

IMPROVING EFFECT OF AGROFORESTRY INTERCROPPING ON THE QUALITY AND YIELD OF EDIBLE CHRYSANTHEMUMS

YANG, F.¹ – SHAO, W. Q.² – LU, X. Y.¹ – YING, L.¹ – HUANG, C.^{3*}

¹*Cultural and Creative College, Anhui Finance & Trade Vocational College, Hefei 230601, China*

²*School of Fine Arts and Design, Hefei Normal University, Hefei 230092, China*

³*School of Forestry and Landscape Architecture, Anhui Agricultural University, Hefei 230036, Anhui, China*

**Corresponding author
e-mail: huangcheng0103@163.com*

(Received 8th Oct 2023; accepted 22nd Dec 2023)

Abstract. As a multifunctional flower with both ornamental and nutritional value, the chrysanthemum has been cherished worldwide. To explore the effects on growth, yield, and quality of *Chrysanthemum morifolium* × ‘Yanshan Jinhuang’ under different agroforestry intercropping models, the growth status, photosynthesis, antioxidant leaf substances, and nutrient content of chrysanthemum flowers under nine *Acer buergerianum* - *Chrysanthemum morifolium* × ‘Yanshan Jinhuang’ intercropping modes, and three chrysanthemum monoculture modes with different planting densities were investigated. The results revealed that the canopy closure of *Acer buergerianum* had significant impacts on the growth of chrysanthemum flowers, and the accumulation of nutrients in major petals. The stomatal conductance and superoxide dismutase activities of leaves were the primary factors that affected the accumulation of secondary metabolites in petals. When the canopy closure ranged from 0.2-0.39 and the chrysanthemum planting density was 40 × 40 cm, the flower yield of chrysanthemum reached 10137 kg·ha⁻¹. This was 34.29% - 38.14% higher than under a single planting mode and the nutrient content was also higher. This study showed that moderate shading improved the flower yield and quality of chrysanthemums in eastern China. Thus, we recommend that growing edible chrysanthemums under forests with proper shade can reduce the investments required for cultivation facilities, while obtaining good quality high-yielding chrysanthemum products.

Keywords: *agroforestry, physiological characteristics, growth, photosynthesis, antioxidant content*

Introduction

As an agricultural production strategy that improves the utilization of land/spatial resources, agroforestry is broadly utilized worldwide (Gu et al., 2021; Fan et al., 2006). In China, agroforestry is typically based on ecology, where perennial woody plants and annual or perennial crops are grown in various combinations on the same land, giving rise to unique management models (Li et al., 2020; Wang et al., 2022).

Intercropping systems play an important role toward increasing crop productivity and maintaining soil fertility (Huang et al., 2022b). Most studies suggest that agroforestry can improve soil quality by reducing soil acidification and inhibiting soil compaction, and that root-to-root interactions effectively inhibit the spread of diseases; thus, reducing pesticide use (Brooker et al., 2016; Wen et al., 2019). On one hand, intercropping enhances soil fertility and increases crop productivity by positively influencing soil microorganisms and increasing enzyme activities (Ma et al., 2017). Further, woody plant canopies may significantly alter the light intensity, temperature, and humidity in the environments of small intercropped systems. For plants that thrive

in shade, intercropped systems provide more comfortable conditions that are conducive to growth (Xu et al., 2023). However, for certain sun-loving leguminous crops (e.g., peanuts and soybeans), intercropping may reduce yields, contingent on the heights of the woody plants. The interception of light by the intercrop canopy can negatively impact the growth of legumes, particularly during germination and flowering (Raza et al., 2019; Wang et al., 2020). It is now known that light exposure affects the phenotype and yields of crops beneath the canopy (e.g., reduced cereal tillers under low light intensity, shorter legume nodules, and fewer pods) (Huang et al., 2022a). Studies have revealed that the quality of intercrops may be improved in intercropping systems through the utilization of light resources, as well as the enhancement of interspecific interactions and soil nutrients (Li et al., 2023). For example, growing green tea plants in orchards can increase their free proline content, reduce their catechin content and polyphenol to amino acid ratio, and improve their quality (Wen et al., 2019).

Potent interactions between species are expected in intercropping systems, which maximize the benefits of individual interactions for different species (Wang et al., 2020). These interspecific interactions depend primarily on species compositions and planting distances. These factors strongly affect the competition for light, water, and soil nutrients between different species, and may have allelopathic effects on other species through the release of rhizospheric exudates (Zhang et al., 2007; Prasad et al., 2005). Species composition is an essential factor that determines the intensity of interactions between species. When herbaceous plants and arbor species are combined the interactions between the two are uneven, as the arbor is in an absolutely dominant position, especially considering the interception of light and rain by aboveground components (Huang et al., 2022a). Therefore, the competitive distances between layouts and crops, and the regulation of competitive relationships between species for resources are key issues that give full play to the advantages of intercropping.

Chrysanthemum is a versatile herbaceous flower that is native to China, and extensively used as a cut flower worldwide (Shimizu-Yumoto et al., 2023). Chrysanthemum petals contain potent antioxidants such as total phenols, flavonoids, chlorogenic acid, and vitamin C, making them both medicinal and an alternative to vegetables or tea, particularly in East Asia (Yang et al., 2023; Chen et al., 2021; Han et al., 2019; Wang et al., 2017). To reduce land use, growers often plant chrysanthemums between trees; however, it is unclear whether intercropping influences the quality and yields of chrysanthemums.

To elucidate the effects of intercropping on chrysanthemum yields and quality, this study selected an intercropped *Acer buergerianum* and *Chrysanthemum morifolium* system as the research model, with the aim of addressing the following questions: (1) Do chrysanthemum flower yields decrease in intercropped systems? (2) How does the quality of chrysanthemums change after intercropping? (3) Which specific intercropping mode is more conducive to the growth of chrysanthemums?

Materials and methods

Study area

The test site is located at the Anhui Mantianhong Ecological Agriculture Co., Ltd. (E117 °25 '31 " , N31 °16 '24 "), in Anhui Province, China, which belongs to a northern subtropical humid monsoon climate zone, with a yellow-brown soil type (Table 1). There is almost no slope, and the soil layer depth is 50-100 cm.

Table 1. Soil characteristics of composite pre-test site

Soil layer (cm)	AN (g·kg ⁻¹)	AP (g·kg ⁻¹)	TN (g·kg ⁻¹)	TP (g·kg ⁻¹)	pH	EC (ds·m ⁻¹)
0-20	2.52 ± 0.88	0.98 ± 0.81	0.62 ± 0.62	1.88 ± 0.08	5.20 ± 0.40	95.31 ± 13.17
20-40	4.1 ± 3.59	0.40 ± 0.37	1.07 ± 0.48	1.61 ± 0.11	5.42 ± 0.51	84.10 ± 13.78

AN, alkali hydrolyzed nitrogen; AP, available phosphorus; TN, total nitrogen; TP, total phosphorus; EC, conductivity value

Five-year-old *Acer buergerianum* species were planted in 2020 at a planting density of 400 plants/ha. Prior to the experiment, the canopy density was adjusted to low (0.20–0.39), medium (0.40 - 0.69), and high (≥ 0.7) via pruning. In early 2022, *Chrysanthemum × morifolium* ‘Yanshan Jinhuang’ (from the Beijing Agricultural Biotechnology Research Center) was planted in the forest, where the planting densities of the three chrysanthemums (30 × 30 cm, 40 × 40 cm, and 50 × 50 cm) were established. Using a chrysanthemum monoculture as a control (Table 2; Fig. 1), the same cultivation and management measures were adopted for different intercropping methods.

