO, N_2O, and N_2 when oxygen is limited. This process generally causes N loss from agricultural soils and contributes toward greenhouse gas N_2O emission (Chen et al., 2012). The Nar enzyme activity of all cropping systems except A1 is enhanced with an increase in the rate of N input, while the Nir enzyme activity first increased and then decreased with an increase in the rate of N input, and legume monocultures had a lower Nir enzyme activity at all N inputs compared with others. The previous study has reported that N addition subsidizes towards an increase in the abundance of denitrifying genes due to a greater nitrate substrate concentration, as both forms of N (ammonium N and nitrate N) have positive relationships with denitrifying gene abundances (Xiao et al., 2021). This could be the result of the stimulation of microbial growth and activity by improved nutrient availability, and improved soil physical properties can make the soil environment more suitable for microbial growth (Ai et al., 2012). Meanwhile, it was quite fascinating to find that the Nar enzyme activity of A1 decreased with an increase in the rate of N input, highlighting that this mixture could be the best choice to improve the soil nutrient balance, but the reason for this is unknown. However, a decreased Nir enzyme activity at N3 input could be attributed to the greater ammonium ion concentration at a high N input rate that resulted to starting the inhibition of Nir enzyme activity (Piotrowska and Wilczewski, 2012). Moreover, it is widely accepted that legumes are natural N fixers and contribute less to environmental pollution *via* ammonia volatilization or leaching—that is why these resulted in lower Nir activities compared with others.

4.2 Response of soil nutrients of different cropping systems to N input

Soil serves as the most important substrate for plant growth and development being a reservoir of many nutrients and a site for the microbial decomposition of plant and animal residues. Soil physical and chemical properties have a substantial influence on the plant community dynamics as a substrate for plant growth and development as well as a critical environmental factor (Li et al., 2022). In this study, the contents of ammonium N and nitrate N were significantly influenced by the N level and cropping system, but total N was not affected by them. The white clover and A1 had a significantly greater ammonium N content at the N2 and N3 inputs (no significance difference) compared with others, highlighting that the soil environment of these treatments allowed urease enzyme to convert urea into ammonium N, along with N addition as substrate for urease enzyme. However, ammonium N decreased or did not influence at a higher rate of N input, which might be because of the absorption of mineral N by soil microorganisms which suppressed the urease activity (Meysner et al., 2006). Nitrate N significantly increased with an increased rate of N input and A2 had the greater nitrate N content at N3 input compared with other cropping systems. This highlights that mixed sowing along with N input is beneficial to enhance the available N in the soil. Ammonium N is the most important substrate for ammonia-oxidizing microorganisms that contribute towards an increase in nitrate N via ammonia oxidation (Taylor et al., 2012), that is why nitrate N increased with increased N fertilization rate. However, the nitrate N content of alfalfa and white clover monocultures first increased and then decreased with an increase in the rate of N input, highlighting the N loss to environment at higher N rates.

4.3 Response of forage yield and nutritional quality of different cropping systems to N input

Forage yield is an important indicator to measure forage resources, which determines the amount of food provided by forage crops for livestock (Kawamura et al., 2008). A general concept prevails that N addition always leads to a greater forage yield. In this study, A1 and A2 had a greater forage yield than their respective monocultures under various N inputs. This result is consistent with the urease enzyme activity which played a crucial role to enhance the available nutrients for plant uptake—a strong positive correlation was found between forage yield and urease enzyme activity in this study. Moreover, the inclusion of legume in the mixture supplies more N to grasses by their N fixing ability, ultimately leading to the better growth and development of grasses (Tahir et al., 2022). In addition, the forage yield of mixtures increased up to a certain level with an increase in the rate of N input (especially up to the N2 threshold level), suggesting that higher N input rates are not beneficial and N can be lost to the environment.

Different planting patterns influence the forage yield and nutritional quality in grassland cultivation (Tahir et al., 2022). The nutritional quality of forage can not only directly affect the growth, reproduction, forage–herbivore interaction, and foraging behavior of livestock and wild herbivores by affecting the difficulty in obtaining nutrients but can also indirectly affect the yield, quality, and economic benefits of livestock products (Cui et al., 2016). From a nutritional value perspective, CP is an essential nutrient for livestock, and its content not only affects the economic benefits of forage but also directly affects the milk yield and milk protein yield of livestock (Yang et al., 2017). In this study, the cropping system significantly affected the nutritional quality parameters (CP, WSC, NDF, and ADF) while the nitrogen level just had a significant effect on CP, highlighting that the cropping system is more critical to control the nutritional quality of forages. The alfalfa and white clover had the greater CP content while having lower fiber contents (NDF and ADF) compared with other treatments, and the CP content slightly increased with an increase in N input, but the fiber contents were not affected. It is well established that legumes had a greater protein content and lower fiber contents compared with the grasses (Klupšaitė and Juodeikienė, 2015)—that is why the legumes were rich in protein content, and the grasses were abundant in fiber contents, and their mixtures were in between as there were negative correlations found between CP and fiber contents. Moreover, white clover had the greater WSC content, followed by A2 than the other treatments.

5 Conclusion

N addition and legume–grass mixed seeding significantly influenced the forage yield, nutritional quality, soil nutrients, and soil enzyme activities. The A1 mixture under N2 had a greater forage yield of 13.88 t ha^{-1} $year^{-1}$ than the other N inputs with higher urease and HAO enzyme activities, which played a significant role to release more available N for plant uptake. Moreover, the A2 mixture under the N3 input had a greater forage yield of 14.39 t ha^{-1} $year^{-1}$ than the N1 input with higher urease and Nar enzyme activities, but it was not substantially greater than the N2 input (13.80 t ha^{-1} $year^{-1}$). Therefore, the growing of legume–grass mixtures under the N input of 300 kg ha^{-1} is recommended, which provides guidance for eco-friendly, sustainable, and cost-effective forage production in Sichuan, China.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

MT: writing—original draft preparation, methodology, and software. XW: methodology, resources, and formal analysis. HL: investigation and data curation. JL: writing—review and editing and visualization. JZ: investigation and data curation. BK: validation and formal analysis. DJ: resources and validation. YY: project administration, funding acquisition, supervision, and investigation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Lingyang Feng,
Peking University, China

REVIEWED BY
Li Xiangyi,
Chinese Academy of Sciences (CAS), China
Wayan Wangiyana,
University of Mataram, Indonesia

*CORRESPONDENCE
Xinhua Zhao
xinhua_zhao@syau.edu.cn
Haiqiu Yu
yuhaiqiu@syau.edu.cn

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Row ratio increasing improved light distribution, photosynthetic characteristics, and yield of peanut in the maize and peanut strip intercropping system

Juntian Lu[1,2], Qiqi Dong[1], Guohu Lan[1], Zecheng He[1], Dongying Zhou[1], He Zhang[1], Xiaoguang Wang[1], Xibo Liu[1], Chunji Jiang[1], Zheng Zhang[3], Shubo Wan[3], Xinhua Zhao[1]* and Haiqiu Yu[1]*

[1]College of Agronomy, Shenyang Agricultural University, Shenyang, Liaoning, China, [2]Maize Research Institute, Dandong Academy of Agricultural Sciences, Dandong, Liaoning, China, [3]Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China

Changes in the canopy microclimate in intercropping systems, particularly in the light environment, have important effects on the physiological characteristics of photosynthesis and yield of crops. Although different row ratio configurations and strip widths of dwarf crops in intercropping systems have important effects on canopy microclimate, little information is available on the effects of intercropping on chlorophyll synthesis and photosynthetic physiological properties of dwarf crops. A 2-year field experiment was conducted in 2019 and 2020, with five treatments: sole maize (SM), sole peanut (SP), four rows of maize intercropping with eight rows of peanut (M4P8), four rows of maize intercropping with four rows of peanut (M4P4), and four rows of maize intercropping with two rows of peanut (M4P2). The results showed that the light transmittance [photosynthetically active radiation (PAR)], photosynthetic rate (*Pn*), transpiration rate (*Tr*), and stomatal conductance (*Gs*) of intercropped peanut canopy were reduced, while the intercellular carbon dioxide concentration (*Ci*) was increased, compared with SP. In particular, the M4P8 pattern *Pn* (2-year mean) was reduced by 5.68%, 5.33%, and 5.30%; *Tr* was reduced by 7.41%, 5.45%, and 5.95%; and *Gs* was reduced by 8.20%, 6.88%, and 6.46%; and *Ci* increased by 11.95%, 8.06%, and 9.61% compared to SP, at the flowering needle stage, pod stage, and maturity, respectively. M4P8 improves the content of chlorophyll synthesis precursor and conversion efficiency, which promotes the utilization efficiency of light energy. However, it was significantly reduced in M4P2 and M4P4 treatment. The dry matter accumulation and pod yield of peanut in M4P8 treatment decreased, but the proportion of dry matter distribution in the late growth period was more transferred to pods. The full pod number decreases as the peanut row ratio decreases and increases with year, but there is no significant difference between years. M4P8 has the highest yield and land use efficiency and can be used as a reference row ratio configuration for maize–peanut intercropping to obtain relatively high yield benefits.

KEYWORDS

maize and peanut intercropping, row ratio configurations, photosynthetically active radiation, chlorophyll synthesis, photosynthetic characteristics

1 Introduction

Intercropping, as a temporally and spatially intensive cultivation technology model, is widely applied by farmers in modern agricultural production across the world due to the efficient utilization of natural resources, higher land equivalent ratio (LER), and ecological benefits (Yu et al., 2015; Raseduzzaman and Jensen, 2017; Nelson et al., 2018). In the reasonable intercropping systems, the high cereal crops intercropping with lower legume crops are usually used to improve the ventilation and light condition of cereal crops and increase nutrient use efficiency and yield (Zhang et al., 2015; Liu et al., 2018). However, the light energy of dwarf legume canopy is limited compared with sole cropping because of the shelter by higher crop canopy (Huang et al., 2022), which leads to restricted photosynthesis and low yield (Keating and Carberry, 1993; Feng et al., 2019). The different row ratio settings and strip widths of dwarf crops in intercropping have important effects on the microclimate environment, crop yield, and economic benefits in cereal and legume intercropping systems (Liu et al., 2018; van Oort et al., 2020; Wang et al., 2021a). Scientific and reasonable ratios can improve light energy interception and utilization efficiency, give full play to the advantages of high-position crops, and stimulate low-light response mechanisms in dwarf crops, thus promoting the yield improvement of intercropping systems to the greatest extent.

