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The effects of cultivar mixtures on insect pest and natural enemy abundance, diseases, and yield in tropical soybean cropping system

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HIGHLIGHTS

- Increasing cultivar mixtures can attract certain groups of natural enemies in soybean field.
- Cultivar mixtures of soybeans have inconsistent effects on insect pests, possibly due to low pest pressure.
- Cultivar mixtures of soybeans do not alter diseases and yield, likely due to the lack of interaction effects between the selected cultivars.
- The selection of cultivars exhibiting interaction effects in cultivar mixtures could be an alternative strategy for biological pest control and sustainable soybean production.

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ABSTRACT

Increasing genotypic crop diversity via cultivar mixtures is a promising sustainable approach to control insect pests and diseases, thereby improving yield. The effects of genotypic diversity have not been studied for many crops. We investigated the effects of cultivar mixtures in a tropical soybean (*Glycine* max L. Merrill) cropping system on i) insect pest abundance, ii) natural enemy abundance, iii) diseases, and iv) yield. In the field trial, three soybean cultivars were used, two commercial and one traditional, with a randomized complete block design. Significant differences among cultivars and some mixtures were found for certain insect pest abundance (whitefly and brown bean bug), but no consistent mixture effects were observed. Significant increases in natural enemies (predatory ant, lady beetle, parasitoid wasp, and dragonfly) were detected in some cultivar mixtures, compared to single cultivars. Higher genetic diversity in cultivar mixtures increased the abundance of certain natural enemies at specific plant stages. The cultivar mixtures did not alter disease symptoms or yield. These results were obtained during a season with very low overall pest pressure, and the effects of cultivar mixtures might be altered at higher pest pressure, which should be further investigated. This study highlights trade-offs in cultivar selection when jointly considering pest and disease abundance and yield, as no single cultivar (or mixture) performed better in all observed aspects. Our study supports the hypothesis that increasing cultivar mixtures can promote the abundance of certain natural enemies, suggesting the potential of cultivar mixture effects for biological control and sustainable agricultural management.

1. Introduction

Biodiversity plays an essential role in ecosystem functioning, and plant diversity, in particular, could support extensive ecosystem services for societal benefit (Quijas et al., 2010). In agroecosystems, plant diversity can enhance biological control of pests and diseases, thereby

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Table 1
Cultivars used in the study and their ratio in mixtures for the treatments.

No	Treatment	Code	Descriptions
1	Santa Cruz	SC	Single cultivar
2	98C81	81	Single cultivar
3	Sbung	SB	Single cultivar
4	Santa Cruz – 98C81	SC-81	1:1 mixture of two cultivars
5	Santa Cruz – Sbung	SC-SB	1:1 mixture of two cultivars
6	98C81 – Sbung	81-SB	1:1 mixture of two cultivars
7	Santa Cruz – 98C81 – Sbung	SC-81-SB	1:1:1 mixture of three cultivars

increasing food production (Letourneau et al., 2011; Ratnadass et al., 2012). Plant species diversity or genotypic plant diversity are recognized to contribute to pest and disease control through bottom-up and top-down effects such as dilution effects (Hambäck et al., 2014), the increased abundance of natural enemies (Cook-Patton et al., 2011), or associational resistance (Malézieux et al., 2009), respectively. In many cropping systems, the relative influence of those mechanisms is not well understood, which hinders the development of sustainable pest management.

In tri-trophic interactions, bottom-up and top-down effects have been regarded as important ecological forces for enhancing integrated pest management (IPM) throughout the past 20 years. Crop resistance and crop diversification are the biotic mechanisms that trigger bottom-up forces and cause negative effects on pests (Han et al., 2022). For instance, plant associational resistances could suppress insect pests via specific physical and chemical barriers in genotypic plant diversity (Dahlin et al., 2018; Malézieux et al., 2009). Additionally, arthropod/insect pest populations can be reduced due to the resource dilution effect, causing difficulties in searching for host plants in crop mixtures, resulting in less damage (Hambäck et al., 2014; Peacock and Herrick, 2000). As top-down ecological forces, diverse genotypic plant diversity have a positive impact on the abundance of natural enemies (Gurr et al., 2016; Han et al., 2022; Lin, 2011). Both, crop species mixtures and cultivar mixtures can be used to achieve beneficial effects on pest control and can affect crop yield (Li et al., 2020; Reiss and Drinkwater, 2018; Tooker and Frank, 2012). Cultivar mixture is defined as “mixtures of cultivars that vary for many characters including disease resistance, but have sufficient similarity to be grown together” (Wolfe, 1985). Using different crop species for mixtures can be time consuming and logistically challenging for the current agricultural production (Lin, 2011; Tooker and Frank, 2012). Hence, cultivar mixtures are more applicable for genotypic plant diversity practice in the current cropping systems. Few studies have addressed the dynamic effects of bottom-up and top-down mechanisms in cultivar mixtures in crop protection and yield; therefore, it is necessary to explore the effects of cultivar mixtures on tri-trophic interactions in the field to identify potential benefits for biological control and sustainable pest management.