Table 2. Experimental design table of different composite modes

Canopy density	Density of chrysanthemums (cm)	Agroforestry pattern
L (0.2-0.39)	30 × 30	P1
	40 × 40	P2
	50 × 50	P3
M (0.4-0.69)	30 × 30	P4
	40 × 40	P5
	50 × 50	P6
H (≥ 0.7)	30 × 30	P7
	40 × 40	P8
	50 × 50	P9
Single cultivation of chrysanthemums	30 × 30	CK1
	40 × 40	CK2
	50 × 50	CK3

L, low canopy density; M, medium canopy density; H, high canopy density

Sampling design

The area specifications of the *Acer buergerianum*/chrysanthemum composite mode and the single chrysanthemum sample were both 15 m × 5 m for a total of 12 squares. Each treatment was repeated three times, with two chrysanthemums representing one group. For each mode, six chrysanthemums with consistent growth and no diseases or pests were randomly selected for tagging, for a total of 72 plants. Organic fertilizer is applied to the woodland (30 t/hm²) 3 months before chrysanthemum planting, and it is fully mixed with the soil using a rotary tiller to meet the growth of chrysanthemum, and no chemical fertilizer is used in the planting process. Place insect traps every 30 m in the plantation. The survival rate of chrysanthemum planting was 99.4%, and the chrysanthemum grew well during the experiment and was not stressed by diseases (Fig. 2; Table 3).

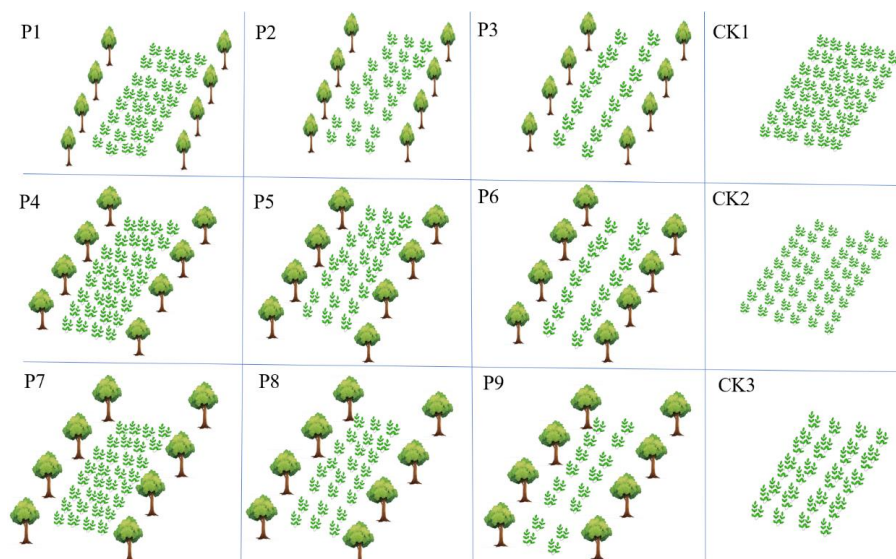


Figure 1. Diagram of intercropping patterns



Figure 2. Plantation photograph

Index measurement

The plant height (H) (accurate to 0.1 cm) and stem thickness (D) (accurate to 0.01 cm) were determined using a tape measure and vernier caliper, and the average cluster diameter (C) of the chrysanthemum flowers was recorded at different periods (August 21th, September 22nd and November 19th). On August 21st, in sunny and windless weather, a portable photosynthetic analyzer (LI-COR, LI-6400 XT, USA) was used to measure photosynthetic indicators from 8:30-11:30 a.m., which was repeated in triplicate to minimize instrumental errors. Healthy and mature functional leaves in the middle and upper portions of the plants were selected, and the light intensity was set to

1600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for instantaneous measurements. Further, the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci), and transpiration rate (Tr) were recorded. On November 9th, the three largest flowers of each chrysanthemum plant were selected and the flower diameter was measured with a vernier caliper (accurate to 0.01 m). All chrysanthemums are harvested at the ripening stage of full bloom (November 19th), stored separately according to flowers, branches and leaves, quickly transferred to the laboratory, and refrigerated at 4°C. Some of these samples were used to detect petal inclusions and leaf antioxidant content, while the remaining samples were employed to count the flower yield (NO.F). The petal TS, TP, TF, TAA, and Vc were quantified using ADS-F-TDX030, ADSO59TC0, ADS-F-KY007-48, ADS147TC0, and ADS141TC0 assays (Jiangsu (China) Aidisheng Biotechnology Co., Ltd). Thiobarbituric acid was used to determine the malondialdehyde (MDA) content (Ma et al., 2015), the guaiacol technique was used to determine the peroxidase (POD) content (Liang et al., 2022), and the nitro blue tetrazolium method was utilized to determine the super oxide dismutase (SOD) content (Banakar et al., 2022).

Table 3. Change of forest microenvironment

Agroforestry pattern	Temperature (°C)		Humidity (%)	
	Surface temperature	Soil temperature	Surface humidity	Soil moisture
P1	32.25 ± 0.12	32.14 ± 0.08	61.35 ± 1.12	73.25 ± 0.43
P2	32.37 ± 0.08	32.26 ± 0.12	60.28 ± 0.98	71.68 ± 0.36
P3	31.39 ± 0.32	32.88 ± 0.09	58.36 ± 0.74	68.73 ± 0.58
P4	32.03 ± 0.13	32.11 ± 0.05	62.12 ± 1.03	74.26 ± 0.44
P5	32.32 ± 0.10	32.25 ± 0.11	60.89 ± 1.07	73.32 ± 0.72
P6	32.34 ± 0.09	32.46 ± 0.07	60.05 ± 0.56	70.17 ± 0.67
P7	31.65 ± 0.14	31.52 ± 0.13	64.27 ± 0.77	75.88 ± 1.04
P8	31.72 ± 0.07	31.79 ± 0.02	63.56 ± 0.24	75.43 ± 0.85
P9	31.73 ± 0.32	32.15 ± 0.06	63.15 ± 1.01	73.35 ± 0.92
CK1	33.14 ± 0.04	32.95 ± 0.08	56.28 ± 0.78	67.54 ± 0.33
CK2	33.15 ± 0.11	33.14 ± 0.04	55.83 ± 0.94	65.31 ± 0.26
CK3	33.14 ± 0.13	33.18 ± 0.07	56.25 ± 0.83	63.12 ± 0.14

Data analysis

Firstly, one-way ANOVA was performed in R software (4.1.3) to compare differences in the chrysanthemum photosynthetic parameters, growth level, flower yield, primary petal nutrients, and antioxidant contents of leaves under different intercropping modes. Canoco 5 was employed to complete the redundancy analysis (RDA), while Monte-Carlo test analysis was used to identify the key factors that affected the main chrysanthemum nutrients.

Results

Correlation of height and cover with aboveground biomass

The intercropping modes had significant effects on the plant height, stem thickness, and bush diameter at different stages of chrysanthemum growth ($p < 0.001$) (Table 4).

Under P7, the chrysanthemum heights were the greatest for the three growth periods (24.33 cm, 44.17 cm, and 51.50 cm, respectively), which were significantly higher than CK1, CK2, and CK3 ($p < 0.05$). The coarse growth of chrysanthemum stems was significantly different under the various intercropping modes. The coarse chrysanthemum stems were largest under the P3 mode at the seedling stage, which increased by 5.06%, 3.66%, and 7.91%, respectively compared with the CK group. The chrysanthemum stem thickness growth rates of the control groups (CK1, CK2, and CK3) accelerated, which were significantly higher than those under most intercropping modes at full bloom (November). The stem thickness of CK3 was the largest, reaching 8.93 mm. The mean chrysanthemum cluster diameter of the nine intercropping modes was significantly lower than that of the control group ($p < 0.05$), with the maximum exhibited by CK2 in August and September, and CK3 being the largest in November (Fig. 3).

