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Research Article

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Posted Date: April 17th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9290990/v1>

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Additional Declarations:

No competing interests reported.

Table 1 and 2 are available in the Supplementary Files section.

Optimizing maize-soybean strip intercropping: effects of row management and maize density on photosynthetic enzyme activity, grain quality, and yield in subtropical China

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Abstract

In subtropical agroecosystem of Guangxi, China, where land availability is constrained optimizing the maize-soybean strip intercropping system (IS) is critical for sustainable intensification of smallholder farming. A two-year field experiment (2022–2023) investigated the effects of two distinct row configurations: four maize rows intercropped with four soybean rows (F) and two maize rows intercropped with two soybean rows (T), under three maize planting densities (D1: 45,000; D2: 52,500; D3: 60,000 plants ha⁻¹). Maize and soybean monocultures were included as controls. Over two growing seasons, key yield components, grain nutritional profiles, photosynthetic enzyme activities, and land equivalent ratio (LER) were measured. Intercropping resulted in a significant increase in maize yield compared to monoculture, with increases ranging from 23.24%–45.34%. The T configuration consistently outperformed the F configuration in terms of system-level productivity. LER for both IS ranged from 0.93 to 1.21, with the TD2 treatment maintaining an LER > 1 in both years, demonstrating enhanced land use efficiency. Additionally, optimized intercropping significantly increased

maize crude protein content, with an average increase of 1.79% to 26.79% across years. In contrast, soybean grain quality improved significantly under intercropping, with soluble sugar, starch, and crude fat content increasing by 10.24% and 13.57%, 14.89% and 19.18%, and 7.39% and 7.97%, respectively. The superior performance of the TD2 was associated with enhanced photosynthetic enzyme activity, particularly the 44.36% increase in RuBisCO activity, and greater post-anthesis dry matter accumulation. Correlation analysis revealed strong positive relationship between dry matter accumulation, photosynthetic efficiency, and yield formation. Among the maize-soybean intercropping combinations tested, TD2 provided the optimal balance between yield, grain quality, and land-use efficiency, offering a sustainable and economically viable solution for enhancing productivity and resource use efficiency for smallholder farmers in subtropical regions.

Keywords:

Maize-Soybean Intercropping; Land Equivalent Ratio; Biological Nitrogen Fixation; Carbon Sequestration; Grain Quality; Resource Use Efficiency

Introduction

Global population growth and shifting dietary demands are expected to increase pressure on the global food system, as the world population is projected to surpass 9.7 billion by 2050, alongside a continued rise in food demand [1]. At the same time, climate change and the loss of arable land limit productive capacity, reducing yield gains in many regions [2]. In this context, accelerating sustainable agricultural practices is essential to ensure crop productivity and food security [3]. Maize and Soybean, as important staple crops, provide substantial

amounts of carbohydrates, protein, and other essential nutrients [4, 5, 6]. The intercropping system, a traditional agricultural practice, can improve resource-use efficiency and yield under limited resources [4, 7]. Among these systems, maize-soybean intercropping, a classic combination of grass and legume crops, plays a vital role [8]. Many studies have shown that maize-soybean intercropping, which involves growing both crops together in specific spatial arrangements, can increase resource use efficiency and improve yield by leveraging complementary differences in growth patterns and spatial requirements, ultimately enhancing nutrient uptake and improving overall productivity and system resilience [7, 9, 10].

Proper canopy structure is crucial for improving light interception and photosynthetic efficiency [11]. Crops in intercropping system (IS) can make better use of resources, including light, which gives them a competitive edge and leads to higher yield. The increased yields in IS are due to improved resource use by both vegetative and grains. However, in systems involving maize and soybean, which have different morphological and physiological attributes, their responses to photosynthetic assimilates can vary. Maize, being taller, can intercept more radiation and maintain better photosynthetic performance [12]. In contrast, soybean is the shorter and more prone to shading, suffers from reduced light exposure in intercropping systems, leading to lower photosynthetic efficiency, weaker stem strength, quicker leaf aging, and less efficient nutrient distribution, all of which increase lodging and yield loss [13, 14, 15]. Previous studies have focused on the photosynthetic capacity of maize under monoculture, but the different light environments created by intercropping systems affect the distribution of carbohydrates and other nutrients, which in turn influences the source–sink relationship of both maize and soybean and their yield formation [16]. Therefore, optimizing intercropping

configurations, particularly row arrangement and plant density, is key to improving productivity in land limited areas [17].

Nitrogen, an essential nutrient, plays a major role in photosynthesis, particularly in leaves, where it is a major component of the photosynthetic machinery [18,19]. However, light interception in the middle and lower canopy layers is often much lower than the nitrogen concentration there, which limits photosynthetic capacity. Martin-Guay et al. [20] highlighted the broader environmental benefits of intercropping systems, such as increased biodiversity and enhanced ecosystem services in intercropped fields. In addition, nutrient synergies in cereal-legume intercropping have been well documented, showing improved nitrogen use efficiency and soil nitrogen availability [21]. The role of deep-rooted legumes in enhancing nutrient cycling within intercropping systems has been emphasized by Odone et al. [22], as legumes can access and recycle nutrients from deeper soil layers. However, despite this evidence, there is still a significant gap in understanding how different intercropping patterns affect maize growth, particularly in different agroecological zones. Previous studies have highlighted the importance of conducting site-specific studies to understand the effectiveness of intercropping strategies in various environments [23, 24]. Furthermore, Wang et al. [25] highlighted the complexity of intercropping effects and limited understanding of how these systems impact photosynthesis, carbohydrate distribution, and yield components.

This research gap offers an opportunity to explore and quantify the benefits of intercropping and nutrient management in maize production. We hypothesize that specific intercropping patterns and improved nutrient management can significantly enhance maize growth, yield, and quality, contributing to improved food security and agricultural

sustainability [26]. Additionally, the study addresses crucial environmental issues, such as soil erosion and biodiversity loss, which are closely linked to agricultural productivity. Nearly 66% of the global population is currently affected by malnutrition [27], and soil degradation is affecting the soil health of cultivated soils [28], highlighting the need for sustainable agricultural practices that prioritize soil health while maintaining high yield.

This research aims to explore the effects of maize-soybean intercropping on key physiological processes and yield outcomes. Specifically, we hypothesize that intercropping configurations, when optimized for light and N utilization, will enhance maize photosynthetic efficiency and N uptake, leading to improved crop productivity. The study will focus on the following objectives: 1) to determine whether maize-soybean intercropping can optimize the interaction between light availability and N assimilation, enhancing photosynthesis and N uptake in maize; 2) to elucidate the physiological changes in maize leaves across different canopy levels in various cropping systems; and 3) to determine whether improved light interception can delay leaf senescence, increase photosynthetic accumulation, optimize the source-sink relationship, ultimately boosting crop yield. The outcomes of this study will deepen our understanding of the dynamics of maize-soybean intercropping systems and inform the development of sustainable agricultural practices that maximize resource efficiency and food security in subtropical regions.

Materials and Methods

Experimental site characteristics and climate overview

A two-year field experiment was conducted at the Agronomy Research Farm of Guangxi University (22°50'N, 108°17'E) during the 2022 and 2023 growing seasons. The experimental

site is located in southern China, characterized by a humid subtropical monsoon climate. The average annual relative humidity is approximately 75–80%, with a mean annual temperature of 22.8°C during the study period. In 2022, the annual precipitation totaled 1097.2 mm, while in 2023 it was 1034.2 mm. Total solar radiation reached 4353.2 MJ m⁻² in 2022 and 4007.9 MJ m⁻² in 2023 (Fig. 1). According to the international soil texture classification, the soil at the experimental site is classified as red loam [29]. Before sowing in 2022, composite soil samples were collected from the top 0–20 cm of soil at the experimental field to determine initial soil physiochemical properties. The following values were measured: soil bulk density 1.45 g cm⁻³, total nitrogen 1.09 g kg⁻¹, available phosphorus of 13.9 mg kg⁻¹, available potassium of 121.6 mg kg⁻¹, soil organic matter of 14.0 g kg⁻¹, pH of 6.30, and field capacity of 48.7%.

Plant Materials and Experimental Design

The maize cultivar Bama Pearl Yellow, widely used in intercropping systems (IS) in the region, and the soybean variety Guichun 15 were selected for this study. The soybean variety Guichun 15 was purchased from Guangxi Zhaohe Seed Industry Co., Ltd. (Nanning, Guangxi, China), and the maize cultivar Bama Pearl Yellow was gifted by the Bureau of Agriculture and Rural Affairs of Bama Yao Autonomous County (Guangxi, China). All seeds were certified and stored at 4°C prior to sowing.

The field experiment included maize monoculture (CKM, 5.25×10^4 plants ha⁻¹) and soybean monoculture (CKS, 2×10^5 plants ha⁻¹) as control treatments. The intercropping treatments were arranged in a randomized complete block design (RCBD) with a split-plot arrangement with three replicates. Two maize-soybean strip intercropping patterns were assigned to the main plots: F (four rows of maize intercropped with four rows of soybean) and

T (two rows of maize intercropped with two rows of soybean). Three maize planting densities were assigned to the subplot: D1 = 4.5×10^4 plants ha⁻¹, D2 = 5.25×10^4 plants ha⁻¹, and D3 = 6×10^4 plants ha⁻¹. In both experimental years, maize and soybean were sown on 1 March 2022 and 16 February 2023, respectively. The row spacing for all treatments was 60 cm with plant spacing within the row as follows: 37.7 cm (4.5×10^4 plants ha⁻¹), 32.3 cm (5.25×10^4 plants ha⁻¹), 28.2 cm (6×10^4 plants ha⁻¹), and 8.4 cm (2×10^5 plants ha⁻¹). Harvesting was performed on 26 June 2022 and 21 June 2023. Plot sizes were 288 m² (20 m × 14.4 m) for the F pattern, 144 m² (20 m × 7.2 m) for T pattern, and 72 m² (20 m × 3.6 m) for both maize and soybean monoculture plots. Guard rows were planted around each plot to minimize border effects.