Recent studies have suggested that cultivar mixture is one of the most promising strategies to reduce insect pests populations (Dahlin et al., 2018; Nboyine et al., 2021; Pan and Qin, 2014; Snyder et al., 2020; Tooker and Frank, 2012). The selection of cultivars for mixtures plays an important role for the efficacy of specific insect pest or disease reduction (Mundt, 2014, 2002). Specifically, identifying cultivars that can benefit from each other can substantially reduce plant acceptance, feeding behaviour, and population growth of widely abundant pests, such as aphids (Dahlin et al., 2018; Kheam et al., 2023). Aside from insect pest population reduction, cultivar mixtures could enhance yield in different crop species (Reiss and Drinkwater, 2018), or reduce the variation of disease severity, compared to monoculture (Vidal et al., 2020). In most cropping systems, the potential interactions between commonly used cultivars are not known, particularly in tropical agroecosystems. A better understanding of how specific combinations of common cultivars respond to insect pests, and diseases, whether they attract natural enemies and increase yield, could improve food security and sustainability of farming systems.

Soybean (*Glycine* max L. Merrill) is one of the most economically important cereal crops globally, including in many developing countries. Soybean production and the commercial demand is increasing every year; however, this crop suffers economic losses mainly caused by insect pests (Musser et al., 2012) and plant diseases (Wrather et al., 2001). Despite several studies on the effects of soybean cultivar mixtures in the field (Grettenberger and Tooker, 2020; Nboyine et al., 2021; Pan and Qin, 2014), limited number of studies have been conducted in a tropical climate in developing countries. For instance, in Cambodia, the average soybean yield is about 1.5 tons per hectare (t/ha) (Belfield et al., 2011), which is lower than the global average of 2.7 tons per hectare (Grassini et al., 2021). The yield can be significantly reduced by insect pests, diseases, weeds, and the use of traditional cultivars, which can have poor disease-resistance and low yield. Although chemical measures are the most effective strategy to control pests and diseases, the integrated pest management (IPM) is widely practiced (Brier et al., 2008). Understanding the effects of potential biological pest management strategies is, therefore, very crucial for ecological and practical perspectives for local food production and sustainable agricultural development in developing countries such as Cambodia and other tropical soybean production systems.

In this field study, we evaluated the use of cultivar mixtures in a soybean (*Glycine* max L. Merrill) cropping system in Cambodia to determine their effects on i) insect pests, ii) natural enemies, iii) diseases, and iv) grain yield. We hypothesized that mixtures of two or three cultivars would result in a reduction of insect pest abundance, an increase in natural enemy abundance, a decrease in disease severity, and an improvement in grain yield when compared to single cultivars.

2. Materials and methods

2.1. Study site and plot descriptions

A soybean field-based experiment was conducted in the research area of Chamkar Leu Upland Crop Seed Farm (12°12′13.9″N, 105°19′08.4″E), at Chamcar Leu District, Kampong Cham province, Cambodia, during the 2022 growing season (June-November). Chamcar Leu, known as “Upper Farm”, is located around 48 km northeast of the provincial capital at Kampong Cham city, and it is a significant farming area for rubber, both for domestic consumption and export. Soybean are also commonly farmed in this area. Chamkar Leu has a tropical climate, with a total monthly precipitation of 83.10 mm, which was high in August (2022). From January to June, the total precipitation for the season was 170.18 mm. The mean monthly temperature ranged from 22.2 °C (in January) to 35.5 °C (in April) in 2022. The climate at Chamkar Leu during 2022 field season was typical.

In 2022, the field experiment was conducted with soybeans following the planting of cassava in a field of approximately 0.3 ha (60 x 42 m). The surrounding area consisted of a mixture of various annual crops, including maize, cassava, and beans, as well as grass margins along with small roads. In the mid-raining season (June-July), the field was ploughed to improve soil quality for the experiment. On July 15th 2022, each soybean treatment was planted in a 30 m^2 per plot, using a randomized complete block design of seven blocks, with seven plots in each block (Supplementary 1). The treatments tested consisted of different mixtures of soybean cultivars (Table 1). Each block contained one replicate of three cultivars: single-cultivar treatments, three two-cultivar mixture treatments, and one three-cultivar mixture treatment. Seven replicates per treatment were used (N = 7). Plot size was 35 m^2 (5 x 7 m) with 1 m wide stretch of bare soil between plots/blocks. All plots were separated from the surrounding area (maize, cassava, and bean fields) by 3 m.