Table 4. Effects of intercropping patterns on growth and yield of chrysanthemum

			df	Sum of squares	Mean squares	F	p-Value
Height	August	Between Groups	11	590.5	53.682	6.455	***
		Within Groups	60	499	8.317		
	September	Between Groups	11	674.354	61.305	6.593	***
		Within Groups	60	557.875	9.298		
	November	Between Groups	11	875.581	79.598	7.464	***
		Within Groups	60	639.883	10.665		
Diameter	August	Between Groups	11	20.743	1.886	5.341	***
		Within Groups	60	21.183	0.353		
	September	Between Groups	11	30.308	2.755	9.066	***
		Within Groups	60	18.235	0.304		
	November	Between Groups	11	44.295	4.027	12.886	***
		Within Groups	60	18.75	0.313		
cluster diameter	August	Between Groups	11	571.569	51.961	15.699	***
		Within Groups	60	198.583	3.31		
	September	Between Groups	11	750.662	68.242	12.594	***
		Within Groups	60	325.108	5.418		
	November	Between Groups	11	441.052	40.096	6.121	***
		Within Groups	60	393.025	6.55		

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level; ***correlation is significant at the 0.001 level. The same applies below

The intercropping patterns significantly affected the chrysanthemum fresh weights, flower diameters, numbers of flowers per plant, and yields ($p < 0.001$) (Table 5). Under the *Acer buergerianum* stands (P7, P8, and P9) with high canopy closure, the heights of the chrysanthemums increased significantly; however, the stem thicknesses were significantly reduced, as were the yields. Interestingly, there were no significant differences in the chrysanthemum growth under the *Acer buergerianum* stands (P1, P2, and P3) with low closure, compared with single planting. However, the number of chrysanthemum flowers, flower diameters, and flower yields increased to varying

degrees. For all intercropping modes, except for P7, P8, and P9, the number of flowers, single fresh flower weights, flower diameters, and yields per unit area were higher than those in the CK group under other intercropping modes. P2 possessed the highest number of flowers, the heaviest single fresh flower weights, the largest flower diameters, and the highest yields per unit area, followed by P3, which was significantly higher than that of the CK group and other intercropping modes ($p < 0.05$) (Fig. 4).

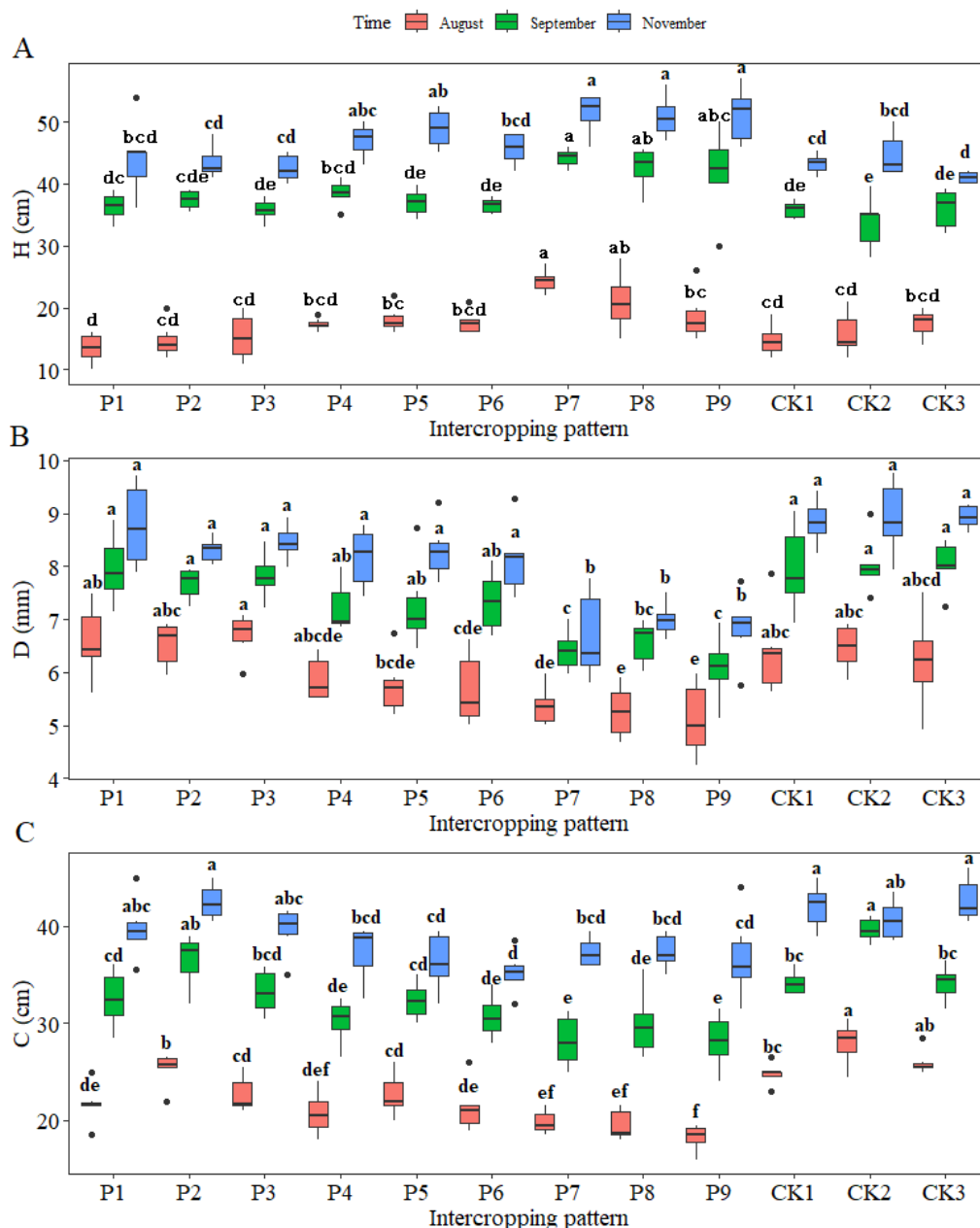


Figure 3. Growth status of chrysanthemum for three different growth stages under different intercropping patterns. (A) Changes in chrysanthemum plant height under different intercropping patterns for different periods. (B) Changes in stem diameter of chrysanthemum for different periods under different intercropping patterns. (C) Changes in chrysanthemum bush diameter for different periods under different intercropping patterns. The significance level was $p < 0.05$, with a 95% mean confidence interval. Letters indicate variations between different treatments. The same applies below

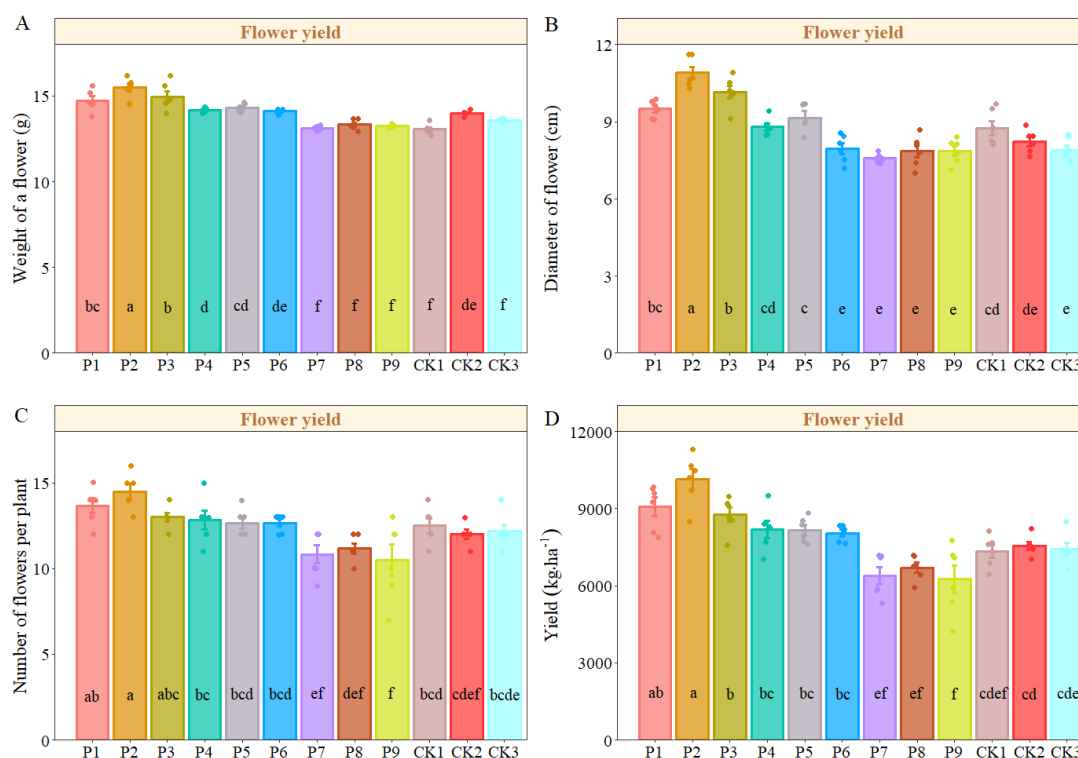


Figure 4. Yield differences of chrysanthemum under different intercropping modes. (A) Chrysanthemum flower weight under different intercropping modes; (B) chrysanthemum flower diameter under different intercropping modes; (C) number of chrysanthemum flowers per plant under different intercropping modes; (D) chrysanthemum flower yield per unit area under different intercropping modes

