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Performance of an Elderberry–Bell Pepper Agroforestry System in the Southeastern US and Insights for Farm Decision-Making

Santosh Paudel[1] | Suraj Upadhaya[1] | Sudha Bhandari[1] | Anuj Chiluwal[1] | Theoneste Nzaramyimana[1] | Srijana Thapa Magar[2]

[1]College of Agriculture, Health, and Natural Resources, Kentucky State University, Frankfort, Kentucky, USA | [2]College of Agricultural and Environmental Sciences, University of Georgia, Griffin, Georgia, USA

Correspondence: Santosh Paudel (santosh.paudel@kysu.edu)

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ABSTRACT

Rising global food demand has intensified agricultural practices that increase yields but often degrade soil, reduce biodiversity, and undermine long-term sustainability. Nature-based solutions such as agroforestry, which integrates perennial shrubs or trees with annual crops, offer pathways for sustainable intensification through income diversification, land-use optimization, and potential ecosystem services, including pollinator presence observed during elderberry flowering. American elderberry (*Sambucus canadensis*) is a multifunctional perennial shrub, yet field-based evidence on shrub vegetable integration and its impacts on crop performance and productivity remains limited. This study evaluated a perennial–annual agroforestry system integrating elderberry with bell pepper (*Capsicum annuum*) under field conditions. A 2-year experiment (2024–2025) compared three varieties of bell peppers: California Wonder, Orange Sun, and Quadrato d'Asti Rosso (QDAR), grown in elderberry-based alley cropping and conventional monocropping systems. We measured growth traits (plant height, leaf area index, stem diameter, number of branches, and number of leaves), physiological traits (chlorophyll and stomatal conductance) at multiple stages (30, 45, 60, and 75 days after transplanting, DAT). Bell pepper and elderberry fruit yields were recorded for both years. Elderberry-based alley-cropping systems improved several growth and physiological parameters and maintained annual crop (bell pepper) yields in the alleys compared with conventional cropping. The California Wonder and QDAR varieties outperform the Orange Sun in terms of growth and yield. The elderberry yield rose from 646 kg/ha (576 lbs/acre) in 2024 to 1625 kg/ha (1450 lbs/acre) in 2025, reflecting establishment and maturity. Mid-season growth and physiological traits measured at 60 DAT, particularly canopy traits such as the number of leaves and LAI, were informative indicators of yield and provided insights relevant to farm-level decision making. Overall, perennial–annual agroforestry demonstrated potential to improve sustainable intensification while generating practical insights into diversified and small to medium-scale farming systems.

1 | Introduction

The increased frequency and intensity of extreme weather events disrupt food production and increase the risk of food insecurity. Global food demand is projected to rise substantially, with world crop calorie production expected to increase by about 47% from 2011 to 2050, driven mainly by yield intensification and moderate cropland expansion (Sands et al. 2023). In

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addition, the global population is projected to reach 9.7 billion by 2050, and it is challenging to feed the expanding population from limited land (United Nations 2019). These pressures intensify the unsustainable agricultural practices, such as excessive use of fertilizers, pesticides, herbicides, excessive tillage, and extensive use of resources. These practices increase yield but degrade the environment due to soil erosion, pollution, climatic stress, biodiversity loss, and more (Akanmu et al. 2023). Farmers are increasingly responding to these pressures through both adaptive and maladaptive actions, underscoring the need to shift toward regenerative, resilient production systems (Upadhaya and Arbuckle 2021).

Nature-based solutions (NbS) are sustainable approaches that work with natural processes to mitigate pressing challenges, such as climate change, food insecurity, biodiversity loss, and land degradation (IUCN 2020; UNEP 2024). By enhancing ecosystem functions, NbS can deliver multiple co-benefits, including improved resilience, environmental sustainability, and human well-being (IUCN 2020). Agroforestry, as a prominent NbS, offers a pathway that incorporates trees and shrubs with crops and/or livestock within the same land management unit (Lundgren et al. 1983). Among agroforestry practices, alley cropping combines trees or shrubs in rows with annual crops grown in inter-row spaces (Garrett et al. 2021). It promotes land optimization through a multifunctional land-use approach. Alley cropping maximizes the productive use of space by combining trees or shrubs with diverse crops to obtain multiple outputs, such as food, fodder, timber, or fruits. This system enables the simultaneous production of annual crops and long-term tree products or shrub berries and fruits, while maintaining the ecological functions of both components.

A growing body of research shows that incorporating trees or shrubs into agricultural landscapes can provide multiple environmental, economic, and social benefits (Bhandari et al. 2025; Castle et al. 2022). These systems provide a diversified income by offering short-term cash flow from annual crops while simultaneously developing medium to long-term products from woody or shrubby components (Jahan et al. 2022; Suwardi et al. 2023). The environmental benefits include trees and shrubs providing shade and creating microclimatic conditions favorable for crops, controlling soil erosion by holding soil tightly, enhancing biodiversity by providing diverse floral resources and habitat for the pollinators and other species (Varah et al. 2020), maintaining soil fertility, improving water quality through reduction of leaching of agrochemicals, and helping in carbon sequestration (Arimi and Omoare 2021). The economic benefits include diverse income sources from trees, shrubs, crops, and all components, as well as reduced risk of income loss (Roshetko et al. 2013). The social benefits include improved food security as diversified crops provide multiple nutrients, a steady food supply, and enhanced community health through access to medicinal plants from agroforestry (Pandit et al. 2019).

With this context, American elderberry (*Sambucus canadensis*) has emerged as a promising perennial shrub for diversified farming systems in the United States. The global elderberry market is experiencing significant growth, with projections indicating an increase of $349.5 million worldwide between 2024 and 2029, representing a compound annual growth rate (CAGR) of 7.6% (Technavio 2025). Furthermore, the elderberry supplements market alone is expected to rise from $1.45 billion in 2025 to $3.42 billion by 2035, with an 8.9% CAGR (FMI 2025). In the United States, elderberry sales as a food ingredient or supplement reached $113 million in 2019, mainly due to its use in immune-boosting products (EastFruit 2024). In addition, the commercial elderberry cultivation in the United States has expanded from 790 acres in 2017 (USDA NASS 2017) to 2627 acres in 2022, with Missouri leading the production (USDA NASS 2022). At the farm scale, established hedgerows of 1000 ft can yield a net annual revenue of $2000–$3000 within 3–5 years, highlighting the crop's economic viability in diversified cropping systems (Brodt et al. 2024). Beyond its market value, elderberry is adaptable to a range of soil and site conditions, can be grown on marginal lands, and begins producing fruit relatively early compared with many tree crops (Bulatovic-Danilovich 2022; Ernst and Painter 2023). These characteristics make elderberry an attractive perennial component for farmers seeking low-risk entry points into agroforestry. Integrating high-value vegetable crops, such as bell peppers, consistently ranks among the most popular vegetables consumed in the United States (USDA NIFA 2022).

Despite this potential, field-based evidence on elderberry-based agroforestry systems remains limited, particularly with respect to integration with high-value annual vegetable crops. Existing research and practice focus on simple alley management, such as sowing cover crops (e.g., grasses or clover) between elderberry rows (Errickson et al. 2025) and typically do not include interplanting high-value annual vegetables like bell peppers in the alleys. Similarly, most elderberry agronomic studies have focused on cultivar trials, berry yields, and soil fertility effects (Thomas et al. 2024). As a result, there is limited empirical understanding of how vegetables perform when grown within elderberry alleys or how these interactions influence overall system productivity. This gap constrains farmers' ability to evaluate elderberry-based agroforestry as a practical option for diversified production.