In line with local agricultural management practices, no irrigation was applied during the growing season, and crops were entirely dependent on natural rainfall. Fertilizer application followed local farming guidelines, using urea (46.4% N), calcium magnesium phosphate (18.0% P₂O₅), and potassium chloride (60.0% K₂O). Prior to sowing, 100 kg ha⁻¹ of P and K fertilizers were applied, along with 250 kg ha⁻¹ of urea, two-thirds of which was applied before sowing and one-third during the trumpet stage.

Sampling and Determination

Aboveground Biomass Sampling

To investigate the factors contributing to grain yield variation under different intercropping patterns and maize planting densities, aboveground biomass sampling was conducted at key developmental stages of maize and soybean. Within each plot, plants were sampled from the central rows to minimize edge effects. For maize, representative plants were collected at

reproductive stages R1 (anthesis), R2 (grain-filling), and R6 (physiological maturity), whereas for soybean, plants were sampled at growth stages R2 (anthesis), R5 (pod-filling), and R7 (initial maturity). The samples, including stems, leaves, and ears for maize and stems, leaves, and pods for soybean were collected at maize growth stages of R1 and R2 and soybean stages R2 and R5, respectively. The samples were then divided into two parts; one was immediately stored at -80°C for enzyme activity assays, and the other was processed for dry matter analysis. For dry matter analysis, the samples were first oven dried at 105°C for 30 minutes to deactivate enzymes, and then further dried at 80°C in the oven until reached a constant weight.

Post-anthesis dry matter accumulation (DMA, g m^{-2}) was calculated using the following formula:

$$DMA = M_m + M_{mg} - M_a \quad (1)$$

Where DMA = dry matter accumulation, M_m = dry weight of stems, leaves, and ear/pods at R6, M_{mg} = grain DMA (dry weight of seeds at maturity), and M_a = dry matter of stems, leaves, and ear/pods at R1.

Leaf Photosynthetic Performance

In addition to biomass data, several critical leaf physiological parameters were monitored to assess canopy photosynthetic performance. Measurements were taken from fully expanded, healthy leaves at different (upper, middle, and lower) during specific growth stages: maize R1 and R2, and soybean R2 and R5.

The activities of key photosynthetic enzymes, specifically ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and phosphoenolpyruvate carboxylase (PEPC), were quantified using commercial assay kits (Solarbio Science & Technology Co., Ltd., Beijing,

China), following the manufacturer's instructions.

Nutritional Quality

For seed nutritional quality analysis, harvested samples were air-dried to a constant weight, then ground to a fine powder using a mill and sieved through a 0.5-mm mesh. The homogenized powder was stored in airtight containers at -20°C until analysis. Crude protein content and total nitrogen content were determined using the Kjeldahl method [30], while starch content was quantified using visible spectrophotometry [31]. Soluble sugars were measured by anthrone-sulfuric acid colorimetry [32], and crude fat content was determined by Soxhlet extraction using petroleum ether as the solvent [33].

Observations Recorded

At harvest, a random area of 2 m^2 was selected from each plot, which included 11 maize plants and/or 38 soybean plants. A comprehensive set of biometric and yield-related observations was recorded to assess crop growth and productivity of the crops. The recorded parameters included:

Yield Attributes: For maize, measurements included plant height, ear length and girth, and grain per cob; and stem node count, branch number, and grain number per plant in soybean.

Overall Yield Metrics: Parameters included actual grain yield (Y , total grain weight harvested), system yield (based on standard intercropping yield protocols), and harvest index, radiation-use efficiency, and LER were calculated using the following equation:

Intercropped System Yield (Y_{IS}): Y_{IS} was calculated as the weighted sum of maize and soybean yields per unit area, accounting for their respective planting proportions in the intercropping system (IS):

$$Y_{is} = \frac{(Y_m \times A_m) + (Y_s \times A_s)}{A_{total}} \quad (2)$$

Y_m and Y_s represent the actual grain yields (kg ha^{-1}) of maize and soybean, respectively. A_m and A_s represent the planting areas (ha) allocated to maize and soybean, while A_{total} is the

total plot area (ha).

Harvest Index (HI): The harvest index was calculated as:

$$HI = \frac{\text{Grain yield}}{\text{Aboveground biomass dry weight}} \times 100 \quad (3)$$

Radiation Use Efficiency (RUE): RUE, which indicates the capacity of crop populations to utilize solar energy, was calculated as:

$$RUE = \frac{H \times W}{Q} \times 100\% \quad (4)$$

Where H = heat production rate per unit mass of grain dry matter ($16.3 \times 10^3 \text{ kJ kg}^{-1}$); W = dry matter mass per unit land area (kg ha^{-1}); Q = total solar radiation during the growth stage (MJ ha^{-1}).

Land Equivalent Ratio: LER was used to quantify land-use efficiency in the maize-soybean intercropping system. LER represents the ratio of land area required under monoculture to produce the same yield as achieved per unit area of intercropping. The system-level LER was calculated as:

$$LER = LER_m + LER_s = \left(\frac{Y_{ISM}}{Y_m} \right) + \left(\frac{Y_{ISS}}{Y_s} \right) \quad (5)$$

Where LER_m and LER_s are partial LERs for maize and soybean, respectively; Y_m and Y_s are monoculture yields; Y_{ISM} and Y_{ISS} are the intercropped yields of maize and soybean.

Biometric observations: These included thousand-grain weight and dry matter accumulation (DMA), both critical indicators of biomass production in plants.

Soil Sampling and Soil Microbial Carbon Determination

During the physiological maturity of maize and soybean, soil samples were collected from 0–20 cm plow layer to quantify soil microbial biomass carbon (MBC). Within each plot, five sampling were selected along an S-shaped transect in the central area to minimize edge effects. At each sampling, soil cores (0-20 cm depth) were taken using a stainless-steel auger after

removing surface residues. The five cores from each plot were thoroughly mixed to obtain a composite sample of approximately 1 kg. Subsequently, the composite soil sample was placed in a sterile resealable bag and immediately transported in an insulated box containing ice packs to the laboratory for MBC analysis. Upon arrival, the samples were sieved (< 2 mm) and stored at 4°C prior to analysis. Soil MBC was determined using the chloroform fumigation-potassium sulfate extraction method [34], and the analysis was completed within three days after sampling to ensure sample integrity.

Statistical Analysis

All data were compiled and organized using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was performed to assess the main effects of intercropping pattern and maize planting density, as well as their interaction across both years. Treatment means were compared using the least significant difference (LSD) multiple-range test. Differences in treatment means were considered statistically significant at $P < 0.05$. Data visualization was performed using Origin 2023b (OriginLab Corporation, Northampton, MA, USA). Error bars and details of statistical tests are provided in the fig. legends.

Results

Yield and System-Level Performance Metrics in Intercropping Systems

Regardless of experimental years and maize planting density, intercropping systems (IS) significantly increased maize grain yield (Y) compared with monoculture. Across the two years, intercropping resulted in an average increase of 23.24%–45.34% in maize Y, harvest index (HI),

and radiation-use efficiency (RUE), relative to monoculture, with values ranging from –6.35%–3.70% for HI, and 61.34%–45.71% for RUE ($P < 0.05$; Table 1). In contrast, soybean grain yield was significantly reduced under IS, with reductions ranging from 13.78%–24.54% compared with soybean monoculture ($P < 0.05$). Under IS, moderate maize planting density generally produced a better balance between maize yield and overall system performance than both low or high densities. Specially, compared to FD2 and FD3, the soybean yields in FD1 were 2.73% and 21.10% lower in 2022, and 17.32% and 27.50% lower in 2023 (Table 1). Similarly, in TD1 configuration, soybean yields were 10.64% and 24.48% lower than those in TD2 and TD3 in 2022, and 10.20% and 27.41% lower in 2023. These results indicate that increasing maize planting density within the same intercropping pattern tends to suppress soybean yield, particularly at the highest maize density (Table 1)

Growth and Yield Traits

The two-year field experiment revealed that different IS treatments significantly affected growth characteristics and yield traits of both maize and soybean relative to their respective monoculture (Table 2). For maize, plant height in CK reached 340 cm at harvest, which was significantly higher than that observed in most IS treatments ($P < 0.05$). However, IS significantly improved ear-related traits, such as ear girth (EG) and number of grain per cob (GNC). The TD2 treatment exhibited the most favorable performance, with EG and GNC reaching 39 mm and 386, respectively, in 2022, and 41 mm and 409, respectively, in 2023, both of which were significantly higher than those recorded in monoculture. The average ear length (EL) of maize under TD2 was 18 cm in both 2022 and 2023, suggesting that EL in TD2 was significantly lower than TD1, but than that under CK in 2023. Overall, the two-row

intercropping pattern improved maize ear traits more consistently than the four-row pattern, particularly under the moderate planting density. For soybean, IS affected several morphological and yield related traits, including plant height (PH) branch number (BN), stem node count (SNC), and grain number per plant (GNP, Table 2). Among the intercropping treatments, TD2 consistently produced the higher BN in both 2022-2023, indicating better a more favorable canopy environment for soybean branching under this configuration. Nevertheless, IS generally reduced soybean PH, SNC, and GNP compared to monoculture, particularly under higher maize planting densities and within the four-row intercropping pattern.