Intercropping results in a more complex canopy structure. The distribution and quality of light in the microclimate environment of crops canopy are crucial to crop photosynthesis and yield (Raza et al., 2019). In the intercropping compound system, the population light distribution and light transmittance have significant differences, which increase the light transmittance of high-position crops and reduce the light transmittance of low-position crops. The studies indicated that the intercropping improved the chlorophyll content and delayed the senescence process of high-position crops and promoted the net photosynthetic efficiency of border rows and nutrition utilization during the symbiotic period (Nasar et al., 2022). However, the negative intercropping productivity caused by interspecific competition has attracted more attention (Wu et al., 2016), especially in inappropriately managed fields. The canopy light extinction coefficient (k) of peanut was significantly decreased when intercropped with maize, while the mean radiation-use efficiency (ε) was significantly higher compared to sole peanut (Awal et al., 2006). Meanwhile, compared with monoculture, the yield of maize was increased by 61.05% in the maize–peanut intercropping system, whereas the yield of intercropped peanut was decreased by 31.80% (Li et al., 2019). Similarly, in the maize–soybean relay intercropping system, the leaves of soybean showed lower leaf mass per unit area, thinner thickness, lower chlorophyll a/b ratio, and lower photosynthetic rate during shade period (Wu et al., 2016). The application of wide strips for dwarf crops in intercropping systems was promoted to improve canopy light radiation and to be suitable for simplified planting (Brooker et al., 2015; van Oort et al., 2020). The light interception (LI) and light use efficiency (LUE) of intercropping peanut strip are significantly affected by the ratio of side rows in the maize and peanut strip intercropping system, and the relative yield of peanut is improved with the strip being wider (Wang et al., 2020). Therefore, it is one of the important ways to obtain yield advantage to improve the light environment through intercropping and row ratio allocation to achieve multi-level and all-around efficient utilization of light resources by the population (Wang et al., 2021a), and improve the efficiency of light energy utilization. Previous research has revealed the yield benefits of wide strips, but the mechanism underlying this improvement in photosynthetic characteristics of dwarf crops has not been well understood (Du et al., 2018).

Under the intercropping mode, there are significant differences in the photosynthetic effective radiation intensity and chlorophyll content of dwarf crop canopy (Kume et al., 2018; Wang et al., 2021b). The low-light environment of intercropping has become an important factor that inhibits the growth, development, and yield improvement of dwarf crops (Liu et al., 2017), because of the decrease of chlorophyll content per unit area and photosynthesis capacity in dwarf crops (Gong et al., 2020). In particular, the ratio of red light to far-red light in intercropping soybean canopy is significantly lower than that of monocropping, which caused soybean shading reaction yield reduction compared with monocropping (Yang et al., 2014). As the main pigment in plant photosynthesis, chlorophyll synthesis is not only regulated by internal genes, but also influenced by external environment. Insufficient and excessive light will inhibit chlorophyll synthesis, resulting in changes in chlorophyll content and composition. Owing to the reflected and absorbed effect by maize plants, the spectral irradiance, R/FR ratio, and photosynthetically active radiation (PAR), δ_{Ro} (the efficiency/probability with which an electron from the intersystem electron carriers was transferred to reduce end electron acceptors at the PSI acceptor side), and φ_{Ro} (the quantum yield for the reduction of the end electron acceptors at the PSI acceptor side) of intercropped soybean leaf were decreased compared with monocrop soybean, which resulted in the lower photosynthetic capacity in the maize–soybean intercropping system (Yao et al., 2017). It was also reported that, although the chlorophyll content and Chla/b of intercropped peanut decreased significantly, more Chla transformed into Chlb, which was conducive to absorbing short wave light, capturing more light energy, and improving the accumulation of photosynthetic products (Gong et al., 2015). The disadvantage of light competition significantly increased the rate of peanut falling and reduced the number of pods per plant and pods yield (Block et al., 2002; Feng et al., 2020). The limitation of photosynthetic synthesis and distribution is the limiting factor for the further improvement of the yield of the maize–peanut intercropping system (Jiao et al., 2021). Therefore, it is necessary to understand the importance of spatial and temporal allocation to improve the production of intercropped peanut and the advantages of intercropping systems (Gao et al., 2022).

In this study, 2 years of field experiments were conducted to explore the effects of different row ratios on the photosynthetic effective radiation, photosynthetic physiological characteristics, chlorophyll content, dry matter accumulation and distribution, yield, and its components of peanut canopy. The purpose is to compare the differences in light environment characteristics of peanut canopy in maize–peanut intercropping systems with different row ratios. The effects of interspecific competition on dry matter accumulation and yield of peanut were analyzed by measuring the photosynthetic physiological characteristics and chlorophyll synthesis law of intercropped peanut. According to the changes of photosynthetic physiological characteristics of peanut and the formation of yield advantage, a theoretical basis was provided for exploring the optimal maize interplanting model.

2 Materials and methods

2.1 Site description

Two-year experiments were conducted in the test field of Northeast Experimental Shenyang Agricultural Observation Station, Ministry of Agriculture and Rural Affairs (40°28′16″N, 124°06′45″E), Dandong city, Liaoning province, China during the 2019 and 2020 growing seasons (May to September). The previous crop was maize, and the soil physicochemical properties were as follows: soil organic matter, 18.4 g kg^{-1}; available phosphorus, 42.2 mg kg^{-1} measured by the Olsen-p method; available potassium, 122.1 mg kg^{-1}; alkaline hydrolyzable nitrogen, 86.0 mg kg^{-1}; and soil pH, 6.4. The field location has a temperate monsoon continental climate, the average annual precipitation was 876.5 mm, and temperature was 10.8°C during the growth stage (Figure 1). Climate data were obtained from the Dandong Meteorological Bureau.

2.2 Experimental design

Field experiments were conducted using a randomized complete block design. This experiment included the following five treatments: sole maize (SM) and sole peanut (SP) consisting of 16 rows, and 4 rows of maize intercropped with 2 rows of peanuts (M4P2), with 4 rows of peanuts (M4P4), and with 8 rows of peanuts (M4P8), with three replicates per treatment, as shown in Figure 2. Maize variety was Liangyu 99 selected by Dandong Denghai Seed Industry Co. Ltd., China, and the peanut variety was Nonghua11 selected by the Peanut Research Institute of Shenyang Agricultural University, China. The row distances of sole crop and intercropping was 0.55 m, and the length of the test plot was 50 m. The planting density of sole and intercropped maize was 7.5×10^4 plants ha^{-1}, and the planting density of sole and intercropped peanut was 1.5×10^5 plants ha^{-1}. Sowing was performed with direct seeding on 5 May 2019 and 7 May 2020. Harvesting was performed on 5 October 2019 and 6 October 2020. For intercropping and sole cropping, the amount of fertilizer applied to maize and peanut was the same. A compound fertilizer (contained 14% N, 16% P_2O_5, and 15% K_2O) was used as a basal fertilizer at a dose of 450 kg ha^{-1} at sowing time. There was no other form of fertilizer input during the growth period.

2.3 Measurements

2.3.1 Photosynthetically active radiation

According to the method recommend by Yang et al. (2014), the average PAR of the middle of the plot and adjacent to maize and peanut canopy (50 cm above the ground) was measured using a light meter (AccuPAR LP-80, United States) between 9:00 and 16:00 h on a clear sunny day. Measurements were taken at the anthesis stage, podding stage, and maturity stage of peanut, repeated three times.

2.3.2 Photosynthetic parameters

According to the method recommend by Wang et al. (2017) at the anthesis stage, podding stage, and maturity stage, photosynthetic parameters (*Pn*, *Ci*, *Tr*, and *Gs*) of the top three leaves on the main stem of peanut were measured at intervals of 2 h from 08:30 to 16:30 h with the LI-6400 XT portable photosynthesis system (LI-COR Inc., Lincoln, USA) equipped with a 2 cm × 3 cm clear chamber on a clear sunny day. The temperature and CO_2 concentration of the leaf chamber resembled the natural environment. The sampling location was in the middle of the plot and adjacent to maize, repeated three times.

2.3.3 Photosynthetic response curve

The photosynthetic response curves of the top three leaves of the peanut main stem were measured using the LI-6400 XT portable photosynthesis system (LI-COR Inc., Lincoln, USA). The parameters were measured on functional leaves from 09:00 to 11:30 h on a clear sunny day. The temperature and CO_2 concentration of leaf chamber were maintained at 25°C and 380 μmol mol^{-1}, respectively. PAR was increased from 0 to 1,500 μmol photons m^{-2} s^{-1} (0, 20, 50, 80, 100, 200, 400, 600, 800, 1,000, 1,200, and 1,500 μmol m^{-2} s^{-1}, 36 min). The sampling location was in the middle of the plot and adjacent to maize, repeated three times.

2.3.4 Chlorophyll content

Chla and Chlb contents were determined using the method of Guo et al. (2018) with slight modifications. Peanut leaves (0.2 g) were added to 80% acetone solution, shaken well, and extracted in the dark for 12 h.