In addition to understanding system productivity, growers require timely information to adjust fertilization, irrigation, labor planning, harvest scheduling, and marketing decisions, ultimately reducing production risks and improving profitability (Kuradusenge et al. 2023; Shafi et al. 2023). Yield prediction research increasingly uses multi-stage growth data to capture crop responses to management and environmental conditions (Paudel et al. 2021; Silva et al. 2023; Zhou et al. 2025). Methods such as LASSO regression have been used for their ability to perform variable selection and identify influential predictors (Khaki et al. 2020; Shahhosseini et al. 2021). The ensemble approaches, like Random Forest (RF), often achieve higher predictive accuracy by capturing nonlinear relationships and interactions across multiple growth stages (Silva et al. 2023; Zhou et al. 2025). Recent work by Bregaglio et al. (2023) showed that embedding phenological heterogeneity via calibrated leaf area index (LAI) dynamics into crop model parameter sets significantly improves regional yield prediction accuracy. Though many yield prediction studies rely on large-scale datasets, limited work has examined the stage-specific prediction in diversified systems, which is critical for management decisions.

Therefore, the objectives of this study were to evaluate a perennial–annual agroforestry system integrating American

elderberry with bell pepper under field conditions. Specifically, this study aimed to (i) quantify the effects of elderberry-based alley cropping on growth, physiological traits, and yield of bell pepper relative to conventional monocropping, (ii) compare varietal performance across cropping systems, and (iii) identify growth stages and traits that provide insights relevant to yield-related farm decision-making. By addressing these objectives, this work provides empirical evidence to inform the design and management of diversified agroforestry systems in temperate production regions such as Kentucky.

2 | Materials and Methods

2.1 | Study Site and Experimental Timeline

We conducted a field experiment at Frankfort, Kentucky, USA (38.2° N, 84.9° W; 750 ft a.s.l.) (Figure 1). The detailed layout of an individual experimental block and the location of sampled plants within the block are shown in Supporting Information S1. Elderberry cuttings were planted as row crops in 2023. We transplanted bell pepper seedlings into alley-cropping and control plots in early June 2024 and late May 2025 and conducted experiments from June to October in 2024 and from May to September in 2025. The site's accessibility, existing infrastructure, proximity to the water source, and its location in an established pasture, which replicates real-world scenarios, make this location favorable for this trial.

2.2 | Soil Properties

Before the start of the experiment, the field was left barren except for a few trees. In 2023, we took eight soil core samples from the middle of the block for a baseline soil sample analysis. We collected soil samples at 0–15 cm depth and measured 15 cm away from the elderberry stem for near-elderberry plots and from the open space for no-elderberry plots, before planting alley crops. The study field has silty loam soil. The soil pH was 6.32, indicating slightly acidic conditions favorable for nutrient availability, while the soil water pH was 7.09, reflecting near-neutral conditions in the soil solution. Soil organic matter averaged 2.4%, and total nitrogen averaged 0.19%, representing a moderate fertility status. Available phosphorus was extremely high (540 mg kg^{-1}), exceeding the sufficiency range of 15–40 mg kg^{-1} typically considered adequate for crop production, while available potassium was also elevated (379 mg/kg), well above the critical threshold of 100–200 mg/kg. These indicate that baseline fertility did not limit crop growth and that observed treatment effects reflected experimental factors (e.g., elderberry presence/absence) rather than baseline soil fertility differences.

2.3 | Climatic Conditions

The study area has a humid subtropical climate with hot and humid summers and cold winters. We obtained the meteorological data from the Kentucky mesonet for Franklin County for

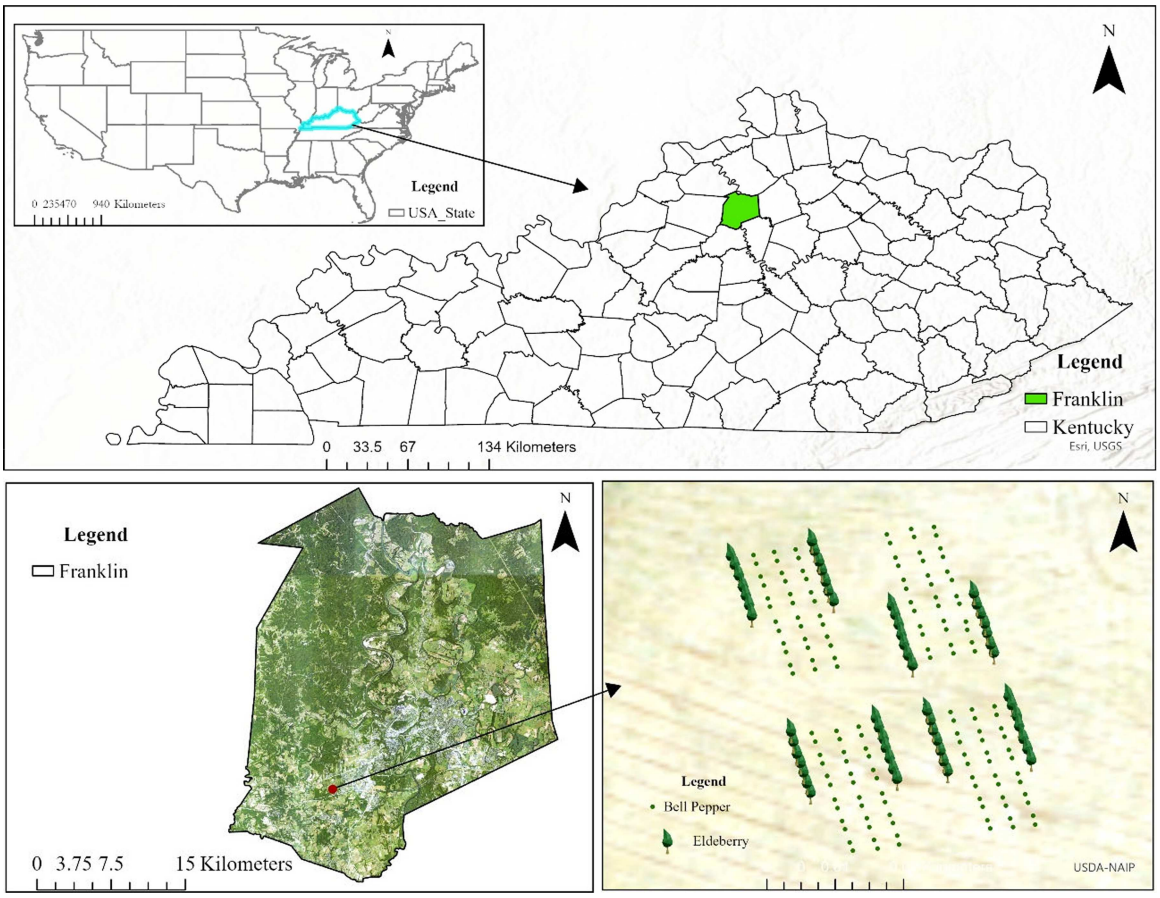


FIGURE 1 | Map of the study area showing alley cropping research at West Farm, Frankfort.

2024 and 2025 (https://www.kymesonet.org). Mean temperatures peaked during July–August, with maximum temperatures exceeding 30°C. Rainfall patterns differed between years, with more evenly distributed precipitation in 2024 and heavier early-season rainfall followed by drier mid-season conditions in 2025 (Figure 2).