Dry Matter Accumulation and assimilates partitioning in maize and soybean under intercropping

Compared with monoculture, IS significantly increased average maize DMA in maize stems, leaves, and ears across years ($P < 0.05$). During the biomass accumulation period, maize grain dry weight ranged from 49 to 76 g plant⁻¹, leaf dry weight from 33 to 46 g plant⁻¹, and stem dry weight from 98 to 113 g plant⁻¹. Among the IS treatments, TD2 maintained the highest total dry matter accumulation, indicating enhanced assimilate production, improved biomass accumulation, and more efficient partitioning of photoassimilates to the grain. In contrast, soybean dry weight was markedly suppressed under IS, particularly at higher maize planting densities during the key developmental stages of R2, R5, and R7. In 2022, average dry weight of soybean stem, leaf, and pod reduced by 25%, 30.77%, and 77.78%, respectively, under IS relative to monoculture (Fig. 2). A similar trend was observed in 2023. Overall, soybean dry matter weight under IS decreased progressively with increasing maize planting density ($P < 0.05$), with the reduction being more pronounced under the F pattern. Total soybean DMA in

2022 (1060.48 g m⁻²) was significantly higher than that in 2023 (879.86 g m⁻²). Notably, both IS patterns and planting density significantly affected DMA of all soybean organs, highlighting the strong sensitivity of soybean growth to canopy architecture, interspecific competition, and resource limitation within intercropping system. However, differences in leaf and pod dry matter accumulation were not statistically significant.

Canopy-level variation in leaf photosynthetic enzyme activities under different intercropping patterns and planting densities

The activities of key photosynthetic enzymes, namely RuBisCO and PEPC, in maize and soybean leaves were markedly affected by IS pattern, planting density, and canopy position (Fig. 3). Overall, enzyme activities tended to increase under the IS compared with monoculture, particularly in the upper and middle canopy layers before anthesis. Although RuBisCO and PEPC activities varied across intercropping configurations and planting densities, the general trend showed that these enzyme activities were significantly increased in maize but decreased in soybean under IS compared with monoculture (Fig. 3). Among the IS treatments, TD2 exhibited the highest RuBisCO and PEPC activities in maize leaves. The average RuBisCO activity in maize leaves under monoculture, TD1, TD2, TD3, FD1, FD2, and FD3 were 9.40, 10.76, 13.57, 11.80, 9.69, 12.16, and 10.78 U/g FW, respectively. These results indicate that maize leaves under TD2 exhibited greater photosynthetic capacity, which may have contributed to improved carbon assimilation and delayed leaf senescence compared with other IS treatments.

Effects of intercropping pattern and planting density on grain nutritional quality of maize and soybean

Planting pattern significantly affected grain nutritional quality of both maize and soybean (Fig. 4). The results showed that, compared with monoculture, soybean crude protein content and maize soluble sugar, starch, and crude fat contents decreased by 3.68%–15.91%, 9.40%–53.71%, 3.65%–16.34% and 1.17%–11.29%, respectively (Fig. 4). In contrast, maize crude protein content increased by 1.79%–26.79% under different IS pattern and maize planting densities (FD1, FD2, FD3, TD1, TD2, and TD3) in 2022 and 2023 relative to monoculture. Soybean grain quality showed a contrasting response. Compared with monoculture, soybean soluble sugar, starch, and crude fat contents increased under IS by 10.24%–13.57%, 14.89%–19.18%, and 7.39%–7.97%, respectively, across years. Moreover, soluble sugar, starch, and crude fat contents were significantly positively correlated with planting patterns under all IS patterns and maize planting densities during 2022–2023.

Soil Microbial Biomass Carbon response to intercropping systems and maize planting density

Intercropping patterns and maize planting density significantly influenced soil microbial biomass carbon (MBC) in both maize and soybean based systems, although the magnitude and direction of the response differed between crops (Fig. 5). In the maize system, IS generally increased soil MBC compared to monoculture. Among all treatments, TD2 consistently recorded the highest MBC values, reaching 130.73 mg kg⁻¹ in 2022 and 138.13 mg kg⁻¹ in 2023, indicating a more favorable rhizosphere environment and greater microbial activity under this treatment. In contrast, maize monoculture and low-density intercropping treatments (FD1, TD1) exhibited the lowest MBC values across both years. Notably, the soybean monoculture treatment maintained the highest soil MBC levels in both years, with values of 103.6 mg kg⁻¹

in 2022 and 135.67 mg kg⁻¹ in 2023. Under intercropping, however, soybean associated soil MBC decreased progressively with increasing maize planting density, indicating a clear negative density dependent effect. The most pronounced reductions were observed under FD3 and TD3 treatments, which consistently produced the lowest MBC values throughout the experimental period. These findings suggest that IS, particularly the TD2, can effectively enhance soil MBC in the maize rhizosphere, whereas excessive maize density under intercropping may suppress microbial biomass in the soybean rhizosphere, likely owing to intensified belowground competition and altered root-zone resource availability.

Effects of intercropping systems and planting density on grain total nitrogen concentration

The results indicated that the IS system significantly influenced the total nitrogen content in grains of both maize and soybean (Fig. 6). In the maize system, intercropping markedly enhanced grain nitrogen accumulation relative to monoculture. Compared with monoculture, both the F and T intercropping patterns increased the total nitrogen content of maize grain, indicating a positive effect of interspecific association on N acquisition and translocation (Fig. 6). In 2022, the FD3 treatment recorded a grain total nitrogen content of 1.80%, representing a significant increase of 32.35% compared to monoculture. In 2023, the TD2 treatment achieved a total nitrogen content of 1.43%, which was 24.35% higher than that in monoculture. These two treatments showed the strongest enhancement in maize grain nitrogen concentration in their respective years. In contrast, maize monoculture consistently showed the lowest nitrogen content across both years, with values of 1.36% in 2022 and 1.15% in 2023. In, soybean system, an opposite trend was observed, Soybean monoculture maintained the highest total nitrogen

content in both years, reaching 6.38% in 2022 and 6.46% in 2023. Under intercropping, the total nitrogen concentration of soybean grain reduced with increasing maize planting density. Among the intercropping treatments, TD3 recorded the lowest soybean grain nitrogen content in both years, with values of 4.82% in 2022 and 5.99% in 2023, corresponding to reductions of 24.45% and 7.28%, respectively, compared with soybean monoculture.

Integrated relationships among biomass accumulation, photosynthetic enzyme activity, yield formation, and grain quality

Maize and soybean exhibited clear differences in yield formation, photosynthetic enzyme activity, and agronomic performance (Fig. 7). Correlation analysis revealed that DMA was key determinant of yield in both crops. In maize, TDW and DMA were strongly and positively correlated with grain yield ($r = 0.97$ and 0.84 , respectively). Similarly, in soybean, the corresponding correlations were $r = 0.93$ and 0.89 . These relationships indicate that DMA and its subsequent allocation are fundamental to yield formation in both maize and soybean. With respect to photosynthetic enzyme activity, RuBisCO activity in maize showed a strong positive correlation with RUE ($r = 0.88$), whereas PEPC activity was moderately correlated with TDW ($r = 0.64$). No significant correlation was observed between RuBisCO and PEPC activities in maize. In soybean, however, PEPC and RuBisCO activities were strongly positively correlated ($r = 0.86$), indicating a more synergistic response within the photosynthetic enzyme system. Several key yield components were also positively correlated with maize productivity. In particular, EL, EG, and GNC were positively correlated with maize yield. Among these traits, EG showed a particularly strong positive correlation with yield ($r = 0.81$) and was also positively correlated with GNC ($r = 0.66$), whereas EL showed only a weak correlation with

yield ($r = 0.31$). In soybean, GNP showed a strong positive correlation with yield ($r = 0.91$), indicating that reproductive sink size was a major contributor to soybean productivity. RUE was strongly and positively correlated with TDW and DMA in both maize and soybean. In maize, the correlation coefficients were $r = 0.98$ and 0.95 , respectively, whereas in soybean they were $r = 0.97$ and 0.90 . These relationships suggest that efficient utilization of incident solar radiation was closely linked to greater biomass production and final yield in both crops. Analysis of agronomic traits further revealed that maize PH was predominantly negatively correlated with ear traits, including EL, EG, and GNC. In both crops, HI was negatively correlated with biomass accumulation indicators, suggesting a trade-off between total biomass production and partitioning efficiency. In soybean, PH was also negatively correlated with yield. The quality trait correlation analysis indicated that, in both maize and soybean, CP was negatively correlated with SCC, SC, and CF, but positively correlated with yield. Conversely, SCC, SC, and CF were all negatively correlated with yield.

Multivariate analysis of treatment effects using PCA and PLS-SEM

Principal component analysis revealed significant differences among treatments in both maize and soybean systems (Fig. 8), indicating substantial treatment-induced variation in the measured traits. In maize system, the first two principal components (PC1 and PC2) together explained 66.89% of the total variance. PC1 mainly associated with crude fat content, RUE, MBC, and total dry weight, whereas PC2 was primarily characterized by HI, CP content, and total nitrogen concentration. In the soybean system, the first two principal components accounted for 83.95% of the total variation. Here, PC1 was mainly defined by total nitrogen content, Rubisco activity, and grain yield, while PC2 predominantly associated with starch

content, total dry weight, and soluble sugar content. Overall, the TD2 treatment exhibited the comprehensive performance in both cropping systems. In maize, the treatment ranking was $TD2 > TD3 > FD2 > TD1 > FD3 > FD1 > CK$.