Table 5. Effects of different intercropping modes on yield of chrysanthemum

		df	Sum of squares	Mean squares	F	p-Value
Weight of flowers	Between Groups	11	41.021	3.729	26.773	***
	Within Groups	60	8.357	0.139		
Diameter of flower	Between Groups	11	71.026	6.457	24.893	***
	Within Groups	60	15.563	0.259		
Number of flowers per plant	Between Groups	11	87.042	7.913	6.261	***
	Within Groups	60	75.833	1.264		
Yield	Between Groups	11	85907620	7809784	13.914	***
	Within Groups	60	33676767	561279.5		

Effects of intercropping mode on photosynthesis of chrysanthemum

There were significant differences in the optical parameters of chrysanthemums under different intercropping modes ($p < 0.001$) (Table 6). The Pn of chrysanthemums under P1, P2, and P3 was significantly higher than that of other cultivation modes ($p < 0.05$) (Fig. 5). The leaf Ci was highest under P7, P8, and P9, which was significantly higher than that of other planting modes; however, the stomatal

conductance under P7, P8, and P9 was the lowest, which was significantly lower than that of the CK ($p < 0.05$). Chrysanthemum leaves had the highest Tr under P1, followed by P2 and CK3.

Table 6. Effects of different intercropping patterns on photosynthetic parameters of chrysanthemum

		df	Sum of squares	Mean squares	F	p-Value
Pn	Between Groups	11	2877.468	261.588	55.918	***
	Within Groups	60	280.682	4.678		
Ci	Between Groups	11	69134.17	6284.924	119.208	***
	Within Groups	60	3163.333	52.722		
Gs	Between Groups	11	187082.2	17007.47	236.955	***
	Within Groups	60	4306.5	71.775		
Tr	Between Groups	11	104.677	9.516	82.232	***
	Within Groups	60	6.943	0.116		

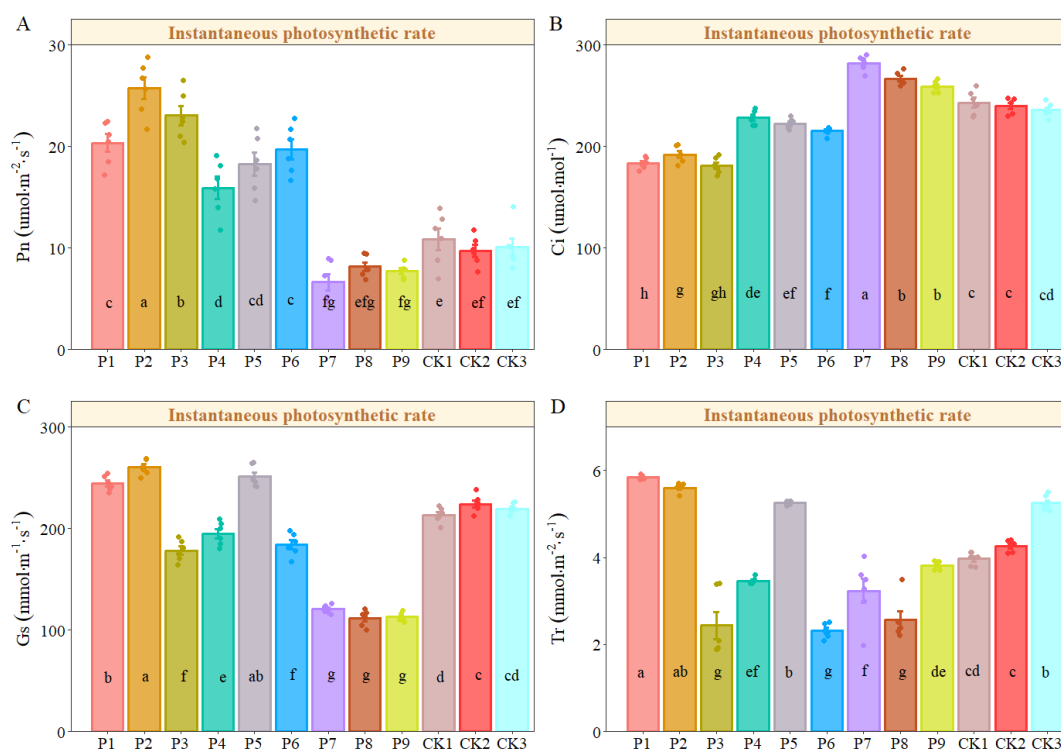


Figure 5. Differences in photosynthetic physiology of chrysanthemum under different intercropping modes. (A) Net photosynthetic rate of chrysanthemum leaves; (B) inter-cellular carbon dioxide concentration in chrysanthemum leaves; (C) stomatal conductance of chrysanthemum leaves; (D) transpiration rate of chrysanthemum leaves

Effects of intercropping mode on the main nutrients of chrysanthemum

The main nutrient contents of chrysanthemum petals were significantly different under different intercropping modes ($p < 0.05$) (Table 7). The TS content of petals was the

highest under P4 (39.00 g/kg), which increased by 8.33% - 69.57% compared with the CK, while the lowest was under P8 (16.00 g/kg). The highest total amounts of TP and TF were under P1 with the lowest under P7 and P9 (Fig. 6), respectively. Under P2, the number of Chrysanthemum petals was the highest, which increased by 8.06%–12.61% compared with the CK, while the lowest was under P5 (10.10 mg/100 g). In all composite modes, the TAA of chrysanthemum petals was lower than that of the CK group, and P9 was the lowest, which decreased by 54.55%–72.22% compared with the CK group.