2.4 | Experimental Design and Treatments

The experiment was a split-plot randomized complete block design. The main plot factor includes the presence/absence of elderberry, and the sub-plot factor includes three varieties of bell peppers. There were four blocks, each measuring 30 × 65 ft, with six plots per block and five plants per plot. The first factor is elderberry integration, which has two levels. The presence of elderberry plants indicates alley cropping, and the absence of elderberry plants indicates conventional cropping. The elderberry shrubs were 2 years old in 2024 and planted generally in a northwest–southeast orientation at a spacing of 4 × 6 ft. A total of seven elderberry plants were planted in each row of the block, creating space between them.

The second factor consists of three varieties of bell peppers, that is, California Wonder, Orange Sun, and Quadrato d'Asti Rosso. Within each block and elderberry treatment, we randomized bell pepper varieties at the plot level rather than assigning them to fixed row positions relative to elderberry shrubs. This approach ensured that all three varieties occurred across rows with differing proximity to elderberry plants within and among blocks, such that row position was not confounded with variety. Shading gradients due to elderberry rows were therefore treated as an inherent component of the alley-cropping system rather than as a separate experimental factor.

We transplanted bell peppers on June 24, 2024, at 2 ft spacing between plants in plots in the first year, and on June 2, 2025, in the second year. The three varieties of bell peppers were randomized within each block. We collected data four times: 30, 45, 60, and 75 days after transplanting (DAT) to assess early (30 DAT), mid (45 and 60 DAT), and late (75 DAT) growth stages' performance of bell-pepper varieties across the elderberry presence and absence sections. Growth and physiological parameters measured included plant height, stem diameter, number of leaves, number of branches, LAI, stomatal conductance, and chlorophyll content. The plant height was measured from the base to the tip of the upper leaves with the help of a wooden ruler. The stem diameter was measured by using a digital caliper at 2 cm above the soil surface. The LAI was measured by using the LAI-2200C plant canopy analyzer (LI-COR Inc., Lincoln, NE, USA). The stomatal conductance was measured using a LI 600 Porometer/Fluorometer (LI-COR Inc., Lincoln, NE, USA) from a fully developed leaf on the upper canopy during midday. Similarly, the chlorophyll content was obtained through a SPAD-502 chlorophyll meter (Minolta Digital, Osaka, Japan). The yield was measured based on the total fruit weight from individual sample plants and from the total plot.

2.5 | Crop Management During the Season

We maintained uniform management practices across treatments. We irrigated the bell pepper plant row and the elderberry shrubs row regularly through a drip irrigation system. This provides a consistent soil moisture to the plants. We did not apply any external fertilizers or soil amendments during the study period, allowing the experiment to rely on the existing soil nutrient status. We did not perform any pruning or pinching in

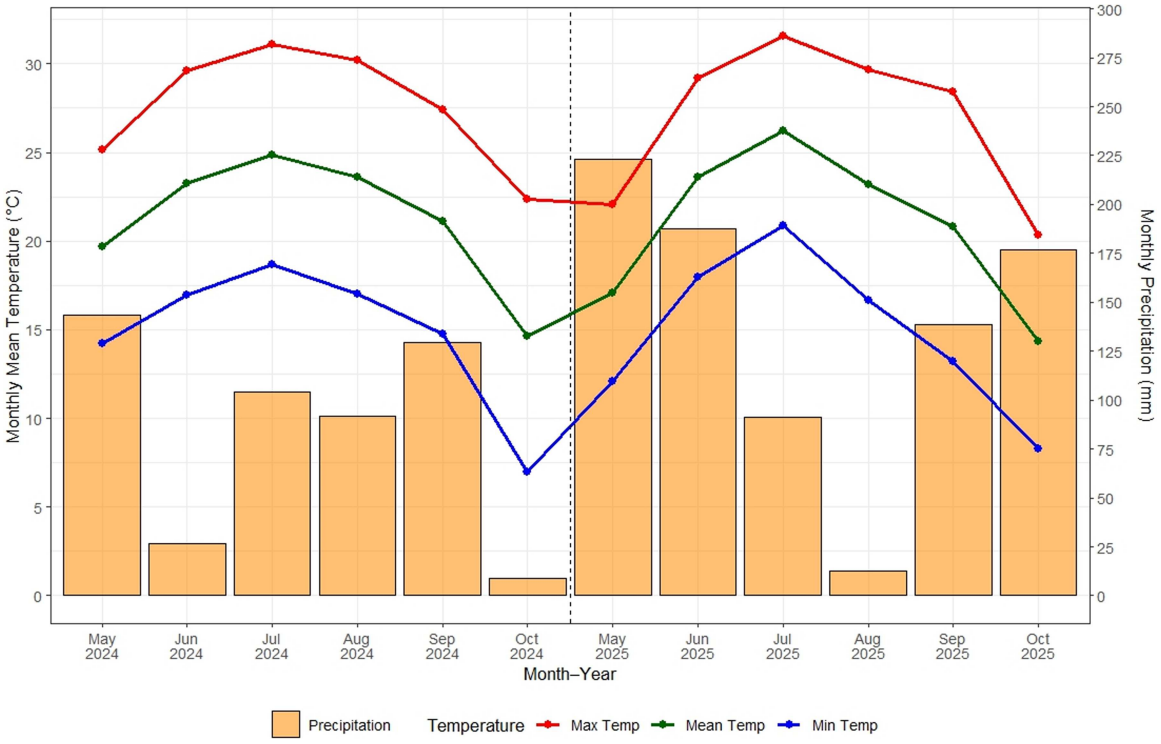


FIGURE 2 | Temperature and precipitation prevailed during the study period (May–October 2024 and 2025).

the case of bell peppers. We conducted the routine field maintenance, such as manual weeding and power weeding, as necessary to reduce competition from unwanted vegetation. During the dormant season (January–February), we pruned elderberry shrubs following recommended practices to promote uniform regrowth (Byers et al. 2022).

2.6 | Crop Performance and Crop/Tree Productivity

We harvested bell peppers multiple times per season once the fruits reached the desired marketable size, firmness, and color development. The peppers reached harvestable maturity roughly 80–90 DAT. We quantified elderberry yield as berry weight per plant to determine the crop productivity. Elderberry harvesting was performed in several rounds per plant due to uneven flowering and fruiting patterns, which resulted in berries ripening at different times.

2.7 | Statistical Analysis

We analyzed the growth and yield parameters to assess the influence of the elderberry-based alley cropping system. A linear-mixed effects model was fitted with the measured variables as the response variable and elderberry presence/absence, variety, and their interaction as fixed effects, while block was included as a random effect to account for the experimental design. Row position was not treated as an independent factor because we randomized bell pepper varieties across rows; therefore, we accounted for spatial variation by including block as a random effect. Type III analysis of variance with Satterthwaite's approximation for degrees of freedom was performed using the lmerTest package. The model assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk test, Q–Q plots, and Levene's test. Tukey's honestly significant difference (HSD) post hoc test was performed through pairwise multiple comparisons using the "emmeans" function from the emmeans package. Compact letter displays (CLD) were generated to represent group differences at $\alpha = 0.05$. Descriptive statistics (mean, standard deviation, and standard error of the mean) were also calculated for each treatment combination. In cases where data did not meet normality assumptions, the Wilcoxon rank-sum test was used as a non-parametric alternative.

Yield prediction models were developed using growth and physiological traits of bell pepper plants measured during the 2024 and 2025 growing seasons ($n = 288$ plants). Traits included plant height, LAI, chlorophyll content (SPAD), stomatal conductance, stem diameter, number of branches, and number of leaves. Elderberry treatment (EB; alley cropping presence/absence) was included as a binary predictor. Yield per plant (lbs) for each year was used as the target variable.