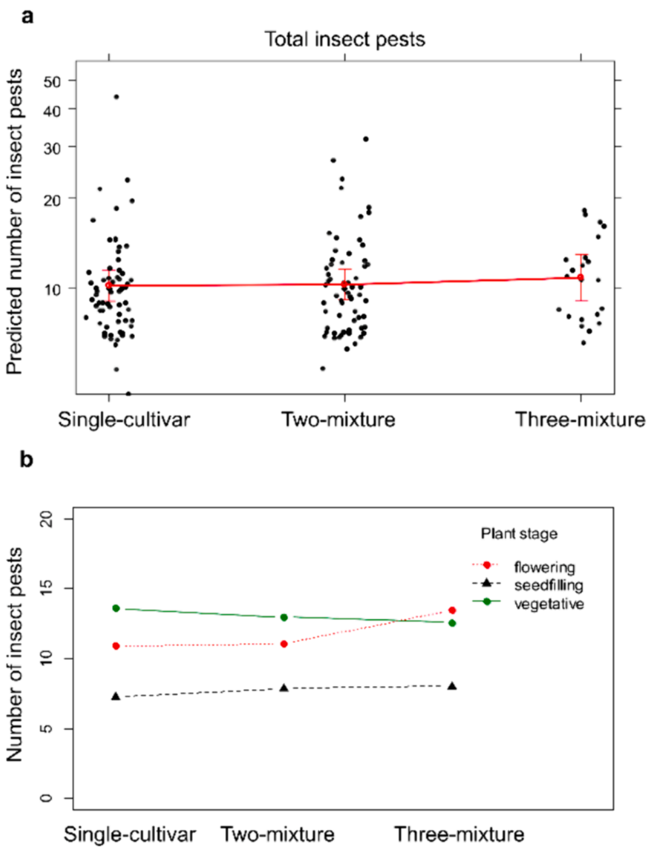

Fig. 1. Overall insect pest abundance in the field. **a)** the predicted effects of insect pests in three different treatments: single-cultivar, two-mixture, and three-mixture. **b)** the number of insect pest at three different plant stages including vegetative (dark green), flowering (red) and seedfilling (black) in single-cultivar, two-mixture and three-mixture. The error lines represent confidence intervals from predictor effect model and the black dots are the predicted number of insect pests. The GLM analyses were used for the statistical significance at $p \leq 0.05$.

2.2. *Soybean cultivars and treatments*

The study employed three soybean cultivars: two commercial cultivars (98C81 and Santa Cruz) and one traditional cultivar (Sbung). The two commercial cultivars were provided by the Conservation Agriculture Service Center (CASC), General Directorate of Agriculture (GDA) of Cambodia. These cultivars are very popular for farmers due to their high yield (mean yield 2.1 t/ha) and good nutritional composition in grain. The two commercial cultivars are known to have moderate tolerance for bacterial pustule (*Xanthomonas axonopodis pv. glycine*) and brown bean bug (*Riptotortus linearis*) (Nget et al., 2022). The traditional cultivar was obtained from a local Orussey Market supplier with a high yield (mean yield = 2.0 t/ha) (Nget et al., 2022). The cultivars have maturity period of 110 days.

Soybean planting in the field followed the soybean planting configuration as described by Belfield et al. (2011). The seed rating of 55 kg/ha was applied for hand planting. The mixtures were equally hand-prepared with a ratio of 1:1 for two cultivars or 1:1:1 for three cultivars (Table 1). The rows were made using tools with 40 cm between rows and 20 cm distance between hills within rows. Four to six seeds were used per hill, resulting in the approximate total of 1,700 plants per plot. Due to the high abundance of weeds in the field, weeds were mechanically removed from the field three weeks after soybean sowing to reduce the potential unwanted effects on the cultivar mixtures. Following two weeks of weeding, weed abundance increased further, so we applied a mixed herbicide, named Sundeak 5 + Domlong 25 (Fomesafen 25 % and quizalofop-p-ethyl 5 %) to minimize the impact of weeds on the treatments.

2.3. *Insect pest assessment*

To assess herbivore populations, ten plants per plot were randomly selected in each week (Grettenberger and Tooker, 2020), and surveyed for insect pests, including aphids, whiteflies, leaf beetles, and brown bean bugs. The selected plants were at the centre of the plots and 1 m away from the edge. The counting began when the plants reached the vegetative stage (V1), 13 days after sowing. The upper and lower surface of leaves and stems were directly observed to count insects. The insect counting stopped when the plants reached the reproductive stage (R6), at which point the economic threshold for soybean aphids is no longer relevant (Ragsdale et al., 2007).

2.4. *Natural enemy assessment*

To investigate the potential impact of cultivar mixtures on the natural enemies of insect pests, we counted predatory ants, lady beetles, dragonflies and parasitoid wasps weekly. Observers walked along the middle row (central line transect) in each plot and closely observed from left to right side of the plot for approximately 5–7 min. The observation was conducted on a weekly basis (once per week, on foot) between 9:00 AM and 12:00 PM, during periods of predominantly warm and sunny weather. The predator survey began at the plants reached V1 and continued until the R6. We recorded the number of observed predator individuals. In order to avoid any potential interference with the presence of natural enemies in the field, no samples were collected for taxonomic analysis. Instead, we relied solely on the visualized morphologies for the counts. As a result, the counts were categorized under a specific group (order) of natural enemies, rather than individual species/family.

2.5. *Disease assessment*

Plant diseases were assessed by counting the number of infected plants with disease symptoms on the leaves in each plot. The incidence of disease was evaluated based on the observed abnormal colours and characteristics of leaves, which were presumably categorized as leaf disease, on each plant within the plots. The assessment were conducted at two reproductive stages: approximately 40 days (R1) and approximately 54 days (R3).

2.6. *Yield assessment*

We determined yield in the plots by harvesting the three selected 1-meter transects in each plot at maturity in the beginning of November 2022. The harvested seeds were dried at 93 °C for 10 days and then weighed to determine dry mass. In this experimental setup, one square metre was subdivided into three rows. Consequently, the total yield from the harvested three metres was equivalent to one square metre. To convert from kilograms per square metre (kg/m^2) to tons per hectare (ton/ha), the following formula was employed: the sum of yield from three meters in kilograms divided by square meters, resulting in observed values of square meters multiplied by 1/1,000 and then by 10,000 to convert to hectares.