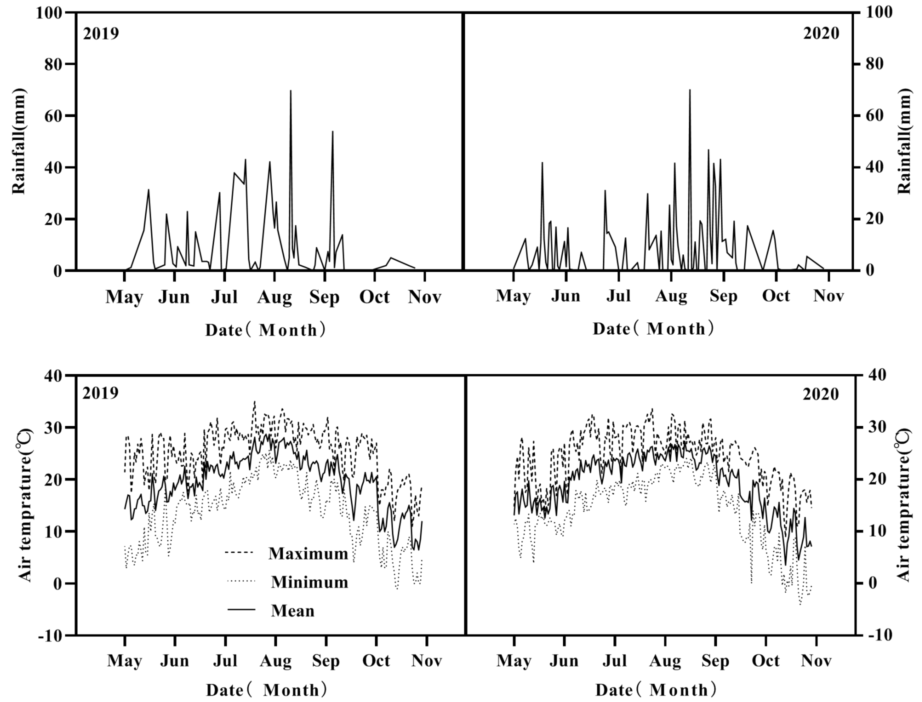

FIGURE 1
The air temperature and rainfall in the growing season of intercropping in 2019 and 2020.

The optical density (OD) values of the extracts were measured at 663 nm and 645 nm using 80% acetone solution as a control to calculate the chlorophyll a, chlorophyll b, chlorophyll (a+b) content, and the chlorophyll a/b values. The sampling was the upper three leaves of the main stem of peanut and was collected at the anthesis stage, podding stage, and maturity stage, respectively, located in the middle of the plot adjacent to the maize, and repeated three times.

2.3.5 Chlorophyll precursor content

δ-aminolevulinic acid (ALA) content was determined according to the method of Kumar Tewari and Charan Tripathy (1998) and Dalal and Tripathy (2012), with a slight modification: Fresh leaves (2 g) were ground with 6 ml of sodium acetate buffer (pH 4.6) in an ice bath, boiled in water for 15 min, centrifuged at 10,000 *g* for 20 min, and washed two times with 4 ml of extract. Supernatant (1 ml) was extracted, four drops of acetyl ethyl acetate was added to a boiled water bath for 15 min, an equal volume of Izod reagent was added, the absorbance value (A) at 553 nm was measured after 15 min, ALA-HCl (Sigma) was used as the standard sample to make a standard curve, and the ALA content (nmol g^{-1} FW) was calculated. The sampling was the upper three leaves of the main stem of the peanut and was collected at the anthesis stage, podding

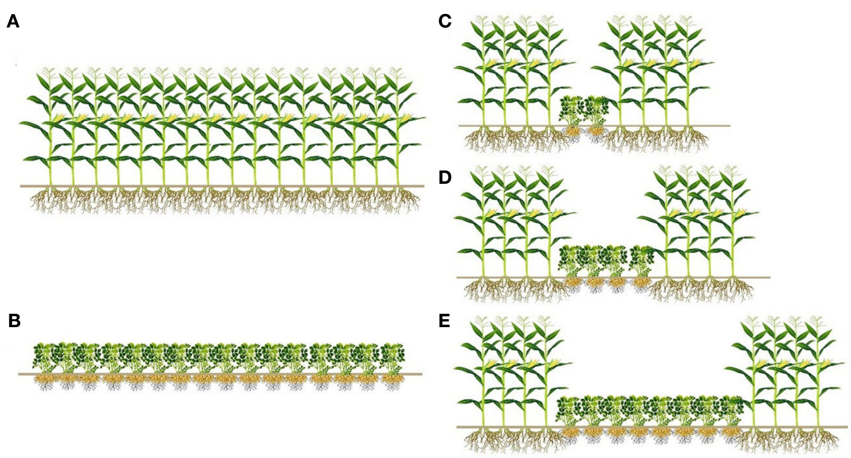

FIGURE 2
The layout of different row ratio configurations in intercropping of maize and peanut. **(A)** Sole maize (SM); **(B)** sole peanut (SP); **(C)** four rows of maize intercropping with two rows of peanut (M4P2); **(D)** four rows of maize intercropping with four rows of peanuts (M4P4); **(E)** four rows of maize intercropping with eight rows of peanuts (M4P8).

stage, and maturity stage, respectively, located in the middle of the plot adjacent to the maize, and repeated three times.

ProtoIX, Mg-ProtoIX, and Pchl content were determined according to the method of Hodgins and Van Huystee (1986) with slight modifications: Take fresh leaves (0.3 g) and place them in a precooled mortar. Add 5 ml of 80% alkaline acetone (acetone: 0.1 mol/L ammonia = 8:1, V/V) and grind in an ice bath. Homogenize at 18,000×*g*, centrifuge at 4°C for 15 min, extract the supernatant, and dilute to 25 ml with 80% alkaline acetone. Then, determine the absorbance values A628, A590 and A575 at 628-nm, 590-nm, and 575-nm wavelengths using a spectrophotometer. Finally, calculate the concentration of each substance according to the following formula and calculate the content in the sample (μ mol g^{-1} FW).

$$\text{ProtoIX} = 0.18016 \times \text{A575} - 0.04036 \times \text{A628} - 0.04515 \times \text{A590}$$

$$\text{Mg} - \text{ProtoIX} = 0.06077 \times \text{A590} - 0.01937 \times \text{A575} - 0.003423 \times \text{A628}$$

$$\text{Pchl} = 0.03563 \times \text{A628} + 0.007225 \times \text{A590} - 0.02955 \times \text{A575}$$

2.3.6 Dry matter

Five representative peanut plants were selected in each treatment at the anthesis stage, podding stage, and maturity stage. The plant samples were divided into roots, stems, leaves, and pods. Then, samples were baked in an oven for 30 min at 105°C, dried to constant weight at 85°C, and weighed. For determination of the dry matter accumulation amount, the dry weight of each sample was measured with an electronic balance (Heeyii JE-301, Hangzhou, China). The dry matter distribution rate (DDR) was calculated using the following formulas described by:

$$\mathbf{DDR}\,(\%) = \frac{\mathbf{DW}}{\mathbf{TDW}} \times \mathbf{100}\,\%$$

where DW is the dry weight of each organ and TDW is the total dry weight of each plant.

2.3.7 Yield and LER

The length of the ridge was 3 m and all middle rows of the intercropped maize and peanut were harvested, whereas the plants of 3 m × 8 rows in the middle of SP and SM were harvested to calculate yields at the maturity stage in 2019–2020. Then, 10 representative peanut plants were selected to measure the number of pods per plant, the number of full fruits per plant, the weight of 100 fruits, the rate of kernels, and the weight of 100 kernels.

$$\text{LER} = (\text{Y}_{\text{im}}/\text{Y}_{\text{mm}}) + (\text{Y}_{\text{ip}}/\text{Y}_{\text{mp}})$$

Y_{im} and Y_{mm} are the yields of the intercropped and sole maize, respectively, and Y_{ip} and Y_{mp} are the yields of the intercropped and sole peanut, respectively. LER > 1 denotes intercropping gain, and LER< 1 indicates intercropping loss.

2.3.8 Data analysis

Analysis of variance (ANOVA) was used to assess treatment effects on yield, dry matter and photosynthetic parameters, and year effect on fitted parameters using SPSS 20 (IBM, USA). Least significant differences (LSDs) were used to separate treatment differences in means at the 0.05 level. The graphs were made using Sigma plot (Version 12, Systat Software).

3 Results

3.1 Changes in photosynthetically active radiation in intercropped peanut canopy

There were significant differences in PAR in the canopy of different peanut row ratios (Table 1). Intercropping decreased the PAR of peanut canopy, and the smaller the peanut row ratio, the greater the PAR reduction. Compared with SP, the M4P8, M4P4, and M4P2 treatments decreased the PAR (mean of 2 years) by 7.34%, 26.28%, and 35.78% at the anthesis stage, 7.32%, 22.71%, and 31.45% at the podding stage, and 7.14%, 21.74%, and 33.71% at the maturity stage, respectively. Compared with M4P4 and M4P2, the PAR of M4P8 has significant advantages, which increased respectively by 25.72% and 38.84%, 20.03% and 35.47%, and 18.69% and 40.08%.

3.2 Changes in chlorophyll content and composition in intercropped peanuts

The peanut row ratio configurations had different effects on the chlorophyll content of peanut leaves (Figure 3). Chla and Chl(a+b) contents of M4P8 were significantly higher than SP, M4P4, and M4P2. Compared with the SP, M4P4, and M4P2 treatments, the M4P8 treatment increased the Chla content (mean of 2 years) by 8.20%, 27.40%, and 30.65% at the anthesis stage, 6.71%, 25.43%, and 29.77% at the podding stage, and 3.28%, 24.33%, and 29.62% at the mature stage, respectively. The Chl(a+b) content (mean of 2 years) increased by 8.10%, 20.26%, and 22.40% at the anthesis stage, 11.06%, 13.11%, and 15.01% at the podding stage, and 3.61%, 11.28%, and 12.37% at the mature stage, respectively. Compared with SP, the Chl(a/b) value (mean of 2 years) of M4P8 had no significant change in the three growth stages, but were 58.42%, 55.58%, and 66.38% and 62.77%, 65.05%, and 85.95% higher than that of M4P4 and M4P2, respectively. Intercropping increased the content of Chlb, but there was no significant difference between M4P8 and SP (except for 15.7% higher than SP at the anthesis stage in 2019), which was lower than that of M4P4 and M4P2.