We built up separate models for each growth stage − 30, 45, 60, and 75 DAT using eight predictors. We evaluated five algorithms: Linear regression, LASSO regression, RF, Gradient Boosting Machine (GBM), and Support Vector Regression (SVM). Predictors were standardized for linear, LASSO, and SVM models, while tree-based models used raw values. Standardization (scaling to zero mean and unit variance) ensures that all predictors contribute comparably to algorithms sensitive to variable scale, whereas tree-based models are scale-invariant and do not require this step.

We assessed model performance using Monte Carlo cross-validation, which was selected to provide stable estimates of model accuracy by averaging over many random training-testing splits. In each iteration, the dataset was randomly divided into 70% training ($\approx$201 samples) and 30% testing ($\approx$87 samples). Models were trained on the training set and evaluated on the unseen testing set. This procedure was repeated 200 times, and model accuracy was summarized as the mean and standard deviation of the coefficient of determination (R^2) and root mean squared error (RMSE) across repeats.

3 | Results

3.1 | Morphological or Physical Growth Parameters of Bell Peppers in Elderberry Presence and Absence Sections

The presence of elderberry consistently influenced the physical growth responses across both study years. The average plant height across the 2 years was significantly higher in elderberry presence plots across all days after transplanting, except for 30 DAT in 2025. Plants in the elderberry presence sections showed steady growth over time, with height advantages ranging from 4 to 6 cm by 75 DAT. Stem diameter was similarly greater in the presence conditions at all DAT in 2024, and particularly at later growth stages in 2025 (Figure 3). This reflects stronger structural development. The LAI also followed a similar trend with higher values under elderberry presence throughout the observation period in both years (Figure 3). This indicates enhanced canopy development. The number of leaves was found significantly higher in elderberry presence plots at 45, 60, and 75 DAT in 2024 and 45 and 60 DAT in 2025. The number of branches was significantly higher at 30, 45, and 75 DAT in 2024 and at 60 DAT in 2025, as in Supporting Information S2.

Plant height increased through the season for all three varieties. The influence of elderberry presence depended on both the variety and the growth stage. In California Wonder, plants growing near elderberry became slightly taller at 45 and 75 DAT, with a few meaningful differences, while early-season values remained similar between treatments. Orange Sun showed the strongest response. Height was consistently higher at 45, 60, and 75 DAT when the peppers were grown in the elderberry plots. Quadrato d'Asti Rosso also showed clear increases at 60 and 75 DAT, although the 30 DAT values were mostly similar across treatments in 2024. Overall, elderberry presence had a greater effect during the mid and late parts of the season when plant growth was most active (Figure 4).

Stem diameter showed a similar pattern. In California Wonder, early-season values were close for both treatments, but differences were clearer at 60 and 75 DAT. The elderberry presence has a negative effect on stem diameter at 60 DAT in the year 2025. Plants in the elderberry plots developed thicker stems at 45, 60, and 75 DAT, and several of these differences were statistically significant. Quadrato d'Asti Rosso showed modest early-season differences, but stem diameter increased

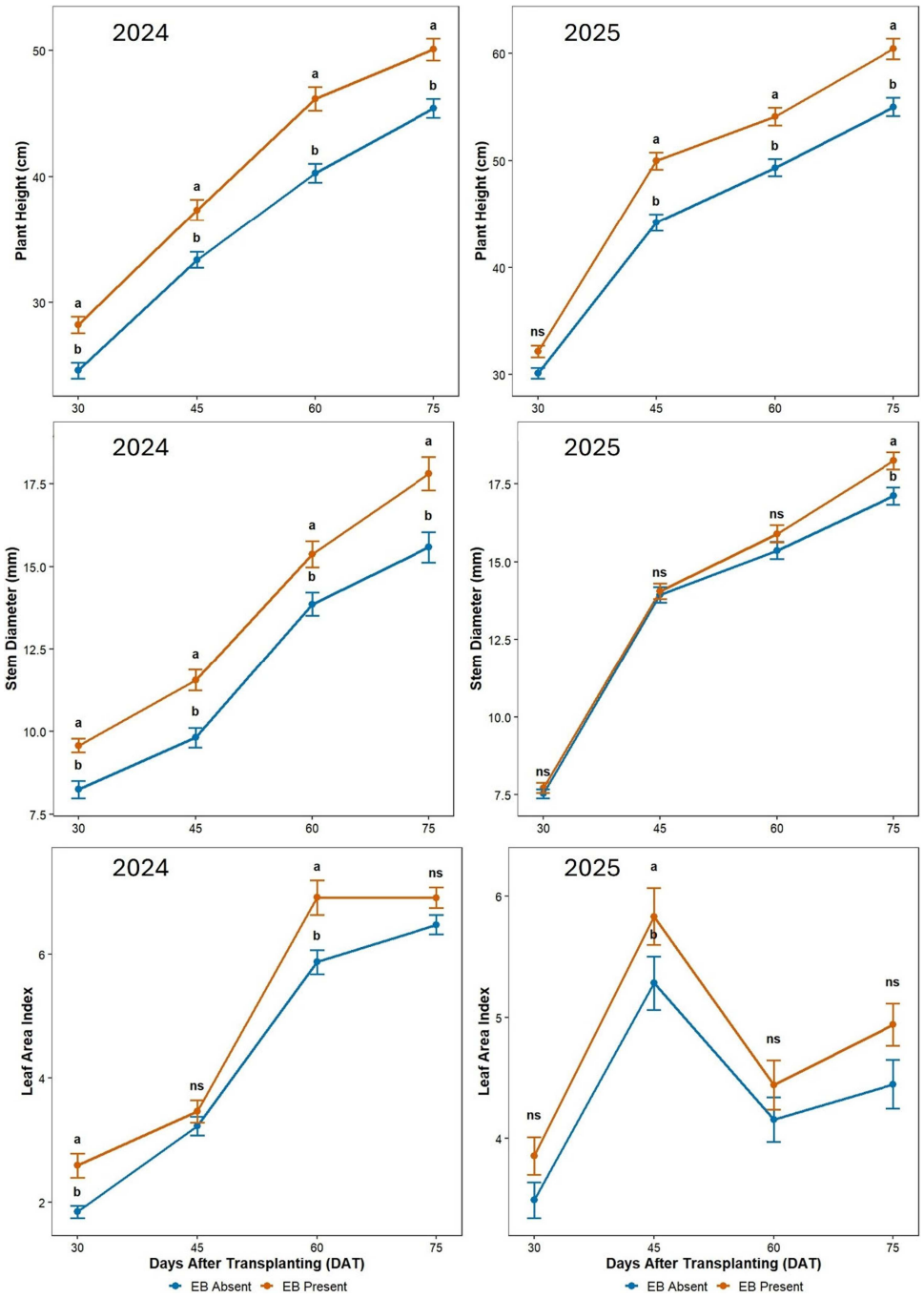


FIGURE 3 | Developmental trends of plant height, stem diameter, and leaf area index in elderberry presence and absence treatments in 2024 and 2025.

noticeably under elderberry presence by 60 and 75 DAT. These patterns suggest that stem development benefits from elderberry presence mainly during periods of rapid vegetative growth (Figure 4).

LAI also shows clear differences between treatments. In California Wonder, LAI was higher at 30 and 45 DAT in the elderberry plots. Orange Sun shows a higher LAI in elderberry presence in 2024, while there is relatively lower LAI in elderberry presence in 2025. Quadrato d'Asti Rosso showed the same trend, with its largest improvement at 60 DAT in 204, and at all DAT in 2025. Across all varieties, the 60 DAT stage consistently appeared to be the point when peppers benefited the most from the modified microclimate created by elderberry, resulting in greater leaf development during peak growth (Figure 4).