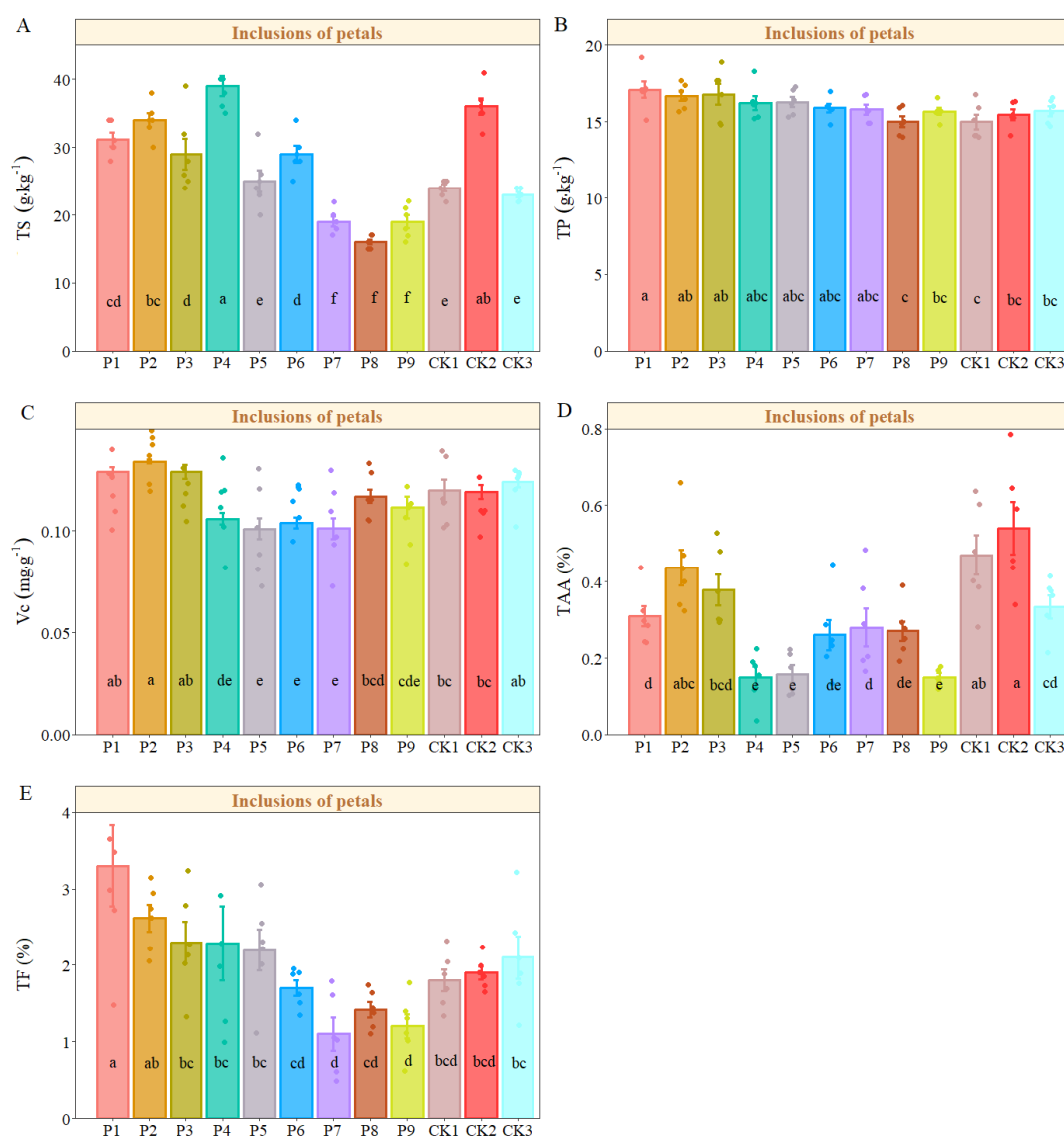


Figure 6. Contents of chrysanthemum petals varied under different intercropping modes. (A) total sugar content, (B) total protein content, (C) vitamin C content, (D) total amino acid content, (E) total flavonoid content

Effects of intercropping mode on antioxidant content in chrysanthemum leaves

The results revealed that the different intercropping modes had significant effects on the SOD and POD activities, and MDA content in chrysanthemum leaves ($p < 0.001$) (Table 8). Under P2, the SOD and POD activities of leaves were the highest, which

increased by 21.65-26.02% and 17.07-27.28%, respectively, compared with CK1, CK2, and CK3. Under P9, the chrysanthemum leaves exhibited the lowest SOD and POD activities but the greatest MDA content, which was significantly higher than that in control and under other intercropping modes ($p < 0.05$) (Fig. 7).

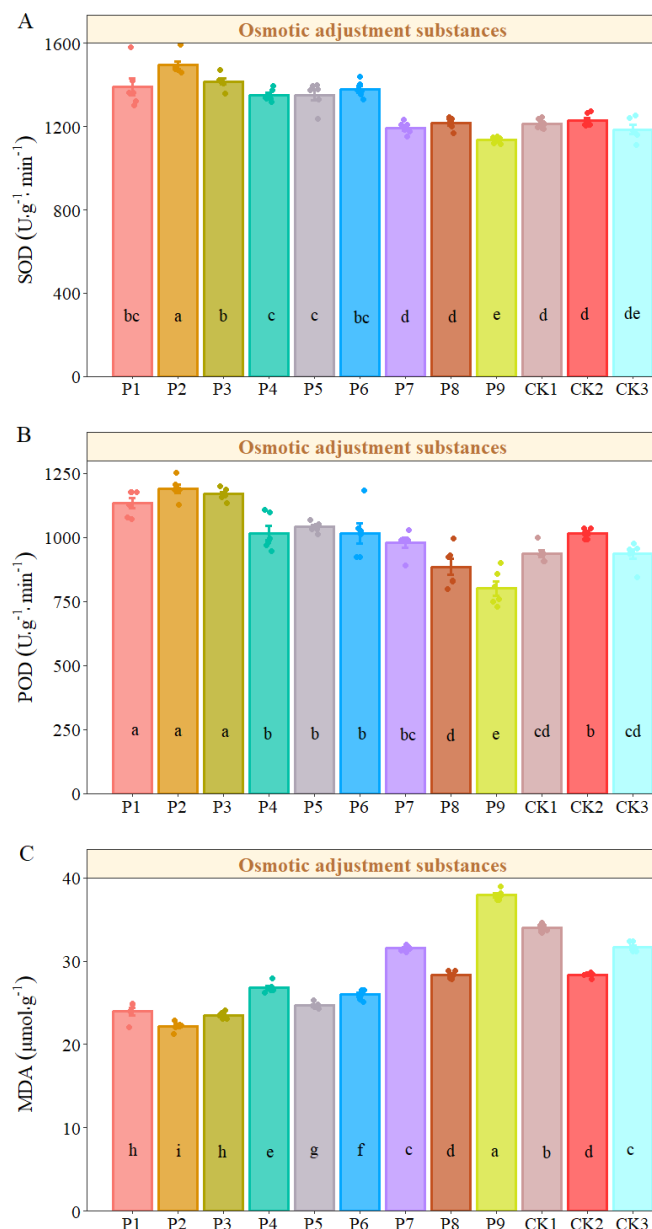


Figure 7. Antioxidant enzyme activities and malondialdehyde content in chrysanthemum leaves. (A) superoxide dismutase activities, (B) peroxidase activit, (C) malondialdehyde content

Eleven factors, including antioxidant enzyme activities, leaf light parameters, leaf N and P contents, flower diameter, and number of flowers per plant were selected as variables, and the main nutrients of five petals were used as response variables for RDA analysis (Table 9). The petal inclusion characteristic values of the first and second axes of the sorting axis were 0.5015 and 0.0098, respectively, and the correlations between

the main petal nutrients and independent variable factors were 0.7196 and 0.6651, respectively. The cumulative explanatory amount of the first two axis independent variable factors on the content of petal inclusions attained 51.12%. The first two axes reflected the relationship between petal inclusions and independent variable factors and were primarily determined by the first axis.

Table 7. Effects of different intercropping patterns on the quality of chrysanthemum petals

		df	Sum of squares	Mean squares	F	p-Value
TS	Between Groups	11	3464.153	314.923	36.702	***
	Within Groups	60	514.833	8.581		
TP	Between Groups	11	30.005	2.728	2.761	*
	Within Groups	60	59.275	0.988		
Vc	Between Groups	11	0.009	0.001	9.107	***
	Within Groups	60	0.005	0		
TAA	Between Groups	11	1.074	0.098	10.379	***
	Within Groups	60	0.564	0.009		
TF	Between Groups	11	25.322	2.302	5.187	***
	Within Groups	60	26.63	0.444		

Table 8. Effects of intercropping mode on antioxidant oxidase activities and malondialdehyde content in chrysanthemum leaves

		df	Sum of squares	Mean squares	F	p-Value
SOD	Between Groups	11	850573.2	77324.84	35.325	0
	Within Groups	60	131338	2188.967		
POD	Between Groups	11	872263.9	79296.72	27.153	0
	Within Groups	60	175223.5	2920.392		
MDA	Between Groups	11	1481.76	134.705	438.29	0
	Within Groups	60	18.441	0.307		

Table 9. Eigenvalue and cumulative explanatory values of the redundancy analysis ranking of chrysanthemum petal contents

Statistic	Eigenvalues	Cumulative explained variation (%)	Pseudo-canonical correlation	Cumulative explained fitted variation (%)
Axis 1	0.5015	50.15	0.7196	97.87
Axis 2	0.0098	51.12	0.6651	99.78
Axis 3	0.0011	51.23	0.3424	99.99
Axis 4	0.0001	51.24	0.3804	100

The two-dimensional ranking plot of petal inclusions and independent variable factors indicated that the first axis was mainly related to Gs, SOD, and the number of flowers per plant, while the second axis was primarily associated with Tr and the leaf P

content (Fig. 8). The TS and TAA were at sharp angles to Gs, SOD, Tr, and P, which implied that the contents of these two petals were mainly affected by Gs, SOD, Tr, and P, and there was a certain positive correlation between the two. The angle between MDA and Ci and the main nutrient content of the five petals were greater than 90°, indicating that the increase in MDA content of Ci affected the accumulation of petal nutrients. The effects of chrysanthemum physiology and biochemistry on the accumulation of main nutrients in their petals were further investigated, and the Monte Carlo test was performed for the respective variables and factors (Table 10). The influence importance of different independent variable factors was $Gs > SOD > Tr > P > POD > Pn > Ci > D.F > N > NO.F > MDA$, and the effects of Gs and SOD on the content of petal inclusions reached a significant level ($p < 0.05$).