3.3 Changes in chlorophyll precursors in intercropped peanuts

Intercropping peanut row ratio configurations affected the synthesis of peanut chlorophyll precursors and their conversion to chlorophyll. Overall, ALA, Proto IX, Mg-Proto IX, and Pchlide

TABLE 1 Effects of different peanut row ratio configurations on PAR of peanut canopy in intercropping of maize and peanut.

Year	Treatment	Anthesis	Podding	Maturity
2019	SP	1,296.03 ± 35.51a	1,372.77 ± 37.29a	1,482.69 ± 25.28a
	M4P8	1,210.56 ± 36.54b	1,269.44 ± 18.00b	1,395.96 ± 38.36b
	M4P4	976.31 ± 10.00c	1,058.92 ± 33.54c	1,159.94 ± 18.44c
	M4P2	871.64 ± 19.46d	968.13 ± 21.10d	991.70 ± 17.74d
2020	SP	1,219.97 ± 24.71a	1,339.43 ± 28.43a	1,457.95 ± 37.01a
	M4P8	1,121.36 ± 30.03b	1,246.80 ± 43.54b	1,335.04 ± 44.13b
	M4P4	879.82 ± 22.80c	1,037.45 ± 22.82c	1,140.83 ± 18.22c
	M4P2	746.63 ± 17.80d	891.81 ± 19.45d	957.70 ± 36.21d
p	Treatment	***	***	***
	Year	ns	ns	ns
	Treatment × Year	ns	ns	ns

Data are expressed as the mean of three replicates ± standard error (n = 3), and different letters indicate statistical difference significance at $p < 0.05$ among the treatments by LSD tests. *** significant at 0.001 level, ns is not significant. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

were significantly higher in M4P8 treatment than in SP; however, it was significantly lower in M4P2 and M4P4 treatment (Figure 4). The ALA content (2-year mean) of M4P8 was 8.51%, 9.46%, and 7.99% higher than SP (Figures 4A, B); the Proto IX content was 8.35%, 8.43%, and 11.14% higher than SP (Figures 4C, D); the Mg-Pto IX content was 7.70%, 8.91%, and 7.52% higher than SP (Figures 4E, F); and the Pchlide content was 7.44%, 7.30%, and 6.87% (Figures 4G, H) higher than SP at the flowering needle stage, pod stage, and maturity stage, respectively.

3.4 Diurnal variation of photosynthetic characteristics in intercropped peanut

Pn in peanut leaves increased rapidly with the increase of light intensity. When PAR reached 600 mol m^{-2} s^{-1}, the increase of *Pn* began to slow down and gradually approached the saturation state (Figure 5). At lower PAR, *Pn* was higher in intercropping peanuts; at higher PAR, *Pn* was higher in sole peanut (Figure 6). The diurnal variation of *Pn* all showed a single peak curve and was affected by the peanut row ratio configurations. Compared with SP, the M4P4, M4P2, and M4P8 treatment showed significantly lower maximum photosynthetic rates (2-year average), whereas the M4P8 treatment exhibited the smallest decrease and a relatively longer duration of high photosynthetic rates. Compared with SP, *Pn*, *Tr*, and *Gs* were significantly decreased in all three cropping patterns, with M4P8 showing the least reduction, followed by M4P4, and M4P2 with the greatest reduction (Figures 7A, B, G, H). Of these, the M4P8 pattern *Pn* (2-year mean) was reduced by 5.68%, 5.33%, and 5.30%; *Tr* by 7.41%, 5.45%, and 5.95%; and *Gs* by 8.20%, 6.88%, and 6.46% compared to SP, at the flowering needle stage, pod stage, and maturity, respectively. Conversely, intercropping promoted an increase in *Ci*, and this increased as the peanut row ratio decreased (Figures 7E, F), with the smallest increase in M4P8, followed by M4P4, and the largest in M4P2. Compared to SP, *Ci* increases by 11.95%, 8.06%, and 9.61% in M4P8 mode at the flowering needle stage, pod stage, and maturity, respectively.

3.5 Changes in dry matter accumulation and distribution rates of intercropped peanuts

The accumulations of per-plant and organ dry matter were significantly varied among the different treatments (Figure 8). The dry matter per plant in each growth stage was SP > M4P8 > M4P4 > M4P2, which reached a significant difference among different treatments. Compared with SP, the dry weight per plant of M4P8 (2-year mean) was decreased by 10.77%, 10.55%, and 15.92% at the anthesis stage, podding stage, and mature stage, respectively.

Intercropping significantly reduced the dry matter of pods, stems, and leaves at each stage, but there was no significant difference in root dry weight between M4P8 and SP treatments (Figure 8). All organs of M4P8 treatment were significantly higher than those of M4P4 and M4P2 treatments (except root dry weight at mature stage). The increase of intercropped peanut rows was beneficial to the accumulation of dry matter per plant and each organ.

The dry matter distribution ratios of organs were significantly different at each growth stage (Figure 9), which was affected by the intercropping peanut row ratio. Compared with SP, there were no significant differences in dry matter distribution ratios among organs in M4P8 treatments at the anthesis stage, podding stage, and mature stage, respectively (Figure 9). However, compared with SP, there were significant differences in dry matter distribution ratios among organs in M4P4 and M4P2. In particular, the dry matter distribution of M4P4 and M4P2 peanut pods was reduced by 12.24% and 15.59% and 16.47% and 21.23% at podding and harvest, respectively.

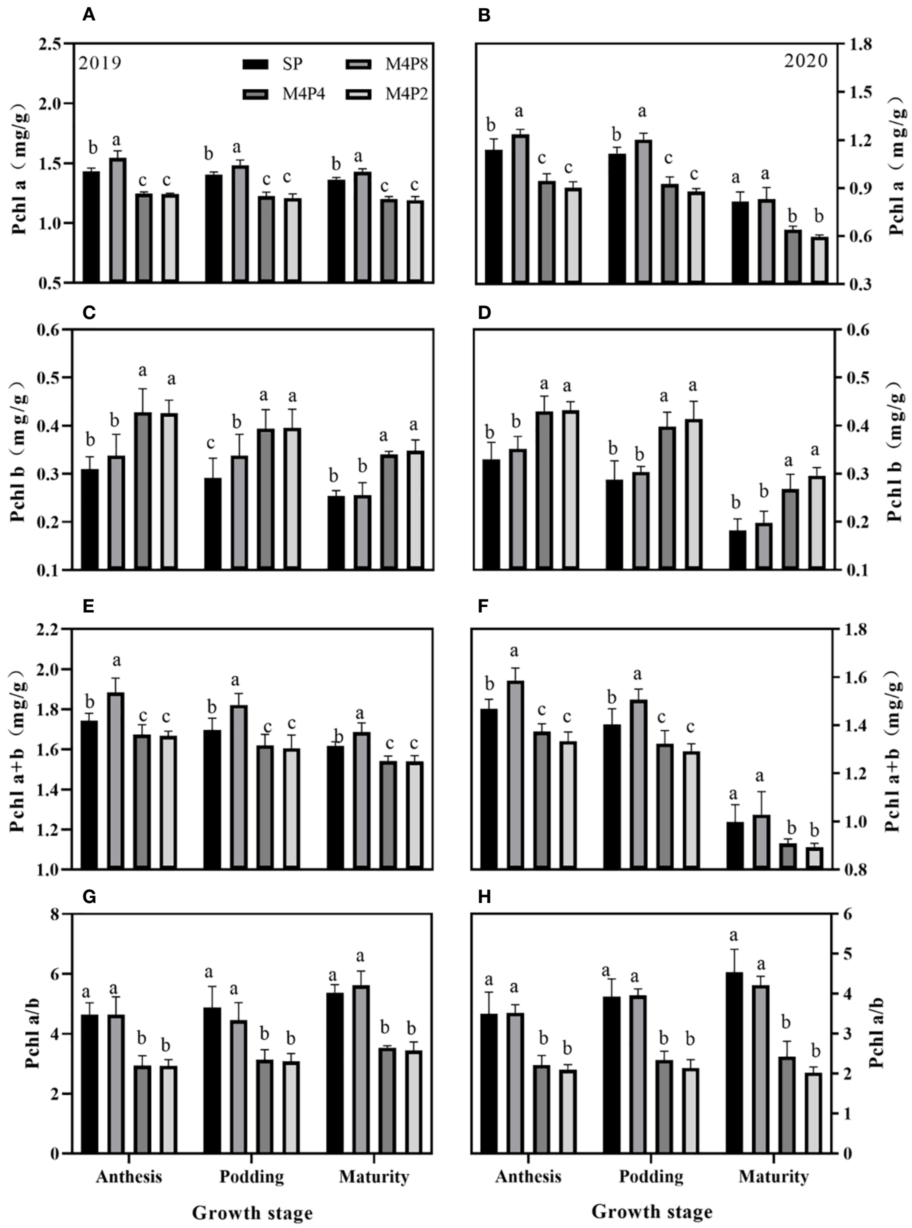

FIGURE 3
Effects of different peanut row ratio configurations on chlorophyll content in intercropped peanut. Different letters indicate statistical difference significance at $p < 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