2.7. *Statistical analyses*

The field data were analysed by using R statistical software (R Core Team, 2021). Generalized linear models (GLMER) were employed (package lme4) to analyse effects of cultivar mixtures on insect pest and natural enemy abundance, disease occurrence and yield. Firstly, we analysed the total insect pest abundance and each pest taxon separately, to test differences between single-cultivar, two-cultivar mixture and

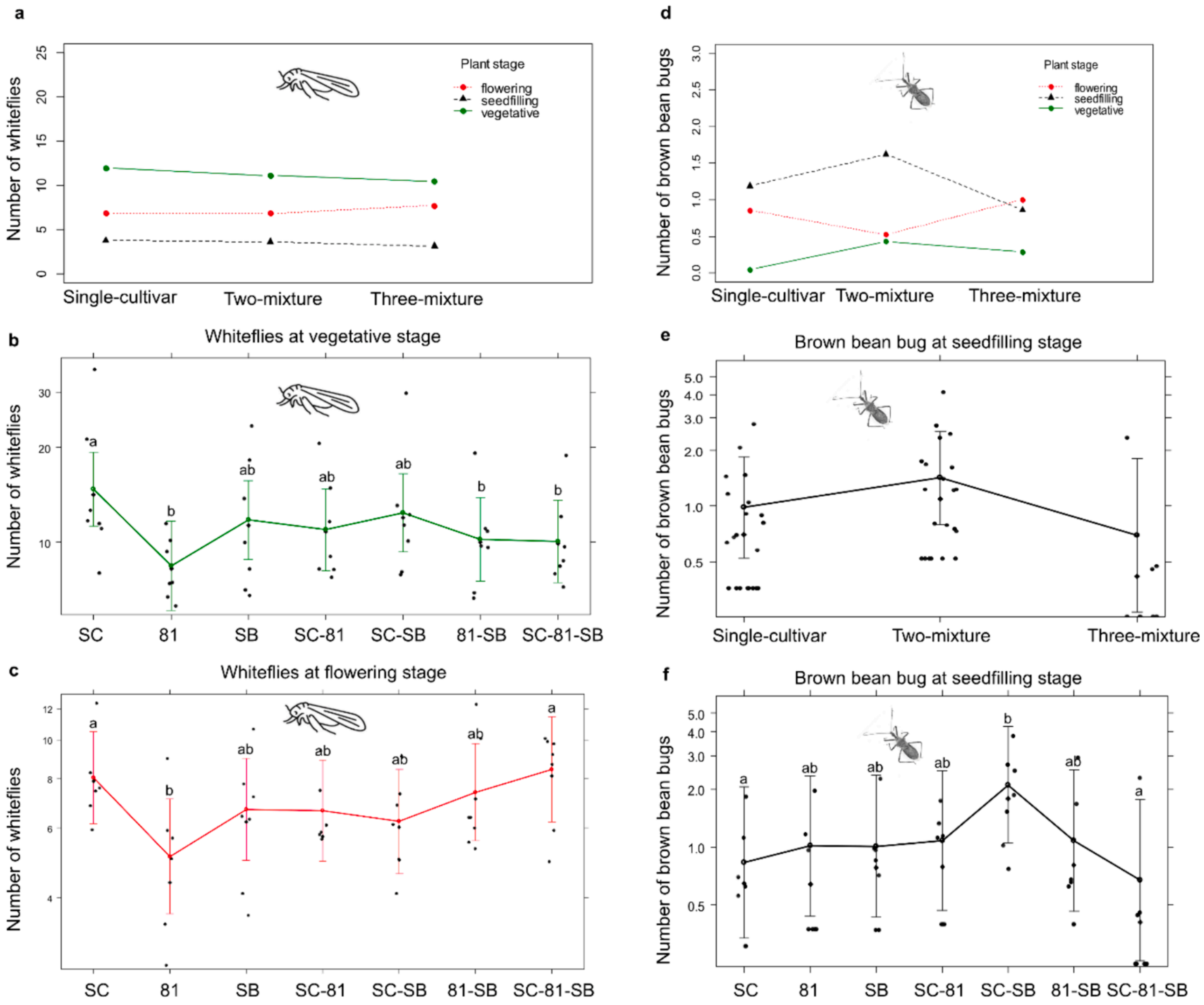

Fig. 2. Whitefly and brown bean bug abundance in different cultivar/mixture treatments in the field. **a)** the different interaction effects of whitefly abundance in single-cultivar, two-mixture and three-mixture at vegetative (dark green), flowering (red) and seedfilling (black) plant stages. **b & c)** the predicted number of whiteflies at vegetative and flowering stage. **d)** the interaction effects of brown bean bug abundance in single-cultivar, two-mixture and three-mixture with three different plant stages. **e)** the predicted number of brown bean bugs in single-cultivar, two-mixture and three-mixture. **f)** the predicted number of brown bean bugs in seven treatments at seedfilling stage. SC: Santa Cruz, 81: 98C81, SB: Sbung, SC-81: Santa Cruz-98C81, SC-SB: Santa Cruz-Sbung, 81-SB: 98C81-Sbung, SC-81-SB: Santa Cruz-98C81-Sbung. Error lines represent confidence intervals from the models and the black dots are the predicted mean numbers. Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