3.2 | Physiological Parameters of Bell Peppers in Elderberry Presence and Control Sections

The chlorophyll content (SPAD) showed a mixed response to elderberry presence depending on the sampling date and year. In 2024, SPAD values were slightly higher under elderberry at 30 and 75 DAT, with significant differences at both timepoints, while 45 and 60 DAT showed no statistical differences. In 2025,

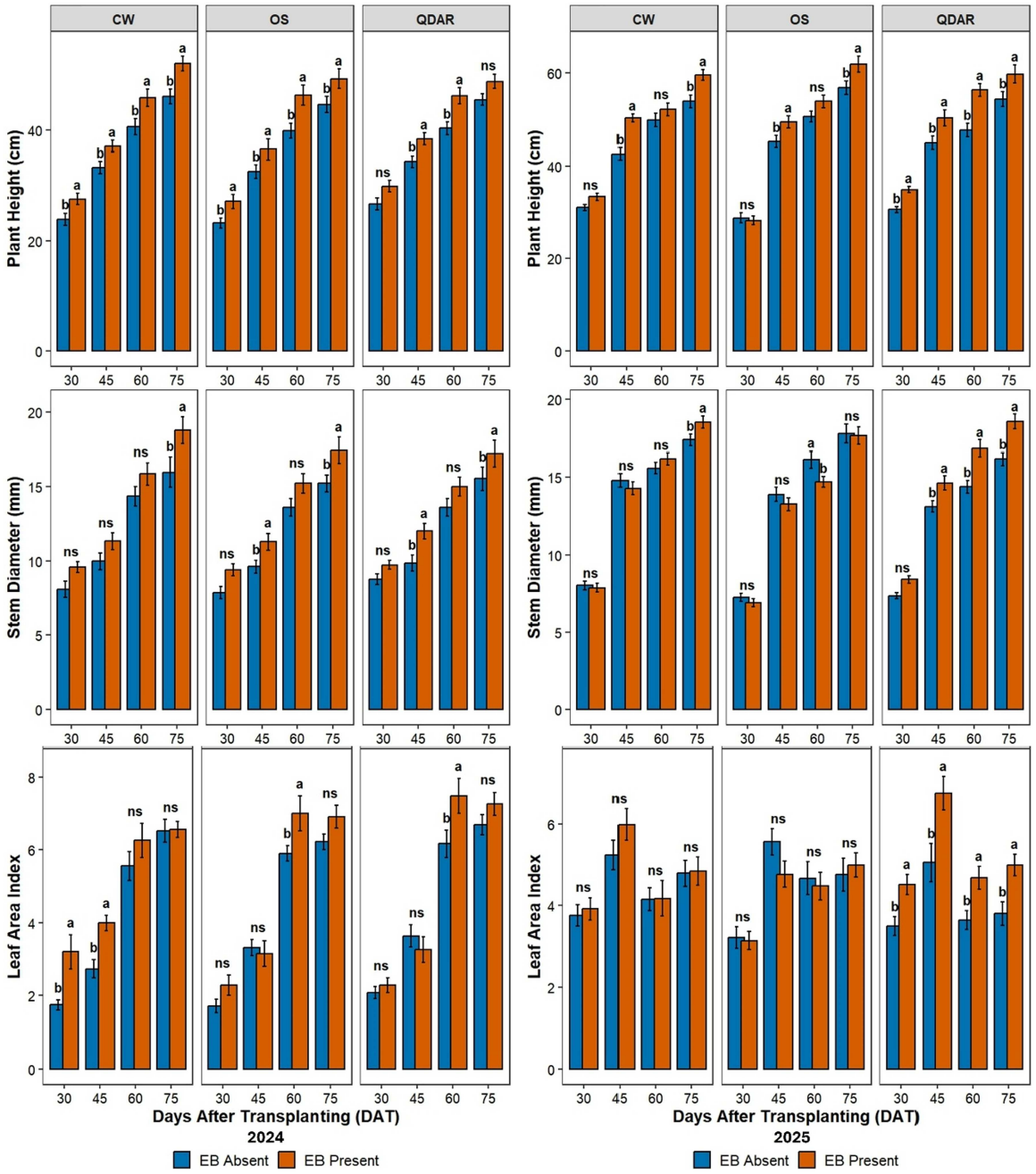


FIGURE 4 | Plant height, stem diameter, and leaf area index of three bell pepper varieties: California Wonder (CW), Orange Sun (OS), and Quadrato d'Asti Rosso (QDAR) under elderberry presence and absence in 2024 and 2025. Error bars indicate standard errors.

chlorophyll remained generally similar between treatments across all sampling dates, with no significant differences detected (Figure 5; Supporting Information S3). Overall, SPAD levels tended to be marginally higher in elderberry-present plots, but these differences were inconsistent across dates and years.

Stomatal conductance also varied by year and sampling date. In 2024, no significant differences were observed between elderberry presence and absence at any DAT, although median values at 60 DAT were slightly higher under elderberry. In 2025, stomatal conductance was significantly higher under elderberry at 30 DAT, while 45, 60, and 75 DAT showed no differences between treatments. These results indicate that elderberry presence influenced stomatal activity only at the earliest growth stage in 2025. Significant differences occurred only at specific dates, such as higher chlorophyll at 30 and 75 DAT in 2024 and higher stomatal conductance at 30 DAT in 2025 (Figure 5).

3.3 | Yield Parameters of Bell Peppers in Elderberry Presence and Control Sections

The yield of bell peppers ranged between 22,000 and 40,000 kg/ha. The crop yield was significantly higher in plots with elderberry presence than in plots without elderberry in