3.6 Changes in yield and compositional of intercropping peanuts

Compared with SP, the yields of intercropped peanut were decreased, while the land use efficiency was significantly increased because the LERs of the three intercropping treatments were more than 1. The highest in M4P8 were 1.34 in 2019 and 1.31 in 2020, which were significantly higher than M4P4 and M4P2 (Table 2). With the increase of peanut row ratio, the pod yield of peanut was increased. Compared with SP, the yield of M4P8 treatment was decreased by 36.65%, but it is increased by 40.99% and 79.01%, respectively, compared with the M4P4 and M4P2 treatments. Compared with SP, the number of pods per plant in the intercropped peanut of M4P8, M4P4, and M4P2 treatments were decreased by 7.56%, 20.11%, and 26.08%. Compared with SP, the number of full pods in the M4P8, M4P4 and M4P2 treatments were decreased by 11.18%, 23.72%, and 30.45%, respectively. Compared with SP, the 100-kernel weight in the M4P8, M4P4, and M4P2 treatments were decreased by 5.93%, 13.44%, and 19.45%, respectively. Compared with SP, 100-seed weight in the M4P8, M4P4, and M4P2 treatments was decreased by 5.84%, 13.14%, and 16.98%, respectively. Compared with SP, the kernel ratio in the

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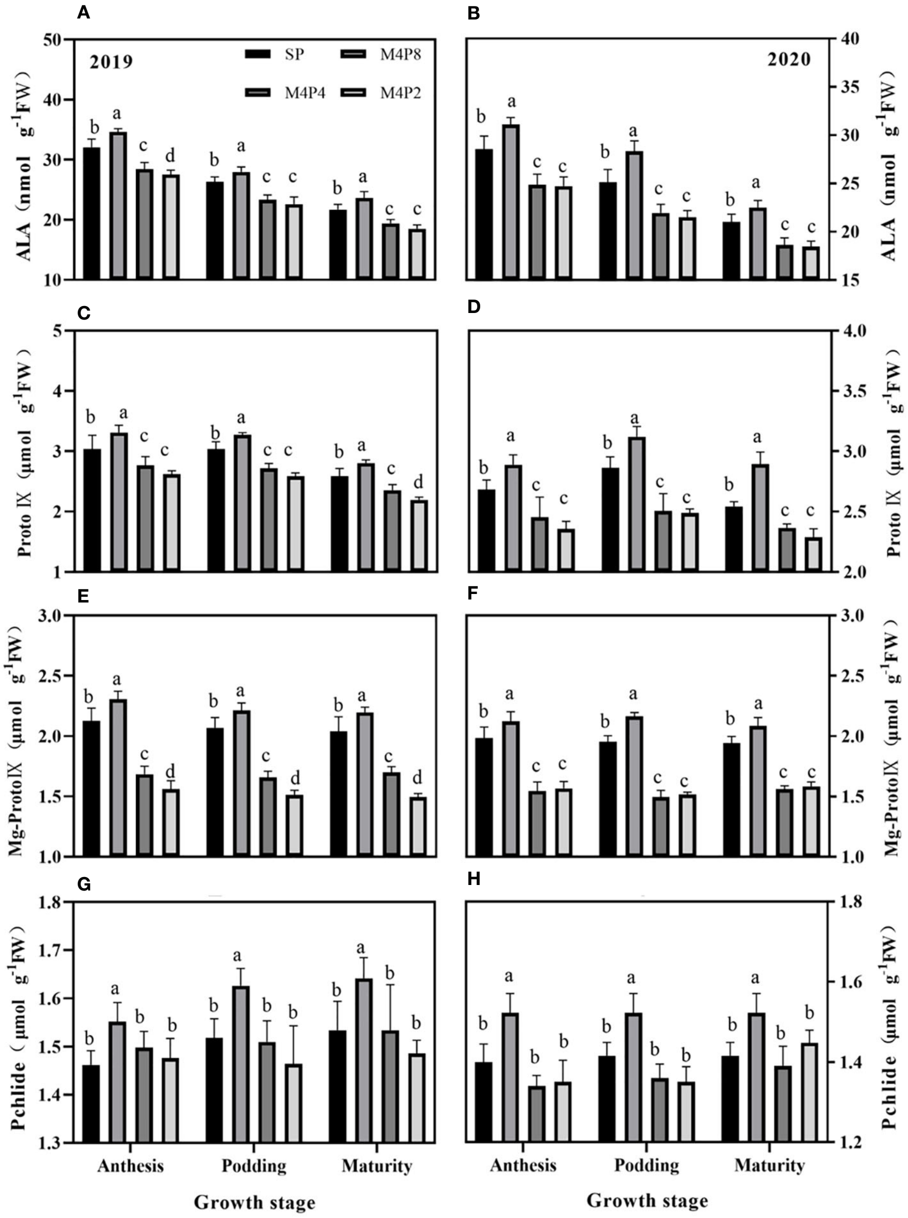

FIGURE 4
Effects of different peanut row ratio configurations on the content of chlorophyll synthesis precursors in intercropped peanut. Different letters indicate statistical difference significance at $p< 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

M4P8, M4P4, and M4P2 treatments was decreased by 8.98%, 16.99%, and 22.64%, respectively.

Compared with 2020, the average values of pod number, kernel ratio, and yield of maize in 2019 were significantly higher, while the 100-kernel weight and yield of peak were significantly lower than those in 2020. There was no significant difference in other indicators. Compared with SP, the full pod number, 100-kernel weight, kernel ratio, and yield of peanut in M4P8 (mean of 2 years) were significantly reduced, while there was no significant difference in the 2-year average values of pod number and 100-seed weight. SP and M4P8 were significantly higher than M4P4 and M4P2.

4 Discussion

4.1 Intercropping changes the light distribution and photosynthetic physiological characteristics of peanut canopy

Light is the most important environmental factor among many external factors that influence the synthesis and accumulation of photosynthetic products of crops (Huang et al., 2022). PAR is the energy source of crop life activities, organic matter synthesis, and

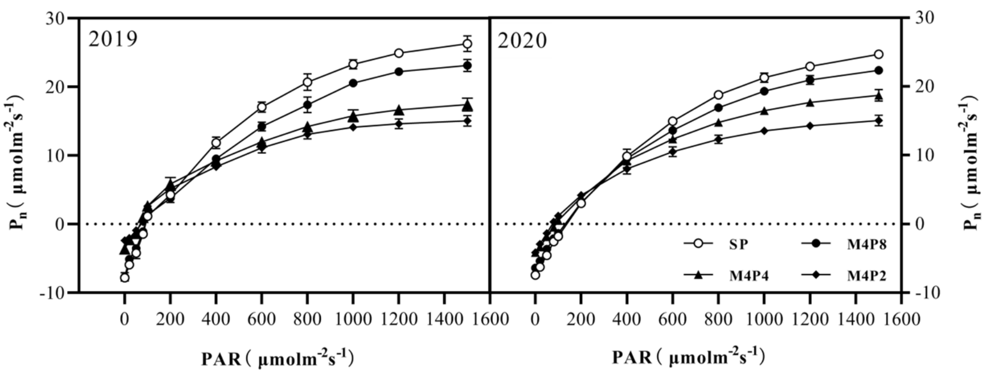

FIGURE 5
Effects of different peanut row ratio configurations on photosynthetic response curve of intercropped peanut. Different letters indicate statistical difference significance at $p< 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

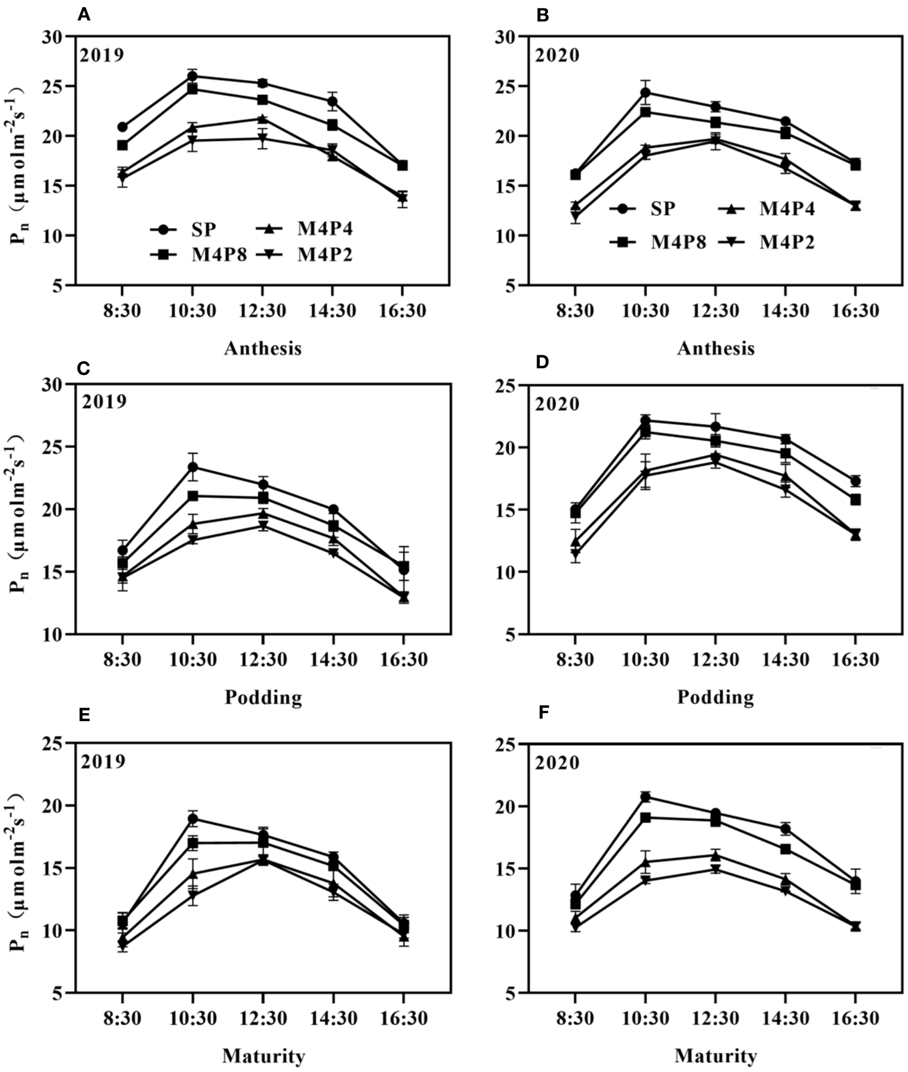

FIGURE 6
Effects of different peanut row ratio configurations on diurnal variation of net photosynthetic rate of intercropped peanut. Different letters indicate statistical difference significance at $p< 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