PLS-SEM further clarified the relationships among the key physiological, agronomic, and soil related variables. In both crops, biomass accumulation exerted a significant positive effect on photosynthetic physiology, indicating that greater biomass production was closely associated with enhanced photosynthetic processes (Fig. 8). In addition, soil microecology had a significant positive influence on grain quality in both maize and soybean, highlighting the important role of soil health in shaping grain nutritional traits. However, the mechanisms of yield formation differed markedly between maize and soybean. In maize, yield components were driven primarily by strong positive effects of plant morphological traits, whereas the direct contributions of biomass accumulation, photosynthetic physiology, and soil microecology to yield were not statistically significant. In soybean, by contrast, yield components were significantly and positively affected by biomass accumulation, photosynthetic physiology, and soil microecology, indicating a more integrated yield formation mechanisms driven by multiple synergistic factors. The relationship between biomass accumulation and soil microecology also differed between the two crops. In maize system, biomass accumulation significantly improved soil microecology, suggesting that vigorous maize growth may enhance the rhizosphere microenvironment. In soybean systems, however, biomass accumulation showed a significant negative effect on soil microecology, potentially reflecting crop-specific differences in root activity, resource acquisition, and rhizosphere regulation. Likewise, the regulation of morphology varied substantially between crops. Soil

microecology exerted a significant positive effect on morphology in maize but a significant negative effect in soybean. These contrasting responses may arise from inherent differences in growth habits, canopy structure, and adaption to underground resource conditions. PLS-SEM further indicated that the superior performance of TD2 treatment was associated with increased biomass accumulation, which directly promoted photosynthetic physiology. Enhanced photosynthetic capacity promoted soil carbon and nitrogen metabolism, improved overall plant growth, and ultimately contributed to higher maize yield and superior grain quality.

Discussion

Interactive Effects of Intercropping Configuration and Planting Density on Key Photosynthetic Enzymes Activity and Dry Matter Accumulation

Intercropping significantly altered photosynthetic performance and DMA in both maize and soybean. In maize, RuBisCO and PEPC activities, together with DMA were consistently higher under IS than monoculture, with the TD2 density (52,500 plants ha⁻¹) showing the most pronounced effect. This pattern suggests that a moderate plant population density enhances canopy light interception, carbon assimilation, and biomass production, which is consistent with the radiation capture advantage of the taller maize canopy in intercropping systems [35]. In contrast, soybean exhibited reduced RuBisCO, PEPC activities, and lower DMA under IS conditions, primarily due to canopy shading imposed by maize [36]. Lowering maize density, particularly under (FD1) partially restored soybean enzyme activity compared to monocropping. However, increasing maize density beyond D2 intensified interspecific light competition, leading to reduced soybean biomass accumulation and a higher risk of lodging [37,38]. Additionally, biological nitrogen fixation by soybean likely contributed to improved soil nitrogen availability, which may have indirectly promoted maize DMA under IS [39]. Thus,

D2 density provide an optimal balance between biomass accumulation, assimilate translocation, and intercrop complementarity [37].

The fixation of CO₂ assimilated during photosynthesis requires optimal and efficient activity of RuBisCo, a key enzyme in the Calvin cycle [40]. Its efficiency is strongly regulated by light availability, which differs markedly between species in intercropping systems due to contrasting plant architecture and canopy structure. In the present study, the higher RuBisCO activity in maize than in soybean is likely due to greater PH of maize, which allows it to intercept more solar radiation and sustain higher rates of carbon assimilates. In contrast, the reduced RuBisCO activity in soybean was likely consequence of light limitation under maize canopy [38]. Planting density plays a critical role in regulating canopy structure, light interception, and resource utilization efficiency in IS system. Higher planting densities reduce light penetration into the lower canopy layers, thereby constraining photosynthetic capacity [41]. The superior performance of TD2 in photosynthetic enzyme activities in both crops compared with other treatments could be attributed to improved light penetration in a moderately populated canopy [37]. Furthermore, the TD2 system is likely to enhance the functional complementarity between maize and soybean, particularly nitrogen fixation by soybean roots, thereby increasing soil nitrogen availability for maize growth. This mechanism probably explains the greater DMA observed in maize under TD2 compared to other treatments. By contrast, highly planting densities in intercropping systems tend to reduce biomass production and increase risk of lodging, due to intensified competition for light, water, and nutrients [37].

Effect of Intercropping Configuration and Planting Density on Grain Nutritional Quality

The IS significantly increased the grain nutritional quality of both maize and soybean, with contrasting responses between the two crops. Intercropping increased maize grain crude

protein content, whereas soybean crude protein content reduced relative to monoculture. A similar response has been reported in other cereal-legume combination, such as maize-cowpea intercropping, particularly at a 2:2 row ratio, significantly increased the total yield and crude grain protein content [42]. In contrast, He et al. [43] reported that the crude protein content of soybean was increased in IS, suggesting that the response of grain protein to intercropping is system-dependent and influenced by crop configuration and environmental conditions. Nitrogen availability is a key driver of maize grain quality, especially crude protein content [44]. The increase in maize protein observed in the study may be partially attributed to IS-induced modifications in rhizosphere microbial communities, including enhanced population of beneficial bacteria and fungi, which improve soil nitrogen availability [45]. In cereal-legume systems, legumes fix atmospheric nitrogen through biological nitrogen fixation, thereby enriching the nitrogen nutrition of the associated cereals and enhancing crude protein accumulation in grains [42]. Evidence from soybean-wheat intercropping indicates that soybean can fix more than twice the atmospheric nitrogen compared with monoculture, although the net effect depends on planting density combinations, with low-density soybean and high-density wheat optimizing nitrogen uptake [46, 47]. Despite these benefits, interspecific nitrogen competition may limit N fixation in soybean under intercropping, thereby constraining protein accumulation. This suggests that the balance between facilitation and competition for nitrogen is a key regulator of grain protein dynamics in intercropping systems. In our study, IS induced opposite effects on the nutritional quality of maize and soybean. In maize, soluble sugar, starch, and crude fat content were reduced under intercropping [48], who reported reduced starch content in maize under reduced nutrient availability. In contrast,

soybean exhibited improvements in several key quality attributes, with a significant increase in soluble sugar, starch, and crude fat content under intercropping [49]. These contrasting patterns highlight that intercropping patterns can differentially regulate carbon and N metabolism in component crops. Such differences are likely driven by species-specific physiological responses, canopy light distribution, and rhizosphere interactions, which collectively influence nutrient allocation and grain quality formation.

Effect of Intercropping and Density on Crop Agronomic Traits

Agronomic traits, including PH and leaf area, are highly responsive to intercropping and planting densities due to the competitive interactions between intercrop components for essential resources [50]. The availability of these resources within an intercropping system significantly affects the morphological responses of the component crops [51]. In this study, variations in agronomic traits, particularly PH, were observed across cropping years, demonstrating the dynamic influence of intercropping and planting densities on crop growth. The reduction in PH in both maize and soybean observed in the first cropping year, relative to monoculture, can be attributed to competition for light, space, and nutrients. These results were supported by [52], who reported that higher planting density tends to suppress agronomic traits in maize and soybean. Nevertheless, an increase in agronomic traits, including PH, was observed in intercropped maize and soybean during the second year. This may be explained by improved nutrient use efficiency in the intercropping system [53]. The improved performance in the second year could be due to better resource allocation and increased intercropping synergies, such as enhanced light capture and root nitrogen fixation. These changes might have caused differences in canopy interception and light intensity availability in both cropping periods, as they are

important indicators affecting crops in intercropping systems [54]. TD2, with a moderate planting density of 52,500 plants ha⁻¹, exhibited superior agronomic traits, particularly in terms of ear girth and cob number, likely due to increased microbial activity, as reflected in higher soil MBC levels. Soybean roots, through biological nitrogen fixation, have the potential to enhance soil microbial activities, which in turn positively affect nutrient cycling and soil health [55]. Therefore, with increased soil microbial activities coupled with better resource use efficiency in terms of light capturing and increased photosynthetic enzyme activity could be responsible for superior agronomic traits in TD2 compared to other treatments. Moreover, maize soybean intercropping fosters microbial activity, including enhanced enzymatic activities and nutrient cycling [55], which directly responsible for enhanced agronomic traits compared to monoculture. Intercropping promotes maize growth by improving light and nutrient use efficiency [53]. While plant and ear height generally increase with planting density, excessive density suppresses both traits [52]. In our study, intercropping reduced plant and ear height in 2022, but increased both in 2023, suggesting that environmental variability played a crucial role. These opposing results reflect differences in light intensity, canopy interception, and shading stress, which are key growth limitations in intensive cropping systems [54, 56]. Environmental factors, such as light conditions and ambient temperature, are known to influence morphogenesis and photosynthetic processes [57]. Even small fluctuations in temperature can significantly impact crop yields [58], and weather variability can delay planting or harvest, reducing biomass and feed value [59]. Furthermore, poor rainfall distribution and inadequate irrigation management, particularly in regions such as India, can exacerbate crop production constraints [60]. Overall, intercropping's effects on maize yields

are jointly regulated by a complex interplay of environmental conditions, system design, and management practices [61].

Carbon and Nitrogen Allocation Mechanisms in IS Based on Source-Sink Relationships

This study systematically elucidated the synergistic mechanism between soil MBC and total nitrogen accumulation in grains within the IS system. The results demonstrated that appropriate intercropping configurations, such as the TD2 treatment, significantly enhanced photosynthetic efficiency in maize by optimizing canopy structure, thereby promoting Rubisco activity [62]. The enhanced photosynthesis performance not only supplied sufficient carbon sources for DMA but also significantly increased soil MBC levels through root deposition [35]. As a core indicator of soil microbial activity, MBC further mediated the integrated regulatory effects of the intercropping system on the stability of soil organic carbon pools, the enhancement of carbon sequestration potential, and the optimization of soil enzyme activities [63]. Among all maize treatments, TD2 exhibited the highest MBC content, with values of $130.73 \text{ mg kg}^{-1}$ in 2022 and $138.13 \text{ mg kg}^{-1}$ in 2023. The activation of microbial communities promoted the mineralization of soil organic matter [64], enhancing nitrogen availability and establishing a synergistic pathway that connected photosynthesis enhancement, DMA, microbial activation, and nitrogen release. This process effectively explains the intrinsic mechanism by which the TD2 treatment achieved a significant increase in grain nitrogen and MBC content. By coordinated regulation of soil enzyme activities, maize-legume intercropping not only directly promoted nutrient uptake and crop growth but also facilitated the transformation of soil organic carbon and nutrient cycling, laying the foundation for sustainable system productivity [65].