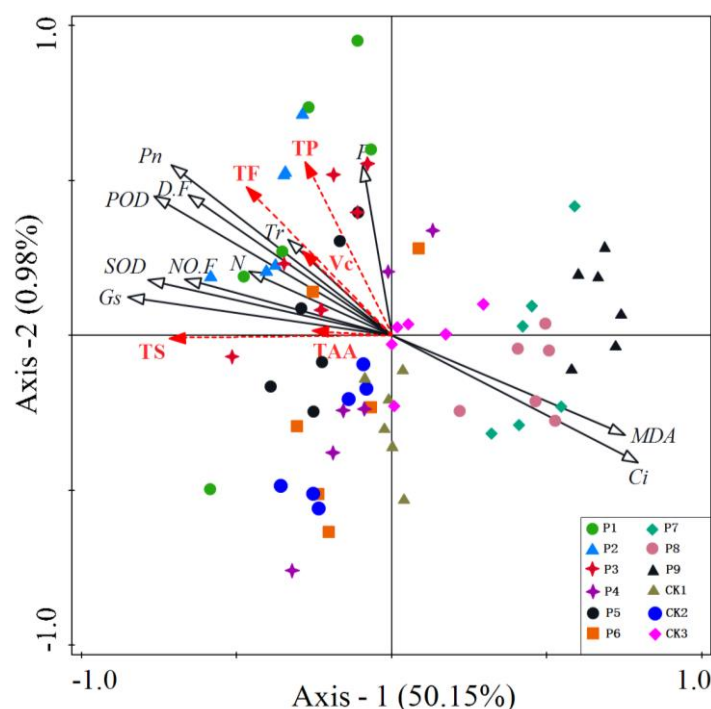


Figure 8. RDA analyzes two-dimensional sequence diagrams

Table 10. Importance ranking and significance test results of factor variable interpretation

Name	Order of importance	Explains (%)	Contribution (%)	pseudo-F	P
Gs	1	36.3	70.8	39.9	0.002
SOD	2	7.4	14.5	9.1	0.006
Tr	3	2.9	5.7	3.7	0.064
P	4	2.1	4.1	2.7	0.078
POD	5	1.2	2.3	1.6	0.228
Pn	6	0.6	1.1	0.8	0.36
Ci	7	0.4	0.8	0.6	0.496
D.F	8	0.2	0.5	0.3	0.642
N	9	< 0.1	< 0.1	< 0.1	0.92
NO.F	10	< 0.1	< 0.1	< 0.1	0.952
MDA	11	< 0.1	< 0.1	< 0.1	0.954

Discussion

Growth and yield of chrysanthemums

Intercropping systems have a significant impact on crop growth, contingent on the species composition of the intercropping system and the interspecific distance (Huang et al., 2022a; Jiao et al., 2021). The influence of species composition on crop growth in intercropping systems is self-evident, and there are multiple competitive or synergistic relationships between species as relates to the acquisition and distribution of resources. A recent experiment showed that intercropping with *Bletilla striata* improved soil conditions and increased yields (Xu et al., 2023). Earlier studies have also shown that intercropping with *Castanea mollissima* improved the soil nutrient availability, and enhanced the quality and yield of *Camellia sinensis* (Ma et al., 2017).

In this study, the growth and flower yields of chrysanthemum differed significantly under diverse canopy density environments; however, the various chrysanthemum planting densities did not translate to significant differences in chrysanthemum growth and flower yields under the same canopy closure conditions. Consequently, differences in light exposure caused by the shading of upper vegetation in intercropping systems may be a critical environmental factor that influences its growth and yields. The flowering physiology of plants is mediated by numerous photoreceptors, and the regulatory mechanism can vary depending on long, short, or day-neutral plants (Lin, 2000). Changes in canopy density affects the light duration and light intensity of the underlying vegetation; thus, for chrysanthemum (as a typical short-day plant) inadequate or excessive light can impact the differentiation of its flower buds, and subsequently affect the flower population. The effects of light quality on chrysanthemum growth were studied, with the results showing that the stem length extension was promoted under low- and far-red light conditions, or was supplemented under far-red light exposure (Jeong et al., 2014; Hisamatsu et al., 2008). The chrysanthemum height was greatest in stands with high closure, which has been typically reported to be consistent with the “equilibrium growth hypothesis” of biomass redistribution. This is caused by low level incident light, where increased shade intensity causes plants to transfer energy supplies from roots to bud growth to stimulate height to escape the shade (Shipley et al., 2002). Therefore, moderate shading can simultaneously meet the chrysanthemum light needs for growth and flower bud differentiation.

For this study, the chrysanthemum plant density did not appear to have a universally significant effect on its growth and yield (Figs. 2 and 3). This was inconsistent with previous studies, which suggested that the expression of most genes that varied in the branching capacity of chrysanthemums were dependent on planting density. In other words, density changes can induce changes in the number of chrysanthemum branches, which in turn also affects flower yields (Sun et al., 2019). It was suggested that the heights of chrysanthemum stems were related to the planting density, where higher densities led to increased chrysanthemum heights (Lee et al., 2009). However, we did not find a similar correlation in our experiments. This may have been because the planting density of chrysanthemums in our experiments was not dense overall. Although there were differences between them, they were more than enough to make them a limiting condition that constrained the vegetative growth of chrysanthemums.

Main factors affecting petal quality

The shading effect of upper vegetation in intercropping systems is an important factor that influences the accumulation of nutrients and the synthesis of secondary metabolites in lower crops (Chen et al., 2017; Zhang et al., 2021). Light quality and intensity have been shown to significantly affect the secondary metabolites of chrysanthemums and rosettes (Ouzounis et al., 2014). As an edible flower, the content of flavonoids, amino acids, and vitamins in the petals is an important parameter that determines its quality (Sun et al., 2023). In this study, chrysanthemum flowers had the highest Pn in the intercropping system under low depression closure, whereas the contents of TP, Vc, and TF in petals were relatively high. This indicated that the strength of chrysanthemum photosynthesis had a positive correlation with the synthesis of petal nutrients (Fig. 7). From the test results, Gs was the main factor that affected the nutrient synthesis of chrysanthemum flowers (Table 7). As described in the literature, the biosynthesis of Vc in plants depended on the regulation of light, in which the photosynthesis-dependent activation of the rate-limiting enzyme GDP-L-galactose phosphorylase (GGP) played a key role in the regulation of Vc synthesis and storage (Takanori, 2022). Studies have found that moderate shading increased the accumulation of C₆C₁ and C₆C₃ phenolic compounds in eleuthero, while severe shading stimulated the accumulation of C₃C₆C₃ phenolic substances (Xu et al., 2020).

The photoperiod is another important environmental factor that regulates the growth and development of medicinal plants, as they typically adopt various physiological modifications to adapt to temporal changes in light exposure, which includes adjusting the synthesis of secondary metabolites (Zhang et al., 2021; Li et al., 2019). Other studies found that different photoperiod treatments on *Linum usitatissimum* cells and *Basella rubra* callus were found to have various sensitivities to light exposure, which also affected the synthesis of phenols and flavonoids (Kumar et al., 2020; Anjum et al., 2017). The canopy density of the upper vegetation directly impacted light exposure for the lower chrysanthemum. This may have been a key factor that led to significant differences in the TF, Vc, and TP contents, as well as other elements in the petals of chrysanthemum under different canopy closures.