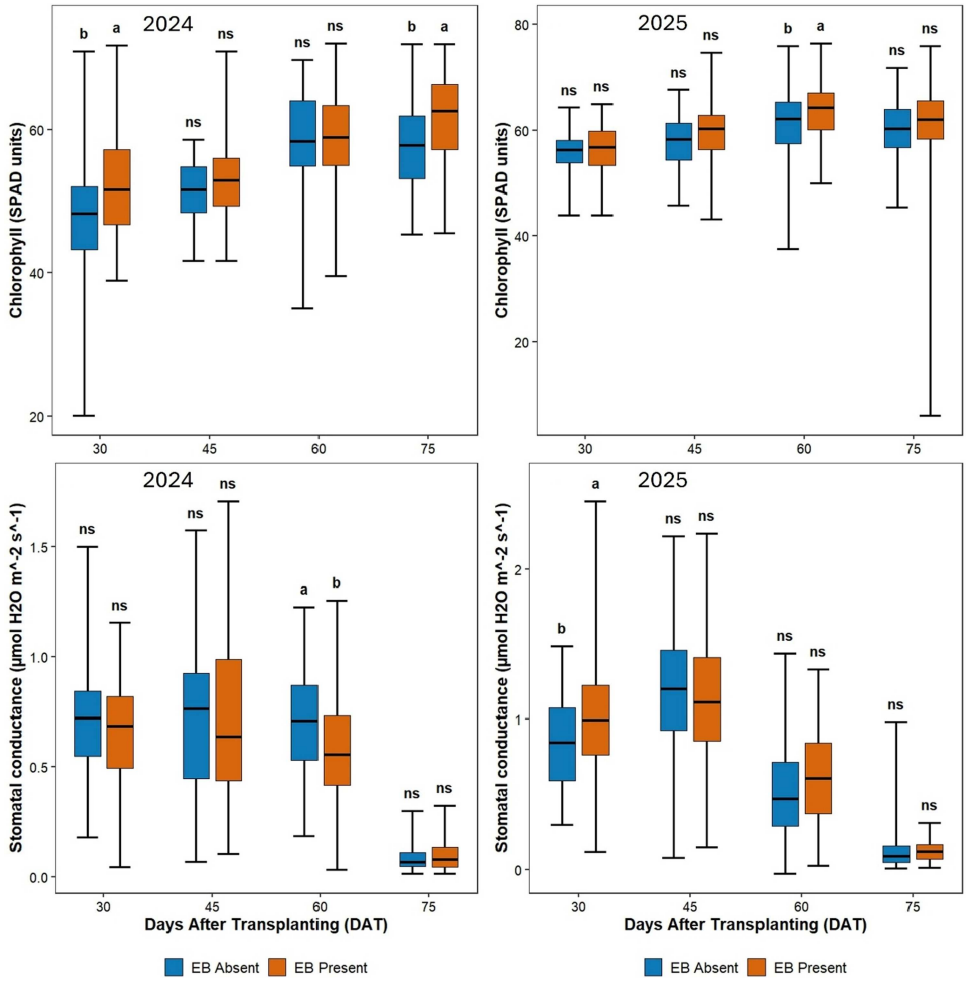


FIGURE 5 | Variation in chlorophyll values and stomatal conductance of bell peppers under elderberry presence and absence treatments across different sampling times in 2024 and 2025.

TABLE 1 | Yield performance of bell pepper varieties as affected by elderberry presence and absence during 2024–2025.

Treatments		Total yields (kg/ha)		
Elderberry (A)	**Variety (B)**	**2024**	**2025**	**Combined**
Presence	CW	40,458^{a}	29,508^{abc}	34,983^{a}
Presence	OS	28,900^{b}	20,631^{c}	24,765^{bc}
Presence	QDAR	38,479^{a}	31,829^{ab}	35,154^{a}
Absence	CW	31,753^{ab}	30,003^{abc}	30,878^{ab}
Absence	OS	24,561^{b}	23,042^{bc}	23,801^{c}
Absence	QDAR	28,108^{b}	34,573^{a}	31,340^{ab}
Mean		32,043	28,264	30,154
LSD (0.05)		7816	11,152	6370
Significance	A	*	ns	ns
	B	***	**	**
	A × B	ns	ns	ns

Note: Means with the same letter are not significantly different; LSD (0.05) = least significant difference; ns = non-significant.
*= significant at 5% probability level.
**= significant at 0.1% probability level.

2024, and the yields were more comparable between treatments in 2025 (Table 1; Supporting Information S4). When the yield data from both years were combined, the yield was higher in the treatment with elderberry present than in the absence treatment (Figure 6). In terms of varietal performance in the elderberry presence and absence sections, the California Wonder and QDAR varieties performed better than the Orange Sun variety in both years.

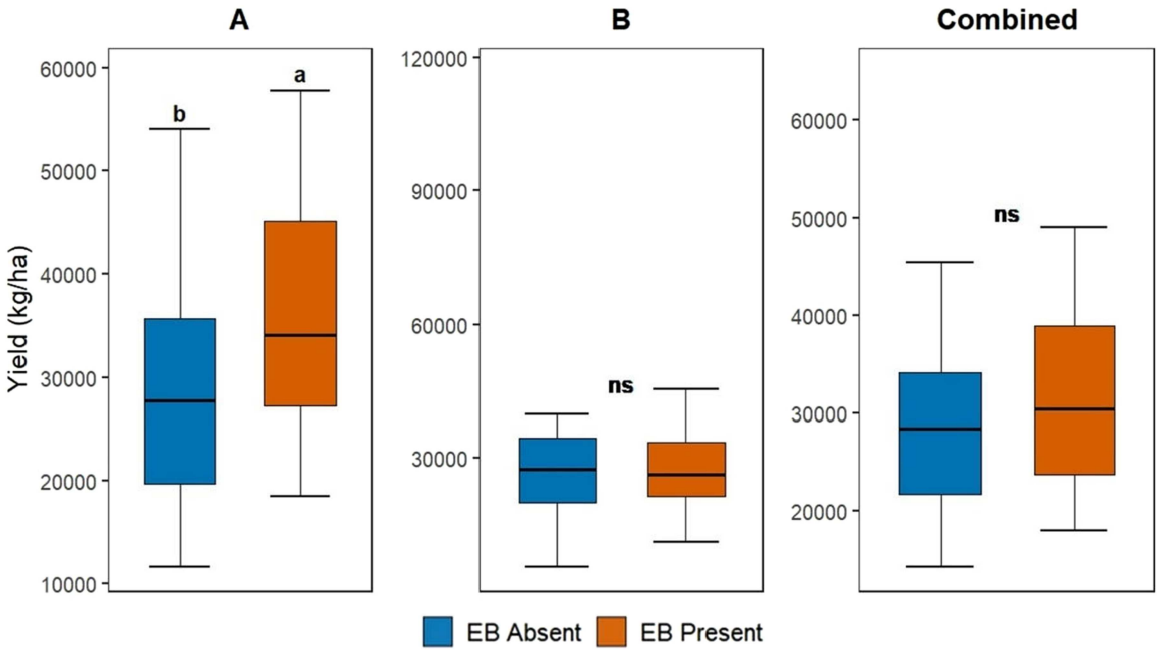


FIGURE 6 | Variation in yield of bell peppers under elderberry presence and absence treatments across 2024 (A), 2025 (B), and the combined 2 years.

3.4 | Yield Prediction Modeling From Growth and Physiological Traits

Machine learning models were used to evaluate the extent to which growth and physiological traits could predict bell pepper yield under elderberry alley cropping. Growth and physiological traits: plant height, LAI, stem diameter, number of leaves, number of branches, chlorophyll, and stomatal conductance were measured at four stages (30, 45, 60, and 75 DAT) across 2 years. The elderberry presence or absence was included as a treatment factor. The model performance was assessed by R^2 and RMSE. The highest predictive accuracy was obtained at 60 DAT, where RF achieved a mean R^2 of 0.29 ± 0.06 with a mean RMSE of 0.50 lbs plant^{-1} across 200 Monte Carlo repeats (Supporting Information S5). Although the explained variance was modest, this indicates that mid-season growth and physiological traits capture a measurable but partial component of yield variability. A large portion of yield variation is likely due to factors such as weather variability, soil parameters, and management practices. Linear regression and LASSO performed comparably at this stage ($R^2 \approx 0.27$), indicating that relationships between mid-season traits and yield were largely linear.

Early prediction at 30 DAT was moderately successful ($R^2 \approx 0.21$–0.24), suggesting that initial canopy development traits provide some signal of final yield. At 75 DAT, models explained ~24%–25% of yield variation, while 45 DAT consistently showed poor performance (R^2 near or below zero), indicating weak trait-yield associations at that stage.

Across models, Linear regression, LASSO, and RF consistently outperformed GBM and SVM. GBM captured at most ~13% of yield variation at 60 DAT, while SVM often produced negative R^2 values, reflecting overfitting and poor generalization.

At 60 DAT, variable importance patterns were consistent across different models (Figure 7). The leaf number (LN) and LAI were the strongest yield predictors, followed by stem diameter (SD). Physiological traits (SPAD and GSW) and plant height contributed modestly, while elderberry presence/absence showed minimal importance, indicating that mid-season canopy traits primarily led yield prediction.