In the soybean system, photosynthetic inhibition induced by intercropping triggered a cascade of carbon and nitrogen metabolic responses. Maize shading reduced light utilization efficiency in soybean, leading to decreased activity of key photosynthetic enzymes and restricted capacity for carbon assimilation, ultimately lowering photosynthetic efficiency [66]. The dynamic interaction between carbon and nitrogen metabolism is crucial for optimal plant performance [67], suggesting that nitrogen availability directly regulates photosynthetic efficiency and carbon fixation [68], while carbon availability influences nitrogen uptake and assimilation [69]. Although plants can optimize carbon allocation strategies to balance source-sink relationships and improve yield, insufficient source strength in this study compelled soybean to prioritize allocating limited carbon resources to storage compounds such as soluble sugars and lipids, rather than to energy-intensive nitrogen assimilation processes [70]. This metabolic shift resulted in reduced root carbon input, lowering rhizosphere MBC levels, which in turn weakened microbial-driven nitrogen transformation functions and a significantly reduced total grain nitrogen content [71].

Impact of Intercropping Configuration and Planting Density on Light Energy Utilization and Crop Yield

The symbiotic growth of maize-soybean intercropping systems, particularly through biological nitrogen fixation and enhanced soil nitrogen availability, plays a crucial role in determining grain yield outcomes [72]. Furthermore, the efficiency light utilization under different planting densities significantly influence grain yield [71]. Both photosynthetic and agronomic traits are jointly responsible for determining the yield of maize and soybean, and maintaining an appropriate balance between these traits is essential for optimizing productivity

[62]. The increased maize grain yield observed under intercropping compared to monocropping can be attributed to maize's physiological attributes, such as long root system and high leaf surface area, which enhance its capacity to effectively utilize available resources. In a maize-soybean intercropping system, increased soil nitrogen supplied by soybean root nodules, which subsequently absorbed by maize roots, further contribute to enhanced maize yield [72]. Moreover, intercropping systems have been shown to increase soil MBC, which can positively influenced maize yield [61].

Conversely, soybean grain yield reduced under intercropping, particularly with increasing planting densities, compared to monoculture. This reduction can be attributed to limited light availability and utilization, as soybean plants are often shaded by maize canopy. Additionally, the observe reduction in photosynthetic enzymes, including RuBisCo, inhibits photosynthetic assimilation, leading to lower grain yield. Despite the ability of soybean root noodles to fix biological nitrogen and enrich the soil, the lower soybean grain yield under IS could be result of competitive pressure between soybean and maize roots for nutrients, which limits nutrient acquisition and ultimately reduces soybean yield potential.

Effect of Intercropping Configuration and Planting Density on Land Equivalent Ratio

The Land Equivalent Ratio (LER) is a key metric for comparing the yield performance of intercropping systems relative to mono-cropping systems, and it reflects how efficiently available resources, including land, are utilized in different cropping systems [73]. A higher LER indicates more efficient land use, which results in higher crop yield without requiring additional land area. In this study, the two-row intercropping system exhibited higher LER values compared to mono-cropping systems, primarily due to enhanced nutrient availability, as

indicated by the higher soil MBC levels observed under intercropping [72]. This suggests that intercropping systems contribute to better stabilization of soil organic matter, likely facilitated by soybean roots, which in turn supports the increased LER. The increased LER observed in this study is consistent with previous research, such as the studies of Wu et al. [74] and Baraki et al. [75], who reported increased LER values in maize-alfalfa and sesame-mungbean intercropping systems, respectively, compared to monocropping systems. While both intercropping row arrangements exhibited higher LER values than the monocropping system, LER was higher in the two-row (T) arrangement compared to the four-row (F) arrangement. This difference can be attributed to better resource utilization, including space and light, in the T-row configuration. Additionally, there may be greater nutrients translocation, including N, P, and K, in the T row arrangement, due to enhanced photosynthetic active radiation [76].

Beside row configurations, planting densities is another critical factor influencing LER in cropping systems [73]. Variations in planting densities alter the way resources, such as land, light, and nutrients are allocated between crops, impacting LER. In this study, the D2 density produced the highest LER, which may be attributed to balanced resource allocation and reduced competition for resources between the maize and soybean [41]. These findings align with the results of Cowden et al. [77], who reported a grain LER of 1.14 and a 10%–14% increase in resource use efficiency in pea-barley intercropping with moderate density. Similarly, a sesame-maize 2:2 intercropping pattern achieved the highest LER [78], validating the importance of optimizing row ratios for enhanced resource use efficiency. In our study, the IS system showed clear land use advantages. The LER of the T-row pattern was 1.08 in 2022 (compared with 1.02 for F-row pattern), and increased to 1.11 in 2023 for both intercropping

configurations. These results are in line with findings by Wu et al. [74], who observed an LER of 1.31 in maize-alfalfa intercropping, and Baraki et al. [75] reported an even higher LER of 1.34 in a sesame-mungbean intercropping. Notably, all treatments in our study had LER values greater than 1, confirming the land-use advantage of intercropping [79]. These results are consistent with Xu et al. [80], who reported 3-year average LER values greater than 1.00 in alfalfa-maize intercropping systems.

Planting density also played a critical role in regulating system performance. In this study, the D2 density generally performed best, producing significantly higher LER values than other densities, except the F treatment in 2023. These findings are consistent with those of Cowden et al. [77], who also reported that moderate density in pea-barley intercropping resulted in improved resource use efficiency and a higher LER.

Conclusions, Future Perspectives, and Limitations

This study demonstrates the complex interactions between maize-soybean intercropping, planting densities, and photosynthetic efficiency that collectively enhance system productivity, resource use efficiency, and nutrient cycling. The results indicate that appropriate intercropping configurations, particularly the TD2 treatment (52,500 plants ha⁻¹), significantly improved maize yield, grain quality, and photosynthetic enzyme activity, driven by enhanced light interception and optimized N availability through biological N fixation by soybean. This treatment also promoted greater soil MBC, which played a central role in enhancing soil health, carbon sequestration, and nutrient cycling.

Conversely, soybean yield was reduced under intercropping, particularly at higher planting densities, due to shading stress from maize and competition for nutrients, which

limited photosynthetic efficiency and carbon assimilation. Despite this, the soybean's ability to fix N contributed to soil N enrichment, benefiting maize growth. The study further showed that moderate planting density (D2) was optimal, balancing the competition for resources between maize and soybean, leading to improved LER, which confirmed the land-use advantages of intercropping systems over monoculture.

In terms of carbon and N allocation, the TD2 intercropping treatment demonstrated enhanced biomass accumulation, photosynthetic capacity, and soil microbial activity, establishing a synergistic relationship between photosynthetic enzyme activities, biomass production, and nutrient cycling. These processes contributed to higher grain yield and improved grain nutritional quality in maize, while highlighting the source-sink relationship and its role in optimizing intercropping system outcomes.

Thus, this study indicates the potential of maize-soybean intercropping systems to enhance resource use efficiency and soil health, providing a sustainable pathway for increasing agricultural productivity while maintaining ecological balance. The results also emphasize the importance of optimizing intercropping configurations and planting densities to achieve higher yields, improved nutrient availability, and better soil health, thus promoting sustainable intensification in agriculture.

Future Research Directions

This study offers valuable insights into maize-soybean intercropping, but several areas warrant further investigation. Future work should focus on the long-term impacts of intercropping systems on soil health and sustainability, particularly examining how intercropping configurations affect microbial diversity, carbon sequestration, and nutrient cycling over

multiple growing seasons. Additionally, exploring multi-species intercropping for enhanced ecosystem services (e.g., pest control, weed suppression, biodiversity) and crop resilience to climate variability is essential.

Further research is also needed on the genetic compatibility and resource use efficiency of intercropped species under varying environmental conditions. Exploring genomic selection for N fixation efficiency in legumes and photosynthetic efficiency in cereals could optimize intercropping performance. The integration of precision agriculture technologies, such as remote sensing and machine learning, could improve spatial arrangement optimization and boost productivity in intercropping systems.

Research Limitations

This study has several limitations. First, it was conducted in a single location (Southern China), limiting the generalizability of the results across different agroecological zones. Future studies should consider regional variability, including soil types, climate, and local farming practices. Second, while this study focused on yield and physiological measurements, a more comprehensive assessment of economic feasibility, labor inputs, and environmental impacts (e.g., greenhouse gas emissions) is needed to fully assess the sustainability of maize-soybean intercropping. Lastly, the long-term effects of intercropping duration on system productivity and soil health were not addressed. Long-term studies are required to understand the temporal dynamics of soil fertility and crop performance in intercropping systems.

Declarations

Abbreviations

ANOVA: analysis of variance; BN: branch number; CF: crude fat content; CK: control

(monoculture); CKM: maize monoculture; CKS: soybean monoculture; CP: crude protein content; D1, D2, D3: maize planting densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively; DMA: dry matter accumulation; EG: ear girth; EL: ear length; F: four-row intercropping pattern (four rows of maize with four rows of soybean); GNC: grain number per cob; GNP: grain number per plant; HI: harvest index; IS: intercropping system; LER: land equivalent ratio; LSD: least significant difference; MBC: microbial biomass carbon (soil microbial biomass carbon); PCA: principal component analysis; PEPC: phosphoenolpyruvate carboxylase; PH: plant height; PLS-SEM: partial least squares structural equation modeling; RCBD: randomized complete block design; RUE: radiation use efficiency; RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase; SC: starch content; SCC: soluble sugar content; SNC: stem node count; T: two-row intercropping pattern (two rows of maize with two rows of soybean); TDW: total dry matter weight; TN: total nitrogen content; Y: grain yield; Y_{IS}: intercropped system yield; Y_m: maize yield; Y_s: soybean yield.