three-cultivar mixture including plant stage as fixed factor in the model. The single-cultivar treatment was obtained by the average of three individual cultivars (SC, 81, SB). The two-mixture treatment was determined by the average of two-cultivar mixtures (SC-81, SC-SB, 81-SB). The three-mixture treatment was derived from three-cultivar mixture (SC-81-SB). These procedures were applied to all analyses concerning the single-cultivar, two-mixture, and three-mixture treatments. Interaction.plot were employed (package interactions) to determine the interaction effects between single-cultivar, two-cultivar mixture and three-cultivar mixture, together with growth plant stages. Secondly, we analysed the total insect pest abundance and each pest taxon separately, to test differences between seven treatments, for all plant growth stages together and for each stage separately. To understand the temporal variation in occurrences of herbivores and predators in the field, we categorized the data into three plant stages, vegetative, flowering and seedfilling, and ran separate analyses for each stage (Supplementary 2). All models were validated by graphic examination of residual plots (Zuur et al., 2010) and over dispersion tests in the DHARMa package. The $\alpha = 0.05$ significance level was applied to test the differences between treatments.

We used GLMER with the Poisson or Negative Binomial family to analyse response variables with count data; these were the number of insect pests: whitefly, leaf beetle, and brown bean bug. Due to large variation in the number of aphids between plots, aphid data were scaled by using the mean value and standard deviation for analysis. Scaled aphid abundance and the continuous data of yield were analysed with a Gamma family. Firstly, we grouped the seven treatments (SC, 81, SB, SC-SB, SC-81, 81-SB, and SC-81-SB) into three main groups: single-cultivar (SC, 81, SB), two-mixture (SC-SB, SC-81, 81-SB), and three-mixture (SC-81-SB). We used groups (single-cultivar, two-mixture and three-mixture) and the total abundance of natural enemies as fixed factors and block as random factor in the models to compare the three group treatments. For the interaction analyses, we used groups and plant stages as fixed factors and block as random factor. Secondly, we used cultivar/mixture (treatments: SC, 81, SB, SC-SB, SC-81, 81-SB, and SC-81-SB) and the total of natural enemy abundance (including: predatory ant, lady beetle, dragonfly, and wasp) as fixed factors to compare the seven treatments. The total number of natural enemies was scaled

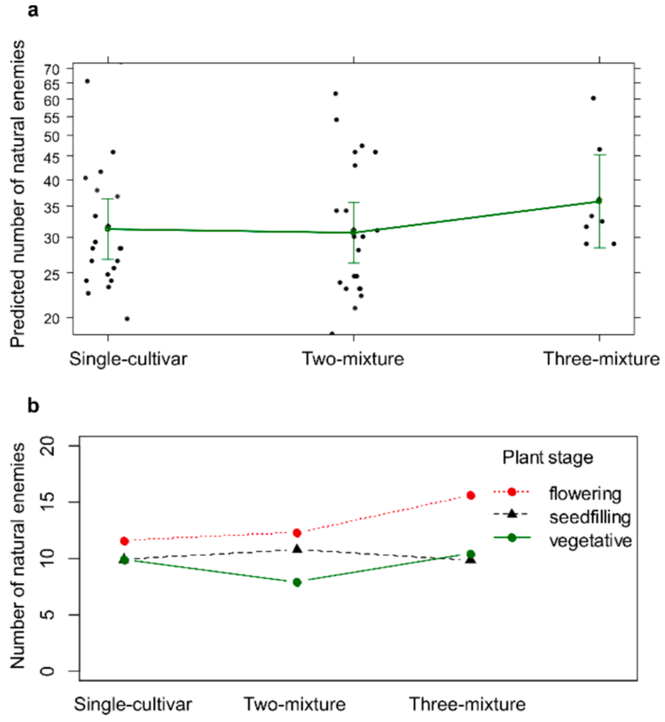

Fig. 3. Overall natural enemy abundance in the field. **a)** the predicted effects of three different treatments: single-cultivar, two-mixture, and three-mixture on the natural enemies. **b)** The number of natural enemies at three different plant stages including vegetative (dark green), flowering (red) and seedfilling (black) in single-cultivar, two-mixture and three-mixture groups. The error lines represent confidence intervals from the models and the black dots are the predicted number of natural enemies. The GLM analyses were used for the statistical significance at $p \leq 0.05$.

when used as explanatory factor in the models for insect pests. Block was used as a random factor in all models.

To analyse the abundance of natural enemies (predatory ant, lady beetle, dragonfly, and parasitoid wasp), we used similar approach as insect pest abundance analyses. We firstly analysed the three group comparisons: single cultivars (SC, 81, SB), two-mixture (SC-SB, SC-81, 81-SB), and three-mixture (SC-81-SB) by using groups as fixed factors and block as random factor in the models. For the interaction analyses, we used groups and plant stages as fixed factors and block as random factor. Then, we used cultivar/mixture (treatments: SC, 81, SB, SC-SB, SC-81, 81-SB, and SC-81-SB) as fixed factors and block as random factor in the models to compare the differences between the seven treatments. The same approach was used for disease and grain yield analyses. We analysed the interaction effects (package interplot) on yield with disease, predator, and insect pest. Overall, single-cultivar group was used as initial reference category for group treatment, and re-run the models with different reference category when needed for other group comparison to get the estimates, standard errors, and p-values for comparisons between groups. We used SC as initial reference category for cultivar treatment, and re-run the models with different reference category, when it was needed to obtain the estimates, standard errors, and p-values for additional comparisons between treatments.