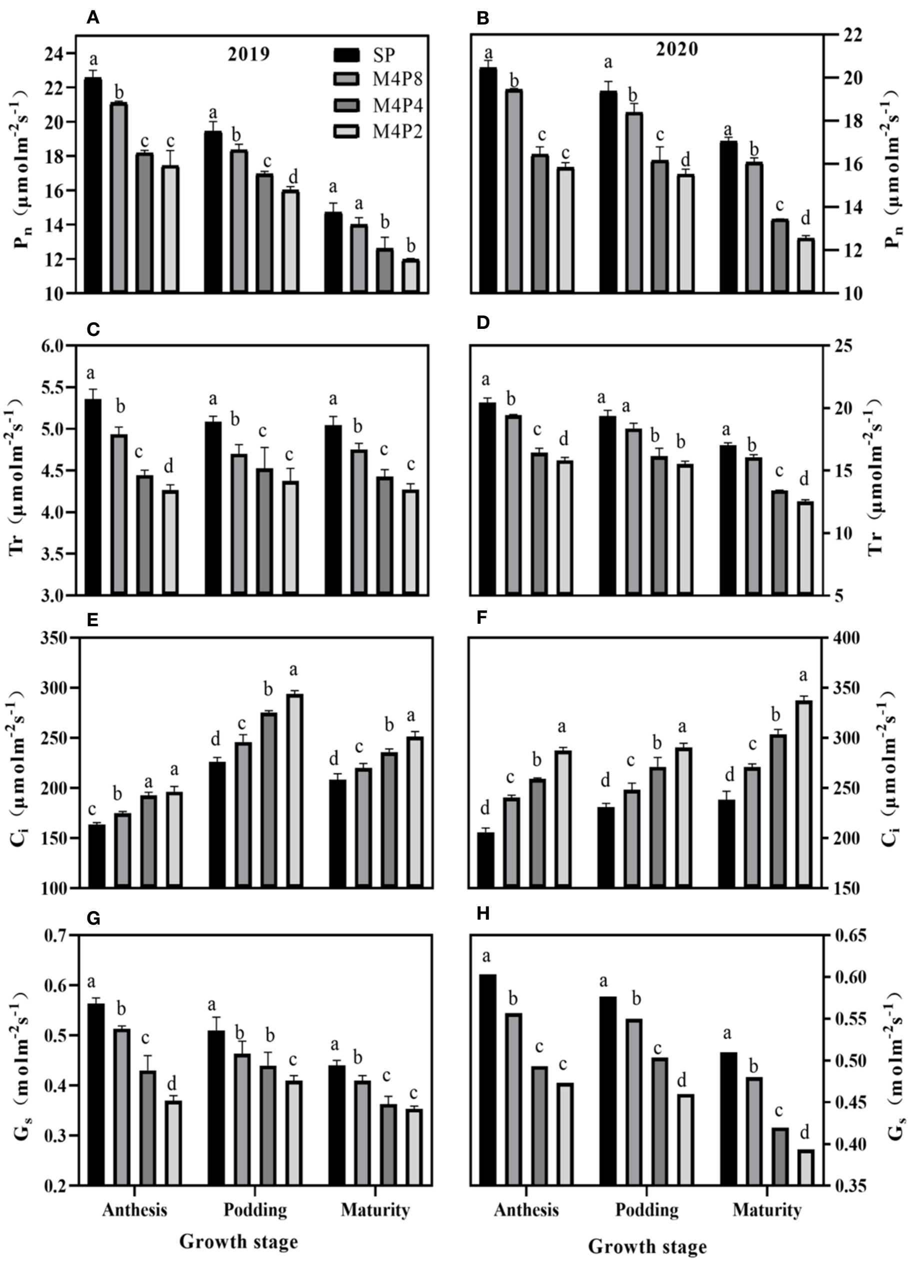

FIGURE 7
Effects of different peanut row ratio configurations on photosynthetic parameters of intercropped peanut. Different letters indicate statistical difference significance at $p< 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

yield formation. Whether PAR is too high or too low, the photosynthetic capacity of plants will be reduced (Abakumova et al., 2016; Gou et al., 2017). The synthesis and distribution of peanut photosynthetic products were directly inhibited by the intercepted light, which is mostly side light, leading to significant changes in the light environment. Previous research reports found that different row ratio configurations (Wang et al., 2021a), row spacing (Zhang et al., 2015), and planting density (Mao et al., 2014; Yang et al., 2021) influenced the light transmittance of intercropping composite populations. Our study found that intercropping reduces the photosynthetic effective radiation reaching the peanut canopy to varying degrees, compared with monoculture. This is due to the fact that maize at a high position would produce a shading effect on peanuts, resulting in significant differences in population light distribution and light transmittance between different planting patterns. In this study, the degree of reduction in PAR in the peanut canopy was strongly correlated with the setting of the peanut row ratio; i.e., there was a significant advantage in light radiation in the M4P8 treatment and the peanut canopy PAR was significantly higher than in the M4P4 and M4P2 models (Table 1). The main reason was that the intercropping system has three-dimensional optical characteristics, which can

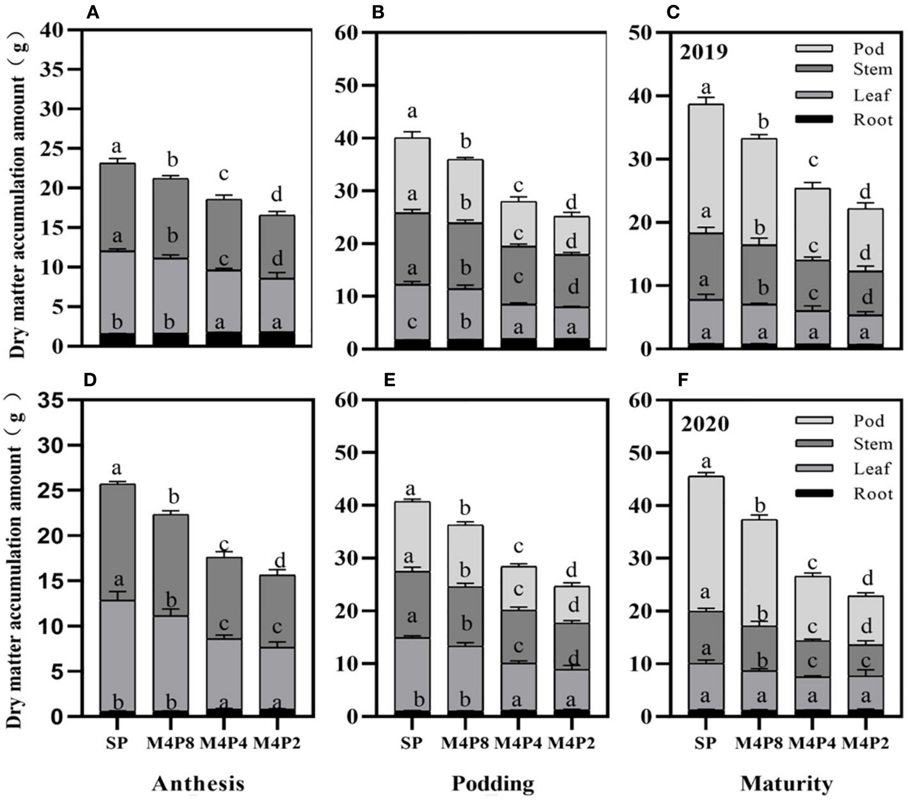

FIGURE 8
Effects of different peanut row ratio configurations on dry matter accumulation in intercropped peanut. Different letters indicate statistical difference significance at $p < 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

promote the utilization efficiency of high-position crops for strong light and low-height crops for weak light, and realize the efficient utilization of light in the system (Wang et al., 2021a). This is consistent with previous studies in strip intercropping systems with different row ratio configurations that have found that the ability of two species to compete for light varies with ecological niche when the light environment of the system is changed (Zhang et al., 2015; Umesh et al., 2023). In the M4P8 pattern, interspecific competition diminished and peanut had a greater advantage in light radiation. These results demonstrate that row-ratio configuration is one of the principal factors that regulate the photosynthetic product synthesis and distribution of intercropped peanut.

Photosynthesis determines the future of agricultural production (Fan et al., 2019). Photosynthetic physiological characteristics have always been an important part of many scholars' research. For instance, Gong et al. (2020) found that the *Pn* values of the upper, middle, and lower layers of the millet canopy were significantly higher than SP by 8.8%–32.5%, 16.0%–46.3%, and 25.0%–114.4% ($p < 0.05$), respectively, under the millet/mung bean intercropping system. Wang et al. (2021a) found that the LI of maize was 23.4% higher than that of the control, and the LI of shaded peanut was 32.2% lower in the study of maize and peanut strip intercropping. LI of intercropped maize increased with the increase of BRP. The LI of peanut decreased with the decrease of BRP, but there was no significant difference between the M6P6 and M8P8 treatment. Our study found that the *Pn*, *Tr*, and *Gs* decreased in intercropped peanut, while *Ci* increased in intercropped peanut (Figure 7). The decline in *Pn*, *Tr*, and *Gs* in intercropped peanut was due to shade from intercropped maize, which inhibited photosynthesis in peanut. While *Pn*, *Tr*, and *Gs* in the M4P8 treatment was lower than in sole peanut, they were significantly higher than in the M4P4 and M4P2 treatments (Figure 7), indicating that the row ratio configuration of the intercropping system was beneficial in alleviating the effects of shade from intercropped maize on the reduction of photosynthetic rates in the canopy leaves of peanut. Compared with sole peanut, *Ci* increased in the functional leaves of intercropped peanut, of which the smallest increase was observed in the M4P8 treatment. These results indicated that the decrease in photosynthetic capacity of intercropped peanut was caused by non-stomatal limitation (Gong et al., 2015; Yao et al., 2017; Han et al., 2022) and that the row ratio configuration of the M4P8 treatment was favorable to the interception and uptake of PAR by peanut leaves in the maize peanut intercropping system, thus improving the photosynthetic characteristics of the leaves.