Ethics approval and consent to participate

Not applicable. This study did not involve human subjects, animal experiments, or genetically modified organisms, and all field experiments were conducted in accordance with the institutional guidelines of Guangxi University and relevant national agricultural research regulations.

Consent for publication

All authors have read and approved the final version of the manuscript, and consent to its publication in *BMC Plant Biology*.

Availability of data and materials

Data will be made available on request from the corresponding authors.

Competing Interests

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

Funding

This work is supported by the Guangxi Key Research and Development Program (AB2506910019), the Bama County Program for Talents in Science and Technology, Guangxi, China (20210049).

Authors' contributions

Conceptualization: LY. Methodology: FG and JL. Formal analysis: TTP. Investigation: FG. Resources: CXS. Data curation: FG. Writing-original draft preparation: JL. Writing-review and editing: LY, MFJ and RGI. Supervision: XBZ. All authors read and approved the final manuscript.

Acknowledgements

We thank the staff of the Guangxi Key Laboratory of Agro-environment and Agro-products Safety for their support during field experiments and data collection. We also thank the Bureau of Agriculture and Rural Affairs of Bama Yao Autonomous County for providing the maize seeds, and Guangxi Zhaohe Seed Industry Co., Ltd. for supplying the soybean seeds.

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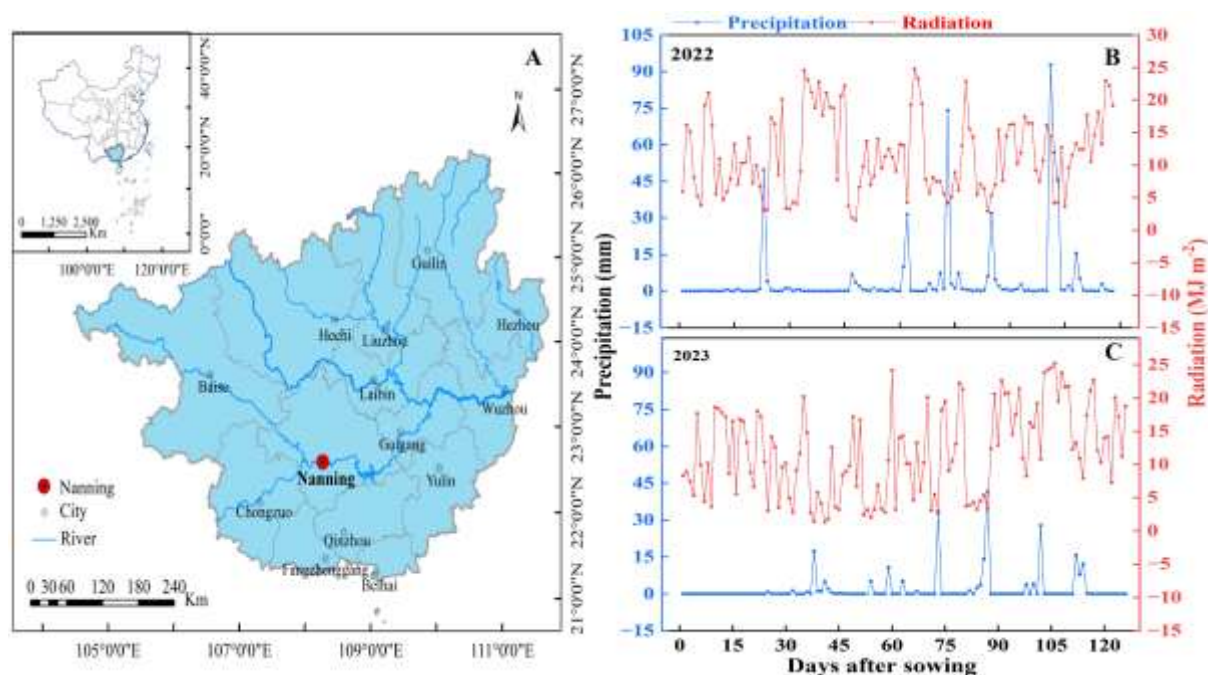
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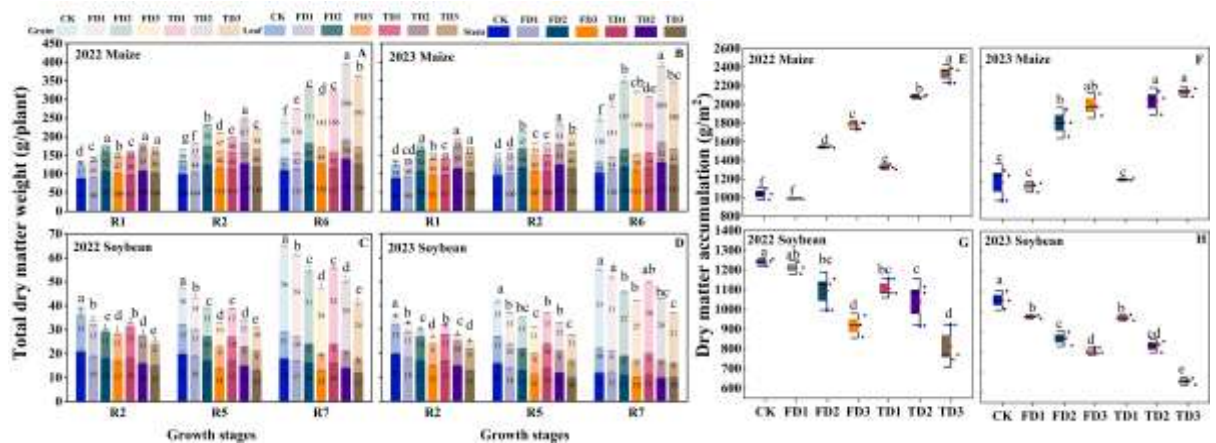
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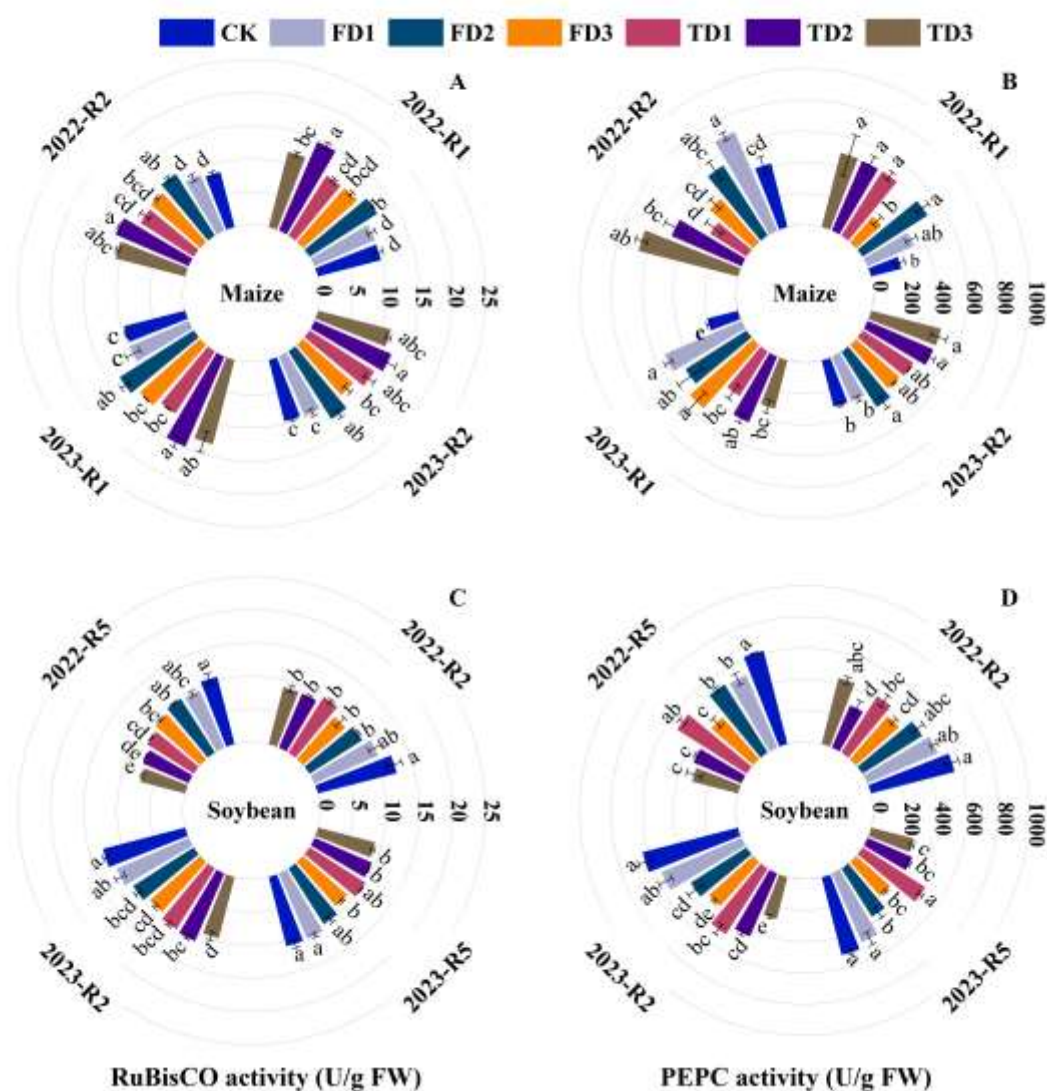
Fig. 1 A, Geographic location of the experimental site; B, Daily precipitation and solar radiation during the growing season in 2022; C, Daily precipitation and solar radiation during the growing season in 2023



83.

84. **Fig. 2** Effect of intercropping row ratio and density management on total dry matter weight and accumulation of maize and soybean at different growth stages (2022–2023).