Furthermore, we did additional analyses for natural enemies (parasitoid wasp at seedfillling stage and dragonfly at vegetative stage) in two specific mixtures (SC and SB), because it became apparent during the initial analyses that cultivar interactions may occur in these specific mixtures. In these analyses, we compared the expected mixture values (calculated as the mean enemy abundance of the cultivars in the mixture) with the observed values for the mixture. We specifically used GLMER with Negative Binomial family with block as random factor in the models.

3. Results

3.1. Insect pest abundance

Four main groups of insect pests were recorded and analysed based on plant stages in this study: aphid, whitefly, leaf beetle and brown bean bug (Supplementary 2). The overall insect pest abundance did not differ between cultivars grown in single cultivars and different mixtures (Fig. 1a). The plant developmental stage had a significant impact on the abundance of pests (Fig. 1b). Insect pest abundance was highest in the vegetative stage, followed by the flowering and then the seedfilling stage (GLM, Estimate = -0.15, SE = 0.07, p = 0.03; Estimate = -0.54, SE = 0.07, $p < 0.001$), respectively. Insect pest abundance was also significantly higher in the flowering stage than in the seedfilling stage (GLM, Estimate = -0.39, SE = 0.07, $p < 0.001$).

Whitefly was observed to be the most abundant herbivore in the field (average 2–15 per plot). The overall number of whiteflies was significantly higher in the vegetative stage, followed by the flowering stage and then the seed-filling stage (Fig. 2a). No significant difference in whitefly abundance was observed between single-cultivar, two-mixture and three-mixture. During the vegetative stage, whitefly was significantly lower in SC-81-SB, 81, 81-SB compared to SC (Fig. 2b), while during the flowering stage, whitefly in SC and SC-81-SB was significantly higher than in 81 (Fig. 2c).

Bean leaf beetle was the second largest group that was observed in this study. However, no difference was detected in leaf beetle abundance between treatments at any plant stage (Supplementary 2). We recorded relatively few brown bean bugs on soybean plants (0 – 3 per plot). No significant differences were observed between single-cultivar, two-mixture and three-mixture (Fig. 2e). The analyses showed that the brown bean bug was most abundant during the seedfilling stage, followed by the flowering and then the vegetative stage (Fig. 2d). During the seedfilling stage (Fig. 2f), the population of brown bean bugs was significantly higher in SC-SB than in SC and SC-81-SB. Aphid populations were generally low, with occasional records of large populations, and no significant difference in aphid abundance was detected between treatments (Supplementary 2).

3.2. Natural enemy abundance

We firstly analysed the total number of natural enemies based on three groups' comparison (single-cultivar, two-mixture and three-mixture). No significant differences were detected between the three treatments, although it was a trend for higher natural enemies in three-mixture treatment (Fig. 3a). We found temporal variation based on plant stages, as natural enemy abundance was significantly higher in the flowering stage compared to the vegetative and the seed-filling stage (GLM, Estimate = -0.31, SE = 0.09, $p < 0.001$; Estimate = -0.19, SE = 0.09, p = 0.03), respectively (Fig. 3b). However, there was no difference in abundance between the vegetative and seedfilling stage.

The study analysed four main groups of natural enemies, namely predatory ants, lady beetles, dragonflies, and parasitoid wasps, across three different plant stages (Supplementary 2). Predatory ants were found to be the most abundant predators in the field, with a range of four-ten individuals per plot. We did not find a significant differences in predatory ant populations between single-cultivar, two mixture and three mixture and no difference was detected between different plant stages (Fig. 4a). However, during the flowering stage the abundance of predatory ants was significantly higher in the mixture of SC-81-SB and 81 than in SC (Fig. 4b).

In the field, parasitoid wasps were the least abundant of natural enemy group The abundance of parasitoid wasps (Fig. 4c) was significantly higher during the flowering stage compared to the vegetative and the seed-filling stage (GLM, Estimate = -2.85, SE = 0.42, $p < 0.001$; Estimate = -0.34, SE = 0.15, p = 0.02). In addition, the number of parasitoid wasps was higher during the seed-filling stage than during the