4.2 Intercropping changed synthesis of chlorophyll and its precursor in peanut leaves

Chlorophyll is the main carrier of plant photosynthesis, and Chla determines photosynthesis, while Chlb determines the breadth

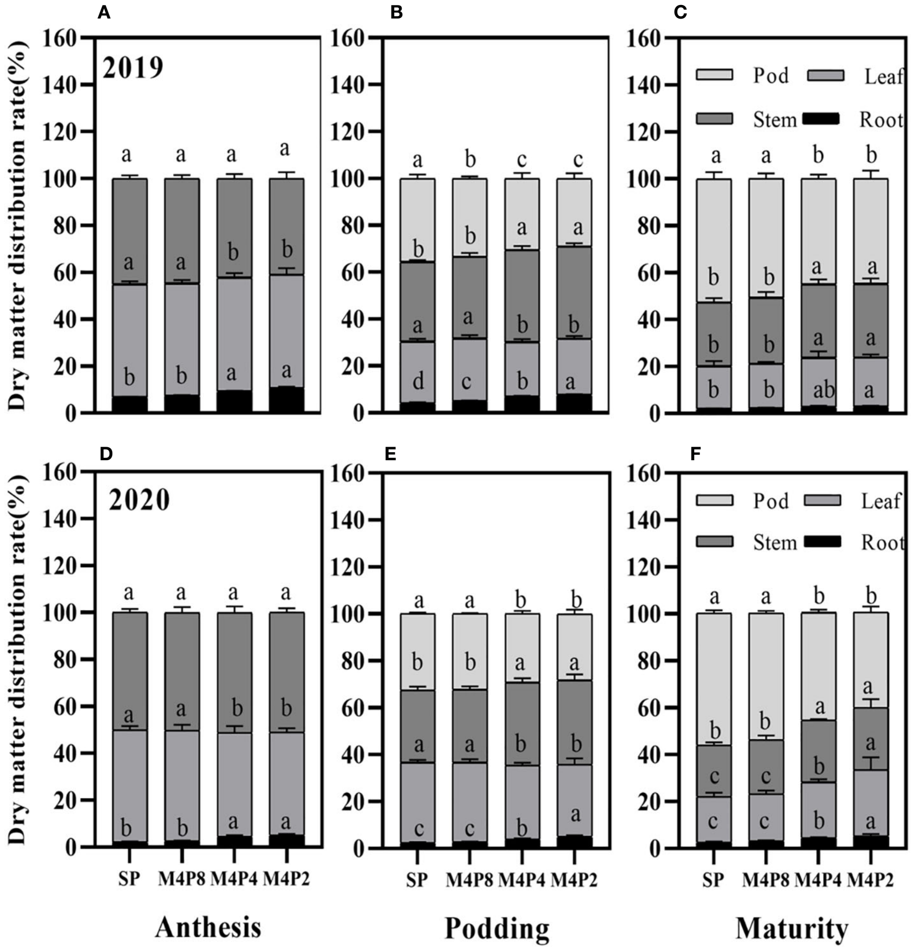

FIGURE 9
Effects of different peanut row ratio configurations on dry matter distribution of intercropped peanut. Different letters indicate statistical difference significance at $p< 0.05$ among the treatments by LSD tests. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

of the spectrum utilized (Brestic et al., 2015). Studies have shown that intercropping increases the relative chlorophyll content of intercropped oats, peanuts, and soybeans (Yao et al., 2017; Bernas et al., 2021; Shi et al., 2021). Our study showed that different row ratio configurations changed chlorophyll content and photosynthetic efficiency in peanut, with the M4P8 pattern increasing the content of Chla, Chlb, and Chla+b in leaves, decreasing the ratio of Chla/b, and enhancing the efficiency of strong and weak light utilization (Figure 3). Under the M4P4 and M4P2 models, the content and proportion of Chlb increased, which enhanced the use of low light, but the Chla and Chla+b content was significantly reduced as a result of the high level and duration of shade, which was detrimental to the use of strong light and reduced photosynthetic capacity. It is clear that the intensity of light has a direct effect on the synthesis, the content, and the distribution of chloroplasts, and that peanut maintains cellular energy balance by regulating the structure and function of its photosynthetic machinery to adapt to changes in the environment (Li et al., 2019).

Chlorophyll synthesis is a series of enzymatic catalytic processes, and insufficient or too much light can affect chlorophyll biosynthesis (Banas et al., 2011; Ashraf and Harris, 2013; Wang et al., 2017). In this study, the change in trend of ALA content and chlorophyll content was consistent (Figures 4A, B), and the difference of ALA content was the important reason for the difference of chlorophyll content in peanut leaves under different intercropping. In SP, excessive illumination causes light inhibition, and Heme accumulation inhibits the synthesis of ALA. In contrast, the M4P8 pattern has relatively little effect of shade from maize to peanut, avoiding strong light inhibition and weak light stress to promote ALA synthesis, providing strong conditions for chlorophyll synthesis in this pattern.

The transformation of Proto IX into Mg Proto IX is an important branch of the chlorophyll synthesis pathway, and Mg proto IX is a sign that Proto IX enters the chlorophyll synthesis pathway (Kopečná et al., 2015; Liu et al., 2018). In this study, the results indicated that the contents of Proto IX and Mg Proto IX in the M4P8 pattern were significantly higher than those in SP (Figures 4C–F), which was consistent with the change in trend of ALA and chlorophyll content, indicating that chlorophyll synthesis did not change under this pattern. With the reduction of peanut row ratio, the decrease of Mg Proto IX content in M4P4 and M4P2 models was significantly greater than that

TABLE 2 Effects of different peanut row ratio configurations on yield and yield components and LER in intercropping of maize and peanut.

	Treatment	Yield components					Yield (kg ha^{-1})		LER
		Pod number	Full Pod number	100-kernel weight (g)	100-seed weight (g)	Kernel ratio (%)	Peanut	Maize	
2019	SP	24.45 ± 0.59a	14.89 ± 0.67a	168.82 ± 4.77a	65.38 ± 2.36a	48.7 ± 0.22a	4,144.73 ± 117.99a	11,077.68 ± 82.85a	1.00
	M4P8	23.10 ± 0.55a	13.11 ± 0.51b	158.71 ± 3.71a	60.68 ± 2.32a	44.6 ± 0.32b	2,634.15 ± 137.82b	7,843.63 ± 563.65b	1.34 ± 0.01a
	M4P4	20.23 ± 0.77b	11.44 ± 0.59c	145.83 ± 3.85b	54.49 ± 1.46b	40.3 ± 0.42c	1,924.42 ± 112.17c	8,399.73 ± 457.21b	1.22 ± 0.01b
	M4P2	19.11 ± 0.67b	10.89 ± 0.45c	137.11 ± 5.58b	53.41 ± 1.81b	38.3 ± 0.12d	1,485.67 ± 82.31d	10,258.87 ± 458.88a	1.25 ± 0.01b
	Mean	21.72	12.58	152.62*	58.49	42.98	2,547.24*	9296.58	1.27
2020	SP	26.78 ± 0.64a	16.00 ± 0.59a	161.34 ± 3.18a	62.62 ± 1.58a	55.6 ± 0.23a	3,771.28 ± 74.65a	13,365.16 ± 560.96a	
	M4P8	25.67 ± 0.50a	14.33 ± 0.35b	151.86 ± 3.16b	59.81 ± 1.47ab	50.3 ± 0.63b	2,381.29 ± 115.76b	9,007.53 ± 423.09b	1.31 ± 0.01a
	M4P4	22.00 ± 0.50b	12.11 ± 0.48c	139.94 ± 2.17c	56.60 ± 2.66bc	46.3 ± 0.52c	1,641.07 ± 96.22c	9,966.32 ± 616.48b	1.18 ± 0.01b
	M4P2	19.89 ± 0.37c	10.56 ± 0.40d	128.88 ± 1.33d	52.82 ± 2.10c	42.3 ± 0.53d	1,317.64 ± 109.29b	11,187.07 ± 458.54a	1.19 ± 0.01b
	Mean	23.58*	13.25	145.51	57.96	48.63*	2,277.82	10881.52*	1.22
Means (2-year average)	SP	25.62 ± 0.66a	15.44 ± 0.44a	165.08 ± 2.87a	64.00 ± 1.35a	52.15 ± 0.98a	3,958.00 ± 90.30a	12,221.42 ± 474.36a	1.00
	M4P8	24.38 ± 0.46a	13.72 ± 0.33b	155.29 ± 2.33b	60.24 ± 1.23a	47.45 ± 1.04b	2,507.72 ± 91.50b	8,425.58 ± 374.07b	1.32 ± 0.01a
	M4P4	21.12 ± 0.48b	11.78 ± 0.37c	142.89 ± 2.22c	55.54 ± 1.40b	43.3 ± 0.56c	1,782.74 ± 82.42c	9,183.03 ± 438.65b	1.20 ± 0.01b
	M4P2	19.50 ± 0.37c	10.72 ± 0.29d	133.00 ± 2.93d	53.12 ± 1.24b	40.3 ± 0.44d	1,401.66 ± 67.59d	10,526.17 ± 351.68a	1.22 ± 0.01b
	Treatment	***	***	***	***	***	***	***	***
p	Year	***	ns	*	ns	***	*	*	ns
	Treatment × Year	*	ns	ns	ns	ns	ns	ns	ns

Data are expressed as the mean of three replicates ± standard error (n = 3). and different letters indicate statistical difference significance at p< 0.05 among the treatments by LSD multiple range tests. * significant at 0.05 level, *** significant at 0.001 level, ns is not significant. SP: Sole peanut; M4P2: four rows of maize intercropped with two rows of peanut; M4P4: four rows of maize intercropped with four rows of peanuts; M4P8: four rows of maize intercropped with eight rows of peanuts.

in M4P8, which may be related to the strong canopy and long shading time, limiting the expression of MgPEC synthase gene and inhibiting chlorophyll biosynthesis. Moreover, studies found that the wheat root acid, citric acid, and other plant iron carriers secreted by maize roots can increase iron absorption of peanuts in maize and peanut intercropping (Xiong et al., 2013; Guo et al., 2014). More Proto IX combines with Fe^{2+} to form Heme. There is an obvious competition between Mg proto IX and Heme in the metabolic process of tetrapyrrole in plants. The heme produced by the combination of Proto IX and Fe^{2+} can regulate feedback and inhibit the synthesis of ALA (Yang et al., 1995). Hence, the conversion efficiency of Proto IX to Mg Proto IX decreased, resulting in the insufficient yield of Mg Proto IX and the accumulation of heme.