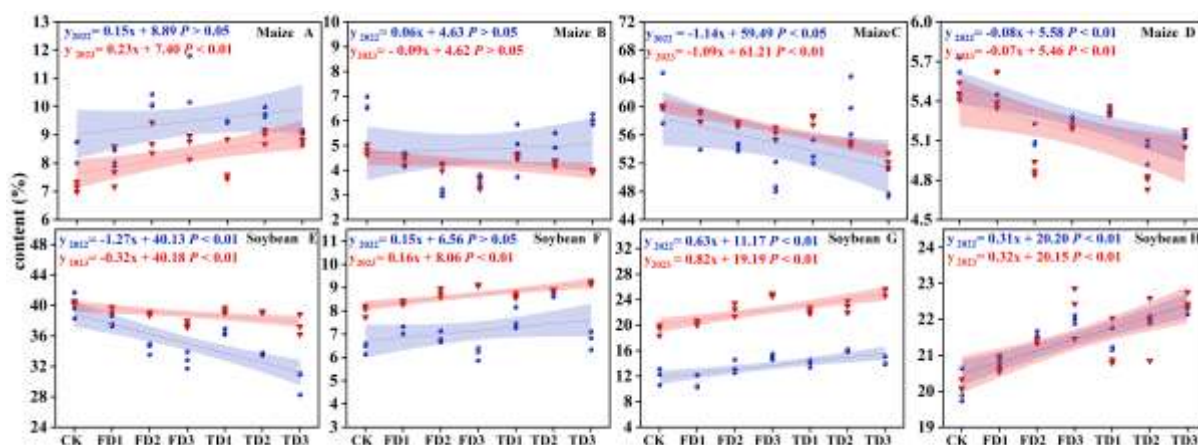
85. Total dry matter weight of maize (A: 2022; B: 2023) and soybean (C: 2022; D: 2023). Dry matter accumulation of maize (E: 2022; F: 2023) and soybean (G: 2022; H: 2023). Maize growth stages: R1, anthesis; R2, grain-filling; R6, physiological maturity; soybean growth stages: R2, anthesis; R5, pod-filling; R7, initial maturity. CK, monoculture maize and soybean; F, four rows of maize intercropped with four rows of soybean; T, two rows of maize intercropped with two rows of soybean. D1, D2, and D3 correspond to maize densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively.



86.

Fig. 3 Effect of intercropping row ratio and maize density management on RuBisCo and PEPC activity of maize and soybean at different growth stages (2022–2023).

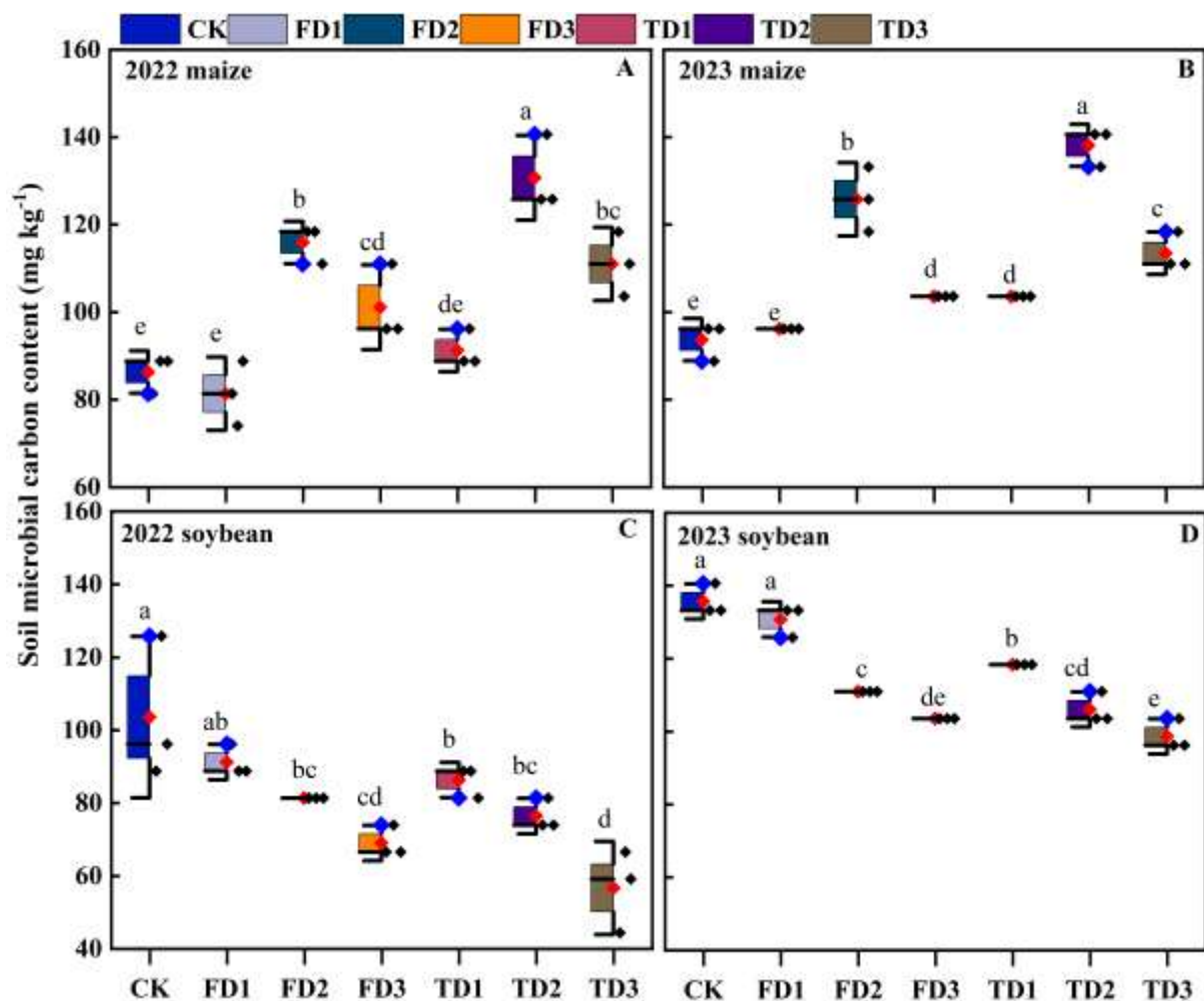
87. Enzyme activities of RuBisCo and PEPC in maize (A, B) and soybean (C, D). Maize growth stages: R1, anthesis; R2, grain-filling; soybean growth stages: R2, anthesis; R5, pod-filling. CK, monocropping maize and soybean; F, four rows of maize intercropped with four rows of soybean; T, two rows of maize intercropped with two rows of soybean. D1, D2, and D3 correspond to maize densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively. Different letters indicate significant differences between treatments ($P < 0.05$).



89.

Fig. 4 Regression analysis between intercropping row ratio and maize density on quality parameters of maize and soybean (2022–2023).

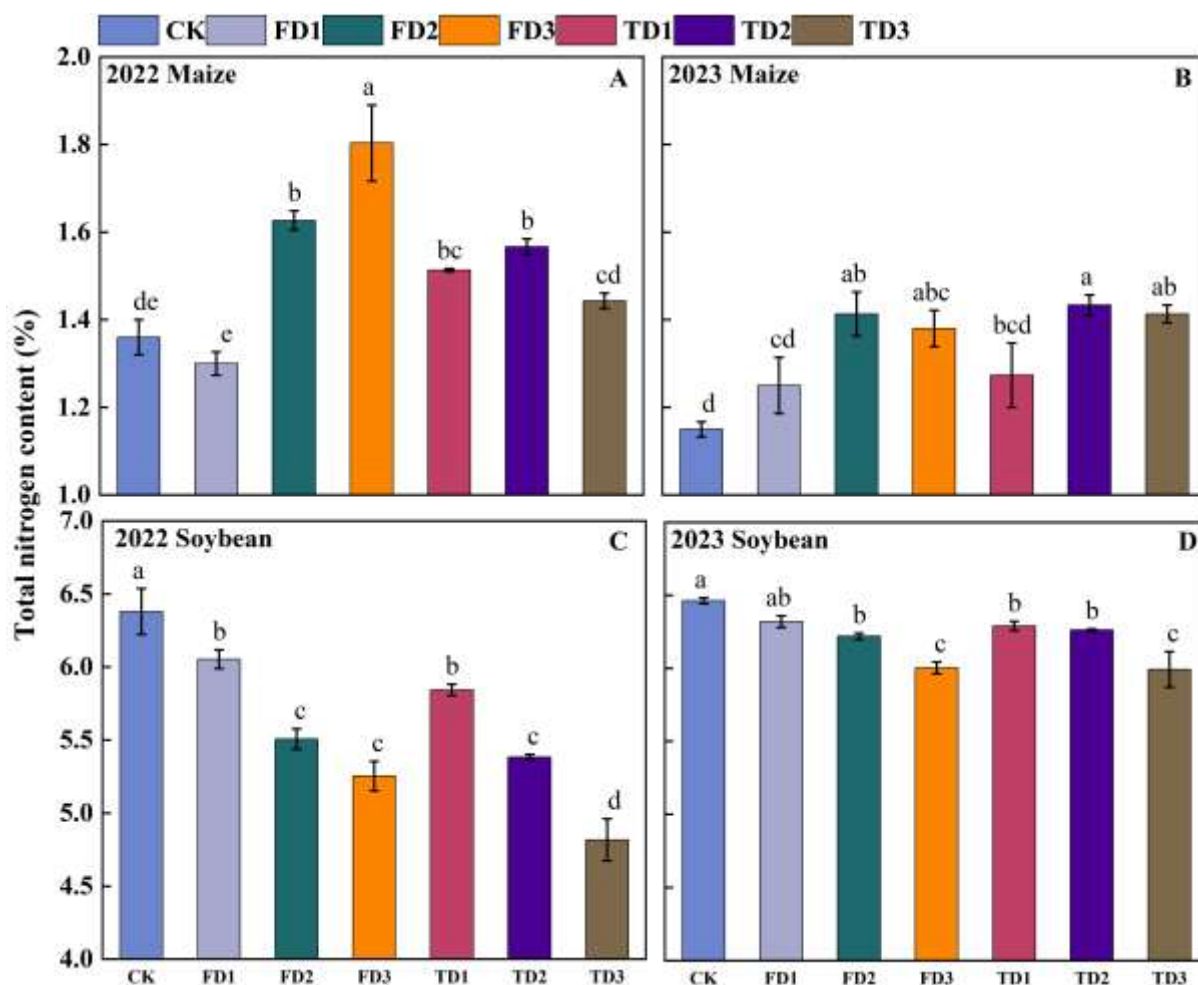
90. Crude protein content (A, E), soluble sugar content (B, F), starch content (C, G), and crude fat content (D, H). Letters A-D and E-H correspond to maize and soybean, respectively. F, four rows of maize intercropped with four rows of soybean; T, two rows of maize intercropped with two rows of soybean. D1, D2, and D3 correspond to maize densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively.