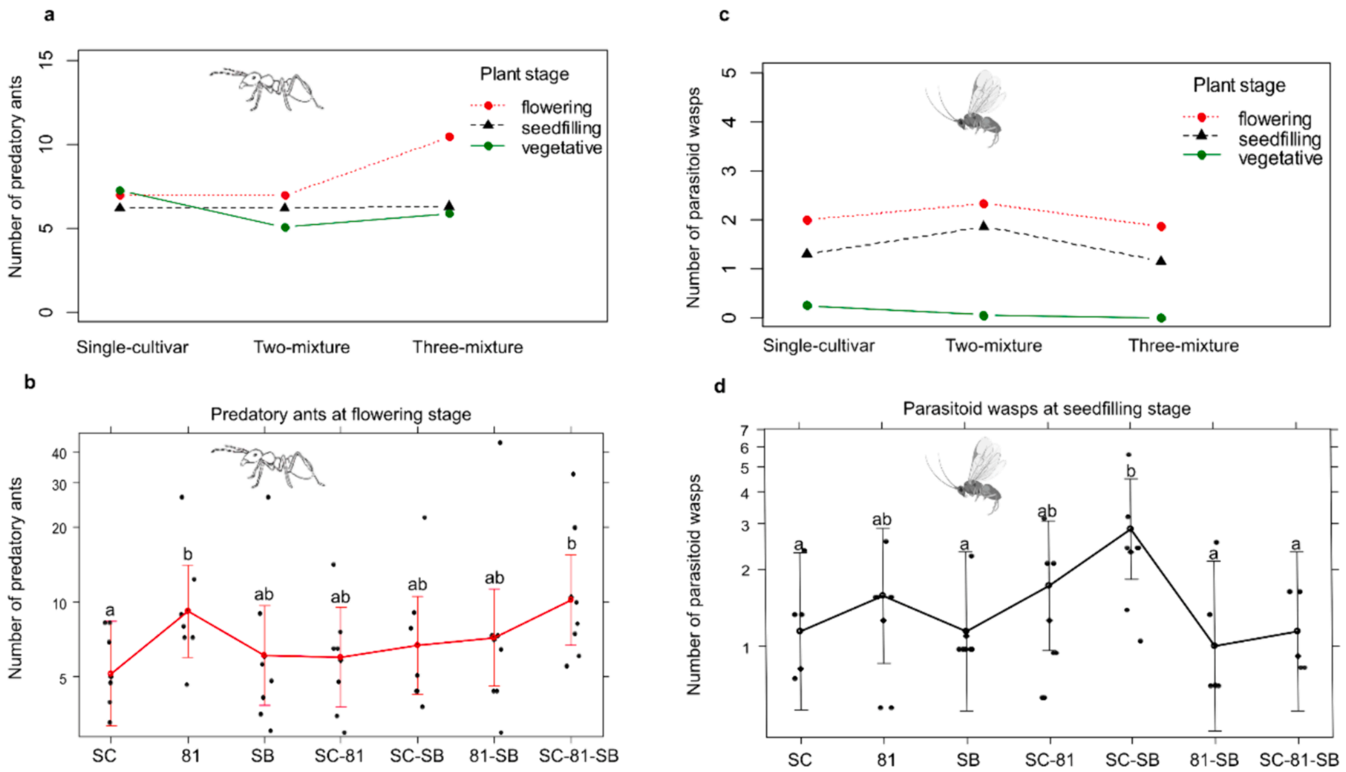

Fig. 4. The abundance of predatory ants and parasitoid wasps in the field. **a)** the interaction effects of predatory ants abundance in single-cultivar, two-mixture and three-mixture at vegetative (dark green), flowering (red) and seedfilling (black) plant stages. **b)** the predicted number of predatory ants at flowering stage. **c)** the different interaction effects of parasitoid wasp abundance in single-cultivar, two-mixture and three-mixture at vegetative stage (dark green), flowering (red) and seedfilling (black) plant stages. **d)** the predicted number of parasitoid wasps at seedfilling stage. SC: Santa Cruz, 81: 98C81, SB: Sbung, SC-81: Santa Cruz-98C81, SC-SB: Santa Cruz-Sbung, 81-SB: 98C81-Sbung, SC-81-SB: Santa Cruz-98C81-Sbung. Error lines represent confidence intervals from the models and the black dots are the predicted mean numbers. Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

vegetative stage (GLM, Estimate = 2.51, SE = 0.42, $p < 0.001$). In the seedfilling stage, the abundance of parasitoid wasps was higher in the SC-SB mixture compared to SC, SB, 81-SB, and SC-81-SB (Fig. 4d).

We noticed that in seedfilling stage the number of parasitoid wasps was higher in the SC-SB mixture than in both single cultivars of SC and SB. The additional analyses showed that the expected value of SC and SB mixture was significantly lower than the observed value of SC-SB (Fig. 6a).

We also found that in the vegetative stage lady beetles were significantly more abundant than in the flowering and seedfilling stages (GLM, Estimate = -0.85, SE = 0.31, p = 0.02; Estimate = -0.85, SE = 0.33, p = 0.009), respectively (Fig. 5a). No difference was observed between the flowering and seedfilling stages. The occurrence of lady beetles was significantly higher in the three-mixture compared to the single-cultivar, but not different from the two-mixture in the vegetative stage (Fig. 5b). The number of lady beetles was significantly higher in the SC-81-SB mixture compared to SC, SB and SC-SB in the vegetative stage (Fig. 5c). Similarly, we found that dragonflies were significantly higher in the flowering stage than in the vegetative and seedfilling stages (GLM, Estimate = -0.41, SE = 0.17, p = 0.02; Estimate = -0.5, SE = 0.18, p = 0.005), respectively (Fig. 5d). No significant difference was observed between the vegetative and seedfilling stages. We further found that in the vegetative stage dragonflies were significantly more abundant in the three-mixture compared to the single-cultivar, but there was no significant different with the two-mixture (Fig. 5e). In the vegetative stage, the abundance of dragonflies was higher in the mixtures of SC-81-SB and SC-SB compared to SC and SB alone (Fig. 5f). Also, the number of dragonflies in 81 was significantly higher than in SC. Since we noticed that the number of dragonfly was higher in the SC-SB mixture than in the single-cultivar of SC and SB, additional analysis of observed versus expected value was performed for this combination. We found that the expected value of SC and SB was significantly lower than the observed value of the SC-SB mixture (Fig. 6b).