4 | Discussion

The consistent improvement in growth and physiological traits of bell peppers, such as plant height, LAI, stem diameter, number of branches, number of leaves, chlorophyll content, and stomatal conductance, in elderberry-based cropping suggests that the elderberry canopy helps moderate the microclimate, reducing heat stress, lowering evaporative demand, and moderating wind exposure. Similar facilitative effects have been reported in temperate agroforestry, where partial shade improves water-use efficiency and reduces physiological stresses in annual crops (Rathore et al. 2022). In line with these results, other studies have found that moderate shading of around 25%–50% in pepper cultivation significantly increased leaf chlorophyll content, stomatal conductance, and fruit yield by alleviating heat stress (Ahmed et al. 2023). Although shading intensity likely varied with proximity to elderberry rows, randomization ensured that these gradients did not systematically bias varietal comparisons. This allowed us to interpret the varietal differences within the context of a functioning alley-cropping system.

The tendency toward increased plant height under elderberry presence, with smaller differences in stem diameter, suggests a shade-avoidance response, where plants prioritize vertical growth under reduced light availability at the expense of stem thickening. Kabir et al. (2019) reported higher plant height of bell pepper and reduced stem diameter with the increment in shade level up to 30%. In the present study, year-to-year variability in weather conditions likely interacted with shading

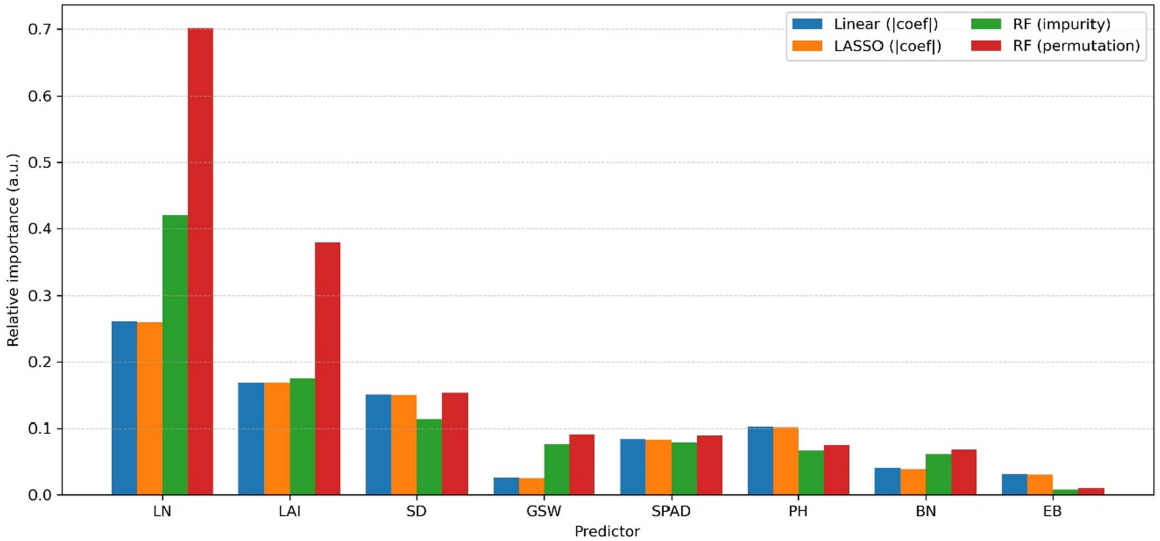


FIGURE 7 | Relative importance of eight predictors for bell pepper yield prediction at 60 DAT across models. (LN: Number of leaves, LAI: Leaf area index, SD: Stem diameter, GSW: stomatal conductance, SPAD: Chlorophyll, PH: Plant height, BN: Number of branches, EB: Elderberry presence or absence). Standardized coefficients are shown for Linear and LASSO regression, while Random Forest importance is presented as impurity and permutation-based scores. Leaf number (LN) and leaf area index (LAI) consistently emerged as the strongest predictors of yield.

effects, contributing to inconsistent LAI responses across different DATs in the orange sun variety in 2025. Further, differences in performance among measured parameters likely reflect resource allocation trade-offs rather than uniform competition effects. In the alley-cropping system, bell pepper plants experienced moderate aboveground competition for light, which altered how assimilates were partitioned among traits. Under partial shade, plants tend to prioritize traits that enhance light capture (e.g., plant height) over those related to structural reinforcement or lateral canopy expansion (e.g., stem diameter and LAI), leading to uneven responses across parameters.

The yield from the alley crop was hypothesized to decrease with the addition of elderberry in the border due to the above and belowground competition. However, we found significantly higher bell pepper yield in 2024 in the elderberry presence section and comparable yield in 2025. The higher pepper yield observed in 2024 under elderberry presence likely reflected more favorable climatic conditions during the peak fruiting period. As shown in Figure 2, 2024 received high rainfall in July and August, coinciding with flowering and fruit development, along with relatively stable mean temperatures around 20°C–25°C. In contrast, 2025 had most precipitation concentrated early in the season (May–June), followed by drier conditions and moderate temperatures during the main reproductive phase, reducing the moisture advantage within the alley plots. Additionally, elderberry plants exhibited greater canopy growth and shading in their second year, which may have increased resource competition for light, water, and nutrients.

The previous studies have demonstrated that bell peppers exhibit remarkable adaptability to partial shade conditions, with studies showing that 30% shade can double marketable yields compared to full sun conditions, while reducing crop losses from 50% to only 10% (Cooper 2012; Ernest 2022; Kabir et al. 2019). The microclimate created by the elderberry plants can provide beneficial growing conditions, including reduced heat stress, improved water retention, and protection from extreme weather events (Ernest 2022; Kabir et al. 2019). Conversely, bell pepper yields often decrease under the canopy of trees or hedgerows due to shade and competition. For example, in a study with a fodder tree (*Grewia optiva*) in northern India, pepper yield was about 33% lower under the tree canopy compared to open-field (no-tree) conditions (Sharma and Thakur 2020). Similarly, peppers grown under closely spaced *Melia composita* trees produced roughly half the number of fruits per plant compared to peppers in sole-crop (open sun) plots (Pant et al. 2020). These examples indicate significant yield penalties when peppers are intercropped with trees, especially at closer tree spacing or dense canopy cover. So, proper canopy management of elderberry is necessary to obtain the optimum benefits from the shading effects.

In contrast to conventional cropping, the presence of elderberry contributes to important ecosystem services within the farm. As a perennial shrub, it improves the soil structure, builds organic matter through litter fall, and contributes deeper root channels that improve porosity and infiltration. Its flowers attract a diverse community of pollinator bees, such as honeybees and bumble bees, which were evident on elderberry flowers during the field visit and monitoring. Frillman (2021) stated that elderberry flowers bloom in late spring with conspicuous umbel-shaped white clusters and are highly attractive to a diverse range of pollinators, including honeybees, bumblebees, ground-nesting bees, and so forth. These ecological values can spill over into annual alley crops. Increased pollinator activity may indirectly support higher fruit set in bell peppers, although this relationship was not measured directly in our study and warrants further investigation. The structural diversity due to elderberry hedgerows or border crops can harbor natural enemies of pests. These regulating and supporting services align with broader evidence that agroforestry systems enhance ecological resilience, nutrient cycling, and microclimate regulation (Jose 2009; Maitra et al. 2025; Paudel et al. 2025). Moreover,

such ecological co-benefits are increasingly recognized in U.S. conservation programs, including the Natural Resources Conservation Program (NRCS), Environmental Quality Incentives Program (EQIP), and Conservation Stewardship Program (CSP), which incentivize producers to adopt diversified perennial systems that improve biodiversity, soil health, pollination, climate regulation, and so forth (Lenhardt and Egoh 2023). Collectively, these ecosystem functions support sustainable intensification by maintaining or improving yield while reducing dependence on external inputs.