91.

92. **Fig. 5 Soil microbial biomass carbon under maize and soybean as affected by intercropping patterns and maize planting densities during the 2022 – 2023 growing seasons.**

93. Soil microbial biomass carbon under maize (A: 2022; B: 2023) and soybean (C: 2022; D: 2023). CK, maize or soybean monoculture; F, four rows of maize intercropped with four rows of soybean; T, two rows of maize intercropped with two rows of soybean; D1, D2, and D3 correspond to maize planting densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively. Different lowercase letters indicate significant differences among treatments at $P < 0.05$ level. Vertical bars represent $\pm$ standard error of the mean (n = 3).

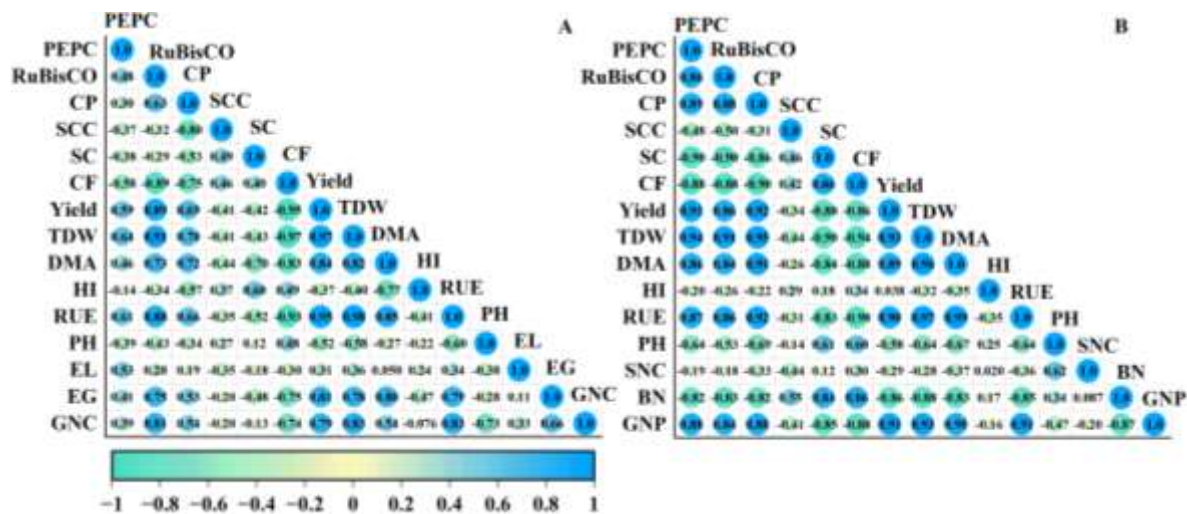


95.

96. **Fig. 6 Grain total nitrogen content of maize and soybean as affected by intercropping patterns and maize planting densities during the 2022 – 2023 growing seasons.**

97. Total nitrogen content under maize (A: 2022; B: 2023) and soybean (C: 2022; D: 2023). CK, maize or soybean monoculture; F, four rows of maize intercropped with four rows of soybean; T, two rows of maize intercropped with two rows of soybean; D1, D2, and D3 correspond to maize planting densities of 45,000, 52,500, and 60,000 plants ha⁻¹, respectively. Different lowercase letters indicate significant differences among treatments at $P < 0.05$ level. Vertical bars represent $\pm$ standard error of the mean (n = 3).

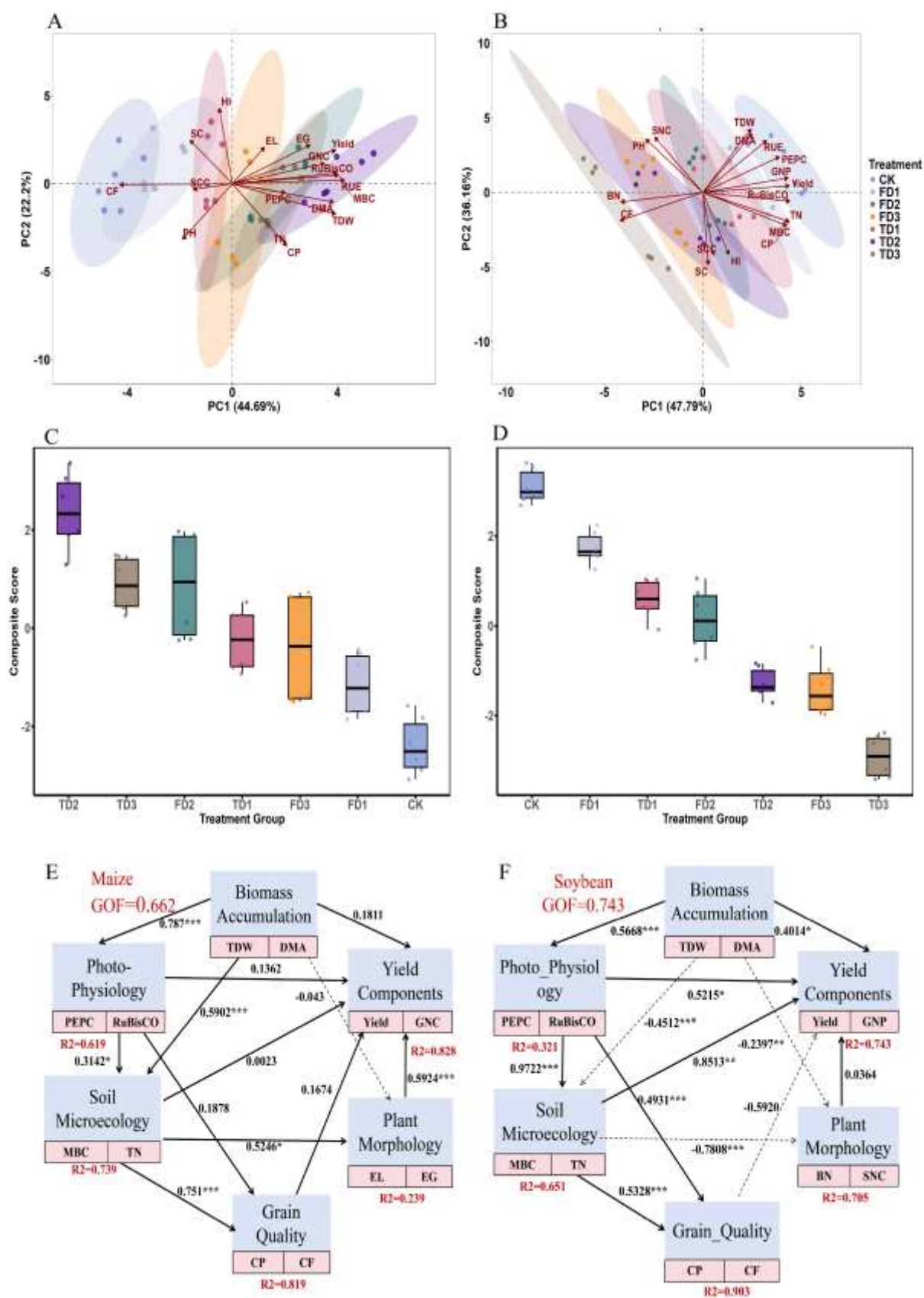
99.



100.

101. Fig. 7 Correlation matrix illustrating the interrelationships among yield components, quality traits, growth parameters, and resource use efficiency in maize and soybean under intercropping systems.

102. Correlation analysis of maize (A) and soybean (B). CP, crude protein content; SCC, soluble sugar content; SC, starch content; CF, crude fat content; TDW, total dry matter weight; DMA, dry matter accumulation; HI, harvest index; RUE, radiation use efficiency; PH, plant height; EL, ear length; EG, ear girth; NC, grain number per cob; SNC, stem node count; BN, branch number; GNP, grain number per plant.



103.

104. Fig. 8 Principal component analysis of maize (A) and soybean (B), comprehensive score ranking of maize (C) and soybean (D), and partial least squares path modeling (PLS-SEM) of maize (E) and soybean (F).

105. CP, crude protein content; CF, crude fat content; TDW, total dry matter weight; DMA, dry matter accumulation; RUE, radiation use efficiency; EL, ear length; EG, ear girth; GNC, grain number per cob; SNC, stem node count; BN, branch number; GNP, grain number per plant. TN, total nitrogen content; MBC, Soil microbial biomass carbon. Solid lines indicate positive effects, while dashed lines indicate negative effects. R^2 represents the variance explained by the structural equation model. The numbers adjacent to the lines are standardized path coefficients, indicating the effect size of relationships, with significance levels denoted as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

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