When the canopy density was low, the SOD and POD contents of the chrysanthemum leaves were high. This implied that the balance of reactive oxygen species in plant tissues were likely affected under low-shade environments, resulting in the synthesis of additional antioxidant enzymes to avoid cellular lipid peroxidation, which was confirmed by the lower MDA content. However, high levels of MDA were detected in chrysanthemum leaves under high forest canopy densities and in the CK, while the activities of SOD and POD were relatively low. It was confirmed that the activities of antioxidant enzymes had important effects on the accumulation of secondary metabolites (Zheng et al., 2017). It may be the case that the synthesis of antioxidant enzymes in chrysanthemums is affected under strong light exposure and excessive shade, resulting in cell peroxidation in plants. Further, SOD activity is one of the important factors that affects the synthesis of major nutrients in chrysanthemums (Table 7).

Conclusions

This study focused on the effects of different agroforestry intercropping patterns on chrysanthemum yields and quality. The results showed that the intercropping of chrysanthemum and *Acer buergerianum* significantly affected chrysanthemum growth,

and ultimately increased the flower yields and quality of chrysanthemum. The canopy density of the upper forest vegetation had a profound influence on the photosynthesis and synthesis of secondary metabolites in chrysanthemum. The Gs and SOD of its leaves were the most important factors that affected the synthesis of secondary metabolites in petals, which ultimately affected petal quality. The experimental results revealed that the yields and quality of chrysanthemums were higher when the canopy density of the upper forest vegetation layer was from 0.2–0.39, and the P2 mode of planting exhibited the best performance. Therefore, this study believes that compared with the single planting of chrysanthemums, proper shade of the upper vegetation in the intercropping system is more conducive to the growth of chrysanthemums and the accumulation of key substances, which can improve the yield and quality of chrysanthemums, and improve the utilization rate of land. Next, we will turn our attention to soil nutrients and microbial community function in intercropping systems, and further explore whether there is interspecific interference and soil function in intercropping systems.

Acknowledgments. This work was supported by the Special Major Science and Technology Project of Anhui Province (202103b06020011), Natural Science Research Foundation of Anhui Province University (KJ2021A1100), and the Special Fundamental Resources Investigation Project of the Ministry of Science and Technology (2019YF101810).

REFERENCES

- [1] Anjum, S., Abbasi, B. H., Doussot, J., Favre-Reguillon, A., Hano, C. (2017): Effects of photoperiod regimes and ultraviolet-C radiations on biosynthesis of industrially important lignans and neolignans in cell cultures of *Linum usitatissimum* L. (Flax). – *J Photochem Photobiol B* 167: 216-227. <http://dx.doi.org/10.1016/j.jphotobiol.2017.01.006>.
- [2] Banakar, M. H., Amiri, H., Ardakani, M. R. S., Ranjbar, G. H. (2022): Susceptibility and tolerance of fenugreek (*Trigonella foenum-graceum* L.) to salt stress: physiological and biochemical inspections. – *Environmental and Experimental Botany* 194: 10. DOI: 10.1016/j.envexpbot.2021.104748.
- [3] Brooker, R. W., Karley, A. J., Newton, A. C., Pakeman, R. J., Schob, C. (2016): Facilitation and sustainable agriculture: a mechanistic approach to reconciling crop production and conservation. – *Funct. Ecol.* 30(1): 98-107. <http://dx.doi.org/10.1111/1365-2435.12496>.
- [4] Chen, L., Liu, Y., Huang, X., Zhu, Y., Li, J., Miao, Y., Du, H., Liu, D. (2021): Comparison of chemical constituents and pharmacological effects of different varieties of *Chrysanthemum flos* in China. – *Chem. Biodivers.* 18(8): e2100206. <http://dx.doi.org/10.1002/cbdv.202100206>.
- [5] Chen, Y. H., Chen, Y., Guo, Q. S., Zhu, G. S., Wang, C. L., Liu, Z. Y. (2017): Growth, physiological responses and secondary metabolite production in *Itpinellia ternata* under different light intensities. – *Pak. J. Bot.* 49(5): 1709-1716.
- [6] Fan, F. L., Zhang, F. S., Song, Y. N., Sun, J. H., Bao, X. G., Guo, T. W., Li, L. (2006): Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. – *Plant and Soil* 283(1-2): 275-286. <http://dx.doi.org/10.1007/s11104-006-0019-y>.
- [7] Gu, C. F., Bastiaans, L., Anten, N. P. R., Makowski, D., van der Werf, W. (2021): Annual intercropping suppresses weeds: a meta-analysis. – *Agriculture Ecosystems & Environment* 322: 11. DOI: 10.1016/j.agee.2021.107658.

- [8] Han, A. R., Nam, B., Kim, B. R., Lee, K. C., Song, B. S., Kim, S. H., Kim, J. B., Jin, C. H. (2019): Phytochemical composition and antioxidant activities of two different color chrysanthemum flower teas. – *Molecules* 24(2): 14. <http://dx.doi.org/10.3390/molecules24020329>.
- [9] Hisamatsu, T., Sumitomo, K., Shimizu, H. (2008): End-of-day far-red treatment enhances responsiveness to gibberellins and promotes stem extension in chrysanthemum. – *J. Hortic. Sci. Biotechnol.* 83(6): 695-700. <http://dx.doi.org/10.1080/14620316.2008.11512446>.
- [10] Huang, B., Zou, X. J., Xu, H. S., Xu, J. Y., Liu, H. Y., Sun, W. T., Gong, L., Niu, S. W., Feng, L. S., Yang, N., Zhang, L. Z., Sun, Z. X. (2022a) Seedling defoliation of cereal crops increases peanut growth and yield in an intercropping system. – *Crop J.* 10(2): 418-425. <http://dx.doi.org/10.1016/j.cj.2021.05.012>.
- [11] Huang, C., Wang, Z. C., Ren, X. L., Ma, X. M., Zhou, M. Y., Ge, X., Liu, H., Fu, S. L. (2022b) Evaluation of soil quality in a composite pecan orchard agroforestry system based on the smallest data set. – *Sustainability-Basel* 14(17): 13. DOI: 10.3390/su141710665.
- [12] Jeong, S. W., Hogewoning, S. W., van Ieperen, W. (2014): Responses of supplemental blue light on flowering and stem extension growth of cut chrysanthemum. – *Sci. Hortic.* 165: 69-74. <http://dx.doi.org/10.1016/j.scienta.2013.11.006>.
- [13] Jiao, N. Y., Wang, J. T., Ma, C., Zhang, C. C., Guo, D. Y., Zhang, F. S., Jensen, E. S. (2021): The importance of aboveground and belowground interspecific interactions in determining crop growth and advantages of peanut/maize intercropping. – *Crop J.* 9(6): 1460-1469. <http://dx.doi.org/10.1016/j.cj.2020.12.004>.
- [14] Kumar, S. S., Arya, M., Mahadevappa, P., Giridhar, P. (2020): Influence of photoperiod on growth, bioactive compounds and antioxidant activity in callus cultures of *Basella rubra* L. – *J. Photochem. Photobiol. B* 209: 111937. <http://dx.doi.org/10.1016/j.jphotobiol.2020.111937>.
- [15] Lee, J. H., Heuvelink, E., Bakker, M. J. (2009): Interaction effects between light level and plant density on plant growth, development and external quality in year-around cut chrysanthemum. – *Korean J. Hortic. Sci. Technol.* 27(3): 391-398.
- [16] Li, C., Hoffland, E., Kuyper, T. W., Yu, Y., Zhang, C., Li, H., Zhang, F., van der Werf, W. (2020): Syndromes of production in intercropping impact yield gains. – *Nat Plants* 6(6): 653-660. <http://dx.doi.org/10.1038/s41477-020-0680-9>.
- [17] Li, C., Stomph, T. J., Makowski, D., Li, H., Zhang, C., Zhang, F., van der Werf, W. (2023): The productive performance of intercropping. – *Proc. Natl. Acad. Sci. U.S.A.* 120(2): e2201886120. <http://dx.doi.org/10.1073/pnas.2201886120>.
- [18] Li, J., Han, X., Wang, C., Tang, L., Zhang, W., Qi, W. (2019): The response of *Achyranthes bidentata* Blume to short-term UV-B exposure. – *Russ. J. Plant Physiol.* 66(1): 160-170. <http://dx.doi.org/10.1134/S1021443719010096>.
- [19] Liang, D. D., Ali, M. M., Alam, S. M., Huang, X. P., Yousef, A. F., Mosa, W. F. A., Orhan, E., Lin, Z. M., Chen, F. X. (2022): Genome-wide analysis of peroxidase genes in passion fruit (*Passiflora edulis* Sims.) and their expression patterns induced by root colonization of *Piriformospora indica* under cold stress. – *Turk. J. Agric. For.* 46(4): 496-508. <http://dx.doi.org/10.55730/1300-011x.3021>.
- [20] Lin, C. (2000): Photoreceptors and regulation of flowering time. – *Plant Physiol.* 123(1): 39-50. <http://dx.doi.org/10.1104/pp.123.1.39>.
- [21] Ma, J., Du, G., Li, X., Zhang, C., Guo, J. (2015): A major locus controlling malondialdehyde content under water stress is associated with *Fusarium* crown rot resistance in wheat. – *Mol. Genet. Genomics* 290(5): 1955-1962. <http://dx.doi.org/10.1007/s00438-015-1053-3>.
- [22] Ma, Y. H., Fu, S. L., Zhang, X. P., Zhao, K., Chen, H. Y. H. (2017): Intercropping improves soil nutrient availability, soil enzyme activity and tea quantity and quality. – *Applied Soil Ecology* 119: 171-178. <http://dx.doi.org/10.1016/j.apsoil.2017.06.028>.