From an economic perspective, elderberry integration provides opportunities for income diversification and risk mitigation. In our study, we obtained 646 kg/ha (576 lbs/acre) of elderberry fruit in 2024 and 1625 kg/ha (1450 lbs/acre) in 2025. The yields are expected to increase as shrubs mature and are managed through regular dormant season pruning (Byers et al. 2022). The establishment costs for sole elderberry were approximately US$4500 acre^{-1}, while annual variable costs ranged from US $1000 to US$3000 acre^{-1} during peak vegetative and productive years, largely driven by labor-intensive harvesting. Gross annual revenue during peak production years was estimated at approximately US$12,500 acre^{-1}, indicating substantial net income potential once the elderberry reaches maturity. The lower initial investment for establishment than big trees and the longevity of productivity make elderberries an attractive perennial crop for integration into alley cropping. Both flowers and fruits can be processed into value-added products such as wines, syrups, jams, jellies, and dietary supplements (Byers et al. 2022), and elderberry's nutritional and medicinal properties are well documented (Senica et al. 2016). Market demand for elderberry products has increased rapidly in recent years (Crothers 2020; FMI 2025). The growing specialty crop market and the USDA's increasing support to strengthen the specialty crop sector create opportunities for rural economic development and local enterprise growth (USDA 2024).

The social dimensions of elderberry-based alley cropping further support its relevance for sustainable agriculture. Food insecurity remains a major public health challenge in the U.S., with 13.5% of households being food insecure (USDA ERS 2025). Integrating perennial and annual crops can provide both short-term food supply and income from alley crops and longer-term nutritional and economic benefits from perennial components. Elderberry-based systems may also support culturally relevant food practices and value-added enterprises, particularly in small-scale, tribal, and peri-urban farming contexts. These attributes contribute to resilient local food systems and rural economic development.

A practical and actionable finding of this study is the identification of 60 DAT as an effective stage for yield assessment when using RF models. Identifying this single, high-value measurement point can substantially reduce labor and monitoring costs for farmers, enabling targeted scouting, better irrigation scheduling, and more efficient fertilizer or pest management decisions. This stage coincides with the crop's transition from vegetative growth to early flowering/fruit-set in bell pepper. RFs are well-suited for this application because they can capture nonlinear relationships and interactions among variables and have been recognized as a top performer in agricultural yield prediction (Jeong et al. 2016; Van Klompenburg et al. 2020). At 60 DAT, canopy-related traits—leaf number (LN) and LAI were consistently the most influential predictors of yield across all models, while stem diameter (SD) and chlorophyll (SPAD) showed moderate effects, and other traits contributed minimally (Figure 7). These findings highlight the central role of canopy development traits in mid-season yield forecasting and align with previous work identifying LAI as a key variable for improving crop yield prediction accuracy (Bregaglio et al. 2023). Focusing field assessments at key growth stages can improve monitoring efficiency and help guide timely management decisions during the growing season. However, the relatively low R^2 values emphasize that trait-based forecasting alone is insufficient for precise prediction. Future research should integrate larger sample sizes, multi-site and multi-year data, and environmental covariates (soil, weather, and topography) to improve robustness. Scaling this approach may be achieved through remote sensing tools (e.g., drones or satellite-based canopy indices), which can measure LAI and canopy traits rapidly across larger areas.

The results demonstrate clear benefits of integrating elderberry into alley cropping systems, but a few considerations remain. The close spacing of shrubs and annual crops in our design restricts the use of mechanical harvesters, which makes the system more favorable for small-scale farmers with the aim of land optimization and sustainable intensification. The large farms can adopt similar practices with wider spacing and modified layouts to accommodate machinery. In addition, this study was conducted at a single location and with the incorporation of only a single crop in the alleys. The multi-location trials are needed to increase the ability to generalize results across varying soils, climates, and management practices. Such research can guide extension recommendations and inform cost-share policies supporting temperate agroforestry adoption. The inclusion of legumes or other complementary crops could further enhance the long-term resilience of the system by enhancing soil fertility, improving nitrogen cycling, or attracting more pollinators. Further study should be conducted under different environmental conditions and cropping combinations. Comparative economic analyses of different cropping systems, including those with a combination of elderberry and sole cropping, would also be valuable for farmers and land managers. This information will be beneficial to find out the most profitable, practical, and sustainable for adoption at different scales.

5 | Conclusion

Based on this study, it is concluded that elderberry-based alley-cropping systems can enhance the growth parameters and maintain the yield of annual crops (bell peppers) in the alleys compared to conventional cropping. The alley-cropping system's productivity is even higher with extra yield from the elderberry. This clearly indicates that the elderberry-based alley-cropping system can enhance the growth and yield of bell peppers, serving as a sustainable agricultural practice that improves crop productivity and promotes ecosystem services. Among the bell pepper varieties tested, California Wonder and QDAR performed better than Orange Sun in terms of growth and yield. Furthermore, mid-season growth parameters measured at 60 DAT predict the yield more accurately using RF models. The canopy-related traits, such as the number of leaves

and LAI, are important predictors of yield that underscore the value of mid-season yield forecasting. This offers a practical tool for management decisions.

Nevertheless, the study has some limitations. The results are based on a short-term establishment phase of the alley-cropping system, and longer-term crop-tree/shrub interactions may differ as the perennial component matures. In addition, microclimate moderation and competition processes were inferred from crop responses rather than directly measured, limiting mechanistic interpretation. The machine learning analysis was also constrained by a relatively small dataset, which may have affected model robustness and generalizability. Incorporating additional explanatory variables, such as soil properties, microclimatic conditions, and weather dynamics, could improve predictive accuracy and enable more reliable yield forecasting at earlier growth stages (e.g., 30–45 DAT).

Despite these limitations, the findings have important implications for sustainable agriculture. Elderberry-based alley cropping offers a promising strategy for small and medium-scale producers to enhance productivity, diversify income, and support ecosystem services such as pollinator activity and soil health. Furthermore, mid-season yield prediction provides a practical decision-support tool for crop management, harvest planning, and marketing. Overall, this study provides empirical support for the adoption of elderberry-based alley-cropping systems and identifies clear pathways for advancing research and on-farm decision-making in diversified cropping systems.

Author Contributions

Santosh Paudel conducted field experiments, curated data, performed all statistical and model analyses, drafted the original manuscript, performed data visualization, and led the revisions. Suraj Upadhaya provided overall supervision and guidance, reviewed and edited the manuscript, and secured research funding. Sudha Bhandari performed data collection, review, and editing of the manuscript. Anuj Chiluwal, Theoneste Nzaramyimana, and Srijana Thapa Magar provide guidance, review, and editing of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Figure S1: Detailed layout of the experimental plot of individual block with three varieties of bell peppers randomized across the block, and encircled dots show the sample plants. **Table S2:** Growth traits of bell peppers in elderberry presence and absence across 2 years (2024-2025) at different DATs. **Table S3:** Physiological traits of bell peppers in elderberry presence and absence across 2 years (2024-2025) at different DATs. **Figure S4:** Variation in yield of different varieties of bell peppers. **Table S5:** Model performance of Random Forest (RF), LASSO regression, Linear regression, SVM, and GBM for predicting bell pepper yield at different data collection times (30, 45, 60, and 75 DAT).