Our study found that compared with SP, the content of Pchlide did not significantly change under M4P4 and M4P2 (Figures 4G, H), but the content of its transformation product Chla and the previous product Mg Proto IX was significantly reduced. In the process of chlorophyll synthesis, a step of synthesis is blocked, its precursor substances will accumulate, and the subsequent precursor substances will decrease (von Gromoff et al., 2008). Our results indicated that Pchlide was blocked in the conversion process of synthetic chlorophyll a resulting in the reduction of chlorophyll content in M4P4 and M4P2, while this phenomenon does not occur in M4P8. The above results show that maize interplanting can promote the effective use of light energy by changing the row ratio configuration, thereby improving the photosynthetic capacity of leaves and the accumulation of the photosynthetic product.

These findings demonstrate that the negative effects on chlorophyll synthesis in peanut due to high intensity and duration of maize shade can be effectively reduced by increasing the number of rows of peanut in a strip intercropping system. Meanwhile, we hypothesize that, in practice, in a strip intercropping system, patterns such as four rows of maize and eight rows of peanuts can promote the effective use of light energy in the peanut canopy, which is conducive to chlorophyll synthesis in intercropped peanut leaves, thus improving the photosynthetic capacity of leaves and photosynthetic product accumulation.

4.3 Dry matter accumulation, distribution, and yield formation of intercropping composite population

The transfer of dry matter from other organs to the pod at the late growth stage determined the peanut yield. This study found that the dry matter accumulation and distribution rate between organs of intercropped peanut were lower than those of monocrop peanut, and these results were like those of intercropped maize and soybean (Yang et al., 2014). Furthermore, intercropping reduces the photosynthetic characteristics of peanut canopy, thereby inhibiting the accumulation and distribution of photosynthetic products, and the dry weight distribution ratio of each organ in M4P8 was significantly higher than that in M4P4 and M4P2 (Figures 8, 9). This was partly due to the fact that proper row ratio configuration improved the photosynthetic characteristics of dwarf crop peanut. These results showed that the M4P8 treatment could increase the accumulation of dry matter among organs, improve the distribution of dry matter among organs, and then promote the transport of dry matter from vegetative organs to grains.

Intercropping had an overall yield advantage and improved the land use efficiency (Martin-Guay et al., 2018). In this study, under different intercropping treatments, LERs were greater than 1, indicating that the total yield of the intercropping system was increased within limited area (Table 2). Wangiyana et al. (2021) found more green leaves and greener leaves of intercropped sweetcorn compared to monocropped ones, which supported higher grain yield under intercropping (Wangiyana et al., 2021); this is consistent with our findings. The yield advantage of intercropping is mainly due to maize. Although the peanut pod relative yield in M4P8 was lower than that in monoculture, the yield and LER in M4P8 were the highest compared with M4P2 and M4P4 (Figure 10). These results indicated that increasing row ratio of intercropped peanut could optimize population structure, reduce shading effects by maize, and improve light energy utilization rate and dry matter accumulation of the population (Wang et al., 2020). It could be seen that the reasonable configuration alleviated the yield reduction caused by the inferior position of dwarf crops in the intercropping system (Zhang et al., 2015). Our study found that the full pod number decreases as the peanut row ratio decreases and increases with year, but there is no significant difference between years. In M4P8, the yield components were significantly better than other intercropping modes, and the optimization effect was relatively ideal. Therefore, it was necessary to appropriately increase the number of peanut planting lines in the maize peanut intercropping system, improve the interspecific competitiveness of peanut, and ensure the yield advantage of the intercropping system.

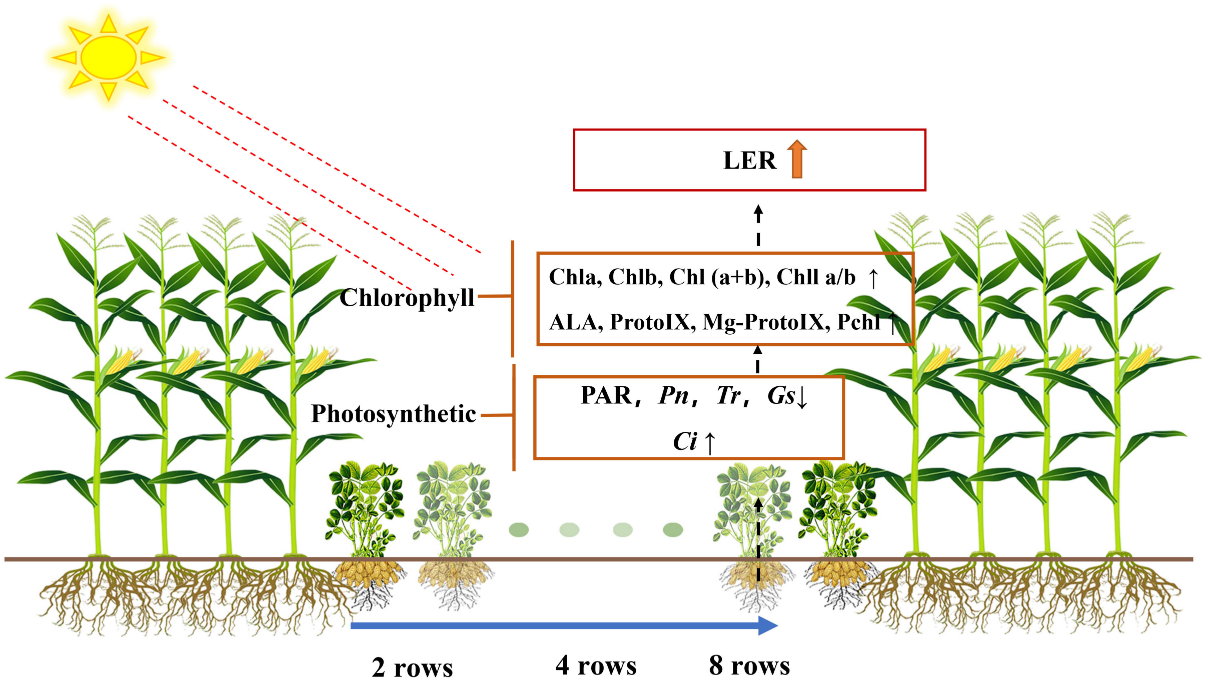

FIGURE 10
Effect of changes in photosynthetic characteristics of intercropped peanut with different row ratio configurations on intercrop yield and land equivalent ratio.

5 Conclusion

Different peanut row ratio settings change the peanut canopy PAR. The difference of PAR under different intercropping modes affects the photosynthetic physiological characteristics of peanut. The very small intercropping peanut row ratio hinders the synthesis of peanut chlorophyll. The insufficient synthesis of ALA, the reduction of the conversion efficiency of Proto IX to Mg-Proto IX, and the obstruction of Pchlide in the conversion process of synthesizing chlorophyll a are the root causes of the difference, thus reducing the photosynthetic capacity of peanut functional leaves, affecting the yield of the intercropping system. As the best row ratio configuration of maize intercropping, the M4P8 model has significant yield advantages, improves land use efficiency, and contributes to sustainable agriculture.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

HY, XZ, and JL designed this study. JL and QD conducted the data analysis and wrote the manuscript. GL, ZH, and DZ carried out the field experiments. HZ helped data analysis. XW, CJ, XL, ZZ, and SW revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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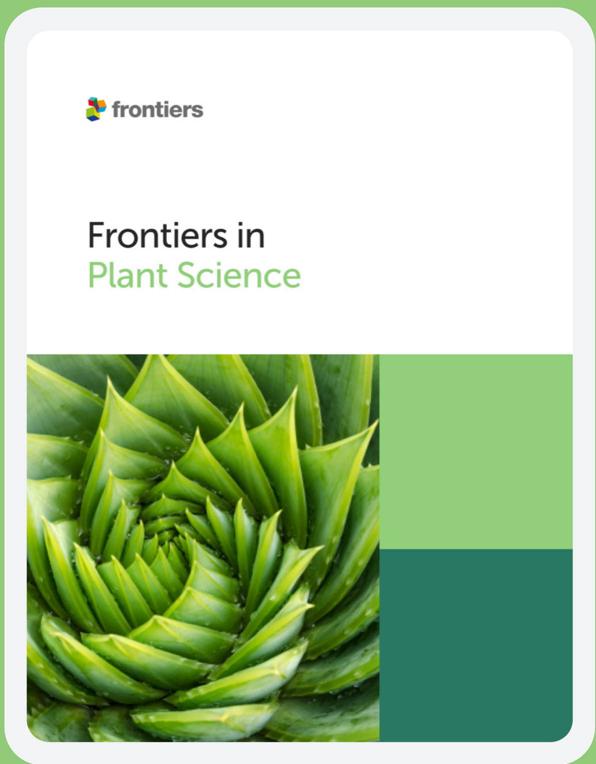

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