- [23] Ouzounis, T., Frette, X., Rosenqvist, E., Ottosen, C., O. (2014): Spectral effects of supplementary lighting on the secondary metabolites in roses, chrysanthemums, and campanulas. – *J. Plant Physiol.* 171(16): 1491-1499. <http://dx.doi.org/10.1016/j.jplph.2014.06.012>.
- [24] Prasad, R. B., Brook, R. M. (2005): Effect of varying maize densities on intercropped maize and soybean in Nepal. – *Exp. Agric.* 41(3): 365-382. <http://dx.doi.org/10.1017/S0014479705002693>.
- [25] Raza, M. A., Feng, L. Y., van der Werf, W., Iqbal, N., Khalid, M. H. B., Chen, Y. K., Wasaya, A., Ahmed, S., Ud Din, A. M., Khan, A., Ahmed, S., Yang, F., Yang, W. (2019): Maize leaf-removal: a new agronomic approach to increase dry matter, flower number and seed-yield of soybean in maize soybean relay intercropping system. – *Sci Rep* 9(1): 13453. <http://dx.doi.org/10.1038/s41598-019-49858-8>.
- [26] Shimizu-Yumoto, H., Yamanaka, M. (2023): Relationship between ethylene and leaf-yellowing induced by darkness in cut small-flowered chrysanthemum. – *Sci. Hortic.* 309: 5. <http://dx.doi.org/10.1016/j.scienta.2022.111676>.
- [27] Shipley, B., Meziane, D. (2002): The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. – *Funct. Ecol.* 16(3): 326-331. <http://dx.doi.org/DOI 10.1046/j.1365-2435.2002.00626.x>.
- [28] Sun, T., Yang, Y., Duan, K., Liao, Y., Zhang, Z., Guan, Z., Chen, S., Fang, W., Chen, F., Zhao, S. (2023): Biodiversity of endophytic microbes in diverse tea chrysanthemum cultivars and their potential promoting effects on plant growth and quality. – *Biology (Basel)* 12(7): 17. <http://dx.doi.org/10.3390/biology12070986>.
- [29] Sun, W., Yang, X. C., Su, J. S., Guan, Z. Y., Jiang, J. F., Chen, F. D., Fang, W. M., Zhang, F. (2019): The genetics of planting density-dependent branching in chrysanthemum. – *Sci. Hortic.* 256: 7. DOI: 10.1016/j.scienta.2019.108598.
- [30] Takanori, M. (2022): How does light facilitate vitamin C biosynthesis in leaves? – *Biosci. Biotechnol. Biochem.* 24(9): 1173-1182. <http://dx.doi.org/10.1093/bbb/zbac096>.
- [31] Wang, F., Miao, M., Xia, H., Yang, L. G., Wang, S. K., Sun, G. J. (2017): Antioxidant activities of aqueous extracts from 12 Chinese edible flowers in vitro and in vivo. – *Food Nutr Res* 61(1): 1265324. <http://dx.doi.org/10.1080/16546628.2017.1265324>.
- [32] Wang, R. N., Sun, Z. X., Zhang, L. Z., Yang, N., Feng, L. S., Bai, W., Zhang, D. S., Wang, Q., Evers, J. B., Liu, Y., Ren, J. H., Zhang, Y., van der Werf, W. (2020): Border-row proportion determines strength of interspecific interactions and crop yields in maize/peanut strip intercropping. – *Field Crops Res.* 253: 10. DOI: 10.1016/j.fcr.2020.107819.
- [33] Wang, Z., Zhou, M., Liu, H., Huang, C., Ma, Y., Ge, H. X., Ge, X., Fu, S. (2022): Pecan agroforestry systems improve soil quality by stimulating enzyme activity. – *PeerJ* 10: e12663. <http://dx.doi.org/10.7717/peerj.12663>.
- [34] Wen, B., Zhang, X., Ren, S., Duan, Y., Zhang, Y., Zhu, X., Wang, Y., Ma, Y., Fang, W. (2019): Characteristics of soil nutrients, heavy metals and tea quality in different intercropping patterns. – *Agroforestry Systems* 94(3): 963-974. <http://dx.doi.org/10.1007/s10457-019-00463-8>.
- [35] Xu, H., Wang, F., Hu, Z., Du, Z., Cheng, L., Xu, X. (2023): Moso bamboo—*Polygonatum cyrtoneura* agroforestry systems: evaluation of soil quality and *Polygonatum* yield. – *Forests* 14(7): 1426.
- [36] Xu, M. Y., Wu, K. X., Liu, Y., Liu, J., Tang, Z. H. (2020): Effects of light intensity on the growth, photosynthetic characteristics, and secondary metabolites of *Eleutherococcus senticosus* Harms. – *Photosynthetica* 58(3): 881-889. <http://dx.doi.org/10.32615/ps.2020.045>.
- [37] Yang, D., Liu, S. S., Teng, F., Zhang, Y. X., Li, M., Yang, Y. C., Zhu, T., Yang, Z. Q., Chen, S., Kang, C., Yang, L. X., Zhu, J. J., Zhan, Z. L., Wang, Z. M. (2023): Morphology and metabolite profiles of southern and northern *Chrysanthemum* in China. – *Ind. Crop. Prod.* 194: 13. DOI: 10.1016/j.indcrop.2023.116250.

- [38] Zhang, L., van der Werf, W., Zhang, S., Li, B., Spiertz, J. H. J. (2007): Growth, yield and quality of wheat and cotton in relay strip intercropping systems. – *Field Crops Res.* 103(3): 178-188. <http://dx.doi.org/10.1016/j.fcr.2007.06.002>.
- [39] Zhang, S., Zhang, L., Zou, H., Qiu, L., Zheng, Y., Yang, D., Wang, Y. (2021): Effects of light on secondary metabolite biosynthesis in medicinal plants. – *Front Plant Sci* 12: 781236. <http://dx.doi.org/10.3389/fpls.2021.781236>.
- [40] Zheng, L., Van Labeke, M. C. (2017): Chrysanthemum morphology, photosynthetic efficiency and antioxidant capacity are differentially modified by light quality. – *J. Plant Physiol.* 213: 66-74. <http://dx.doi.org/10.1016/j.jplph.2017.03.005>.