3.3. *Disease severity*

Although we did not specifically evaluate individual disease symptoms on the leaves, we observed four main groups of diseases including: bean yellow mosaic virus, mungbean yellow mosaic virus, rust and soybean vein necrosis virus in our study. The leaf disease was analysed by comparing three treatment groups and two reproductive stages (R1 and R3). We found that the disease occurrence on the leaves was higher in the R3 than in the R1 plant stage (Fig. 7a). However, there were no significant differences were observed between cultivars grown in single-cultivar, two-mixture and three-mixture. The overall disease occurrence varied between treatments with the lowest occurrence in SC. Disease in SB was significantly higher than in SC-81-SB (Fig. 7b).

3.4. *Grain yield*

No differences in grain yield were observed between single-cultivar, two-mixture and three-mixture treatments (Fig. 8a). The analyses of individual treatments showed differences in yield. The yield in SB was significantly lower than in SC and 81 (Fig. 8b). No correlation was observed between insect pests and diseases (Fig. 8c). We found that yield has a negative correlation with disease, indicating that the higher disease levels results in lower yield (Fig. 8d). However, there was no correlation between predators/insect pests and yield.

4. Discussion

Our study indicates that increasing genetic diversity by mixing

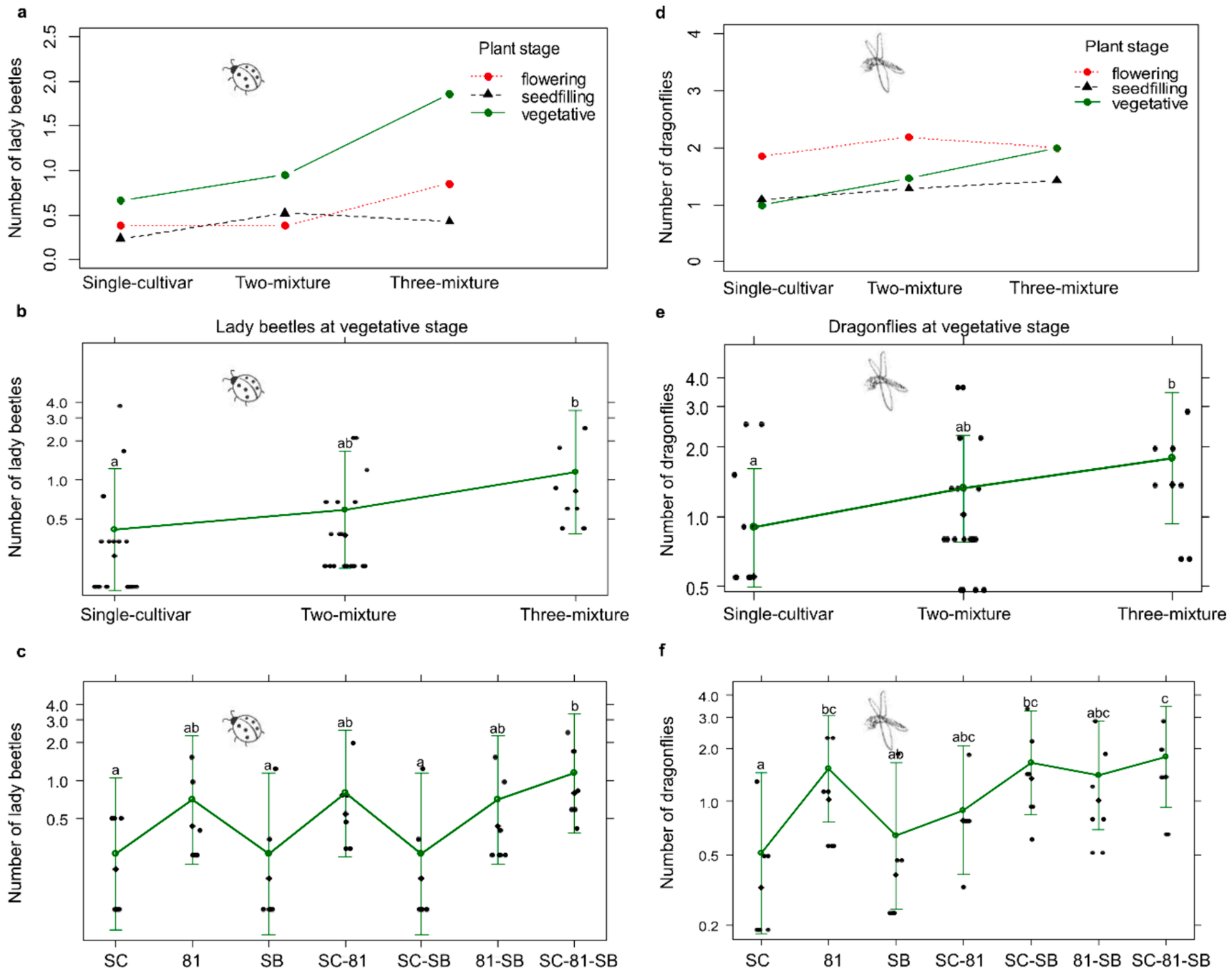

Fig. 5. The abundance of lady beetles and dragonflies in the field. **a)** the different interaction effects of lady beetles abundance in single-cultivar, two-mixture and three-mixture at vegetative (dark green), flowering (red) and seedfilling (black) stages. **b)** the predicted number of lady beetles in single-cultivar, two-mixture, and three-mixture at the vegetative stage. **c)** the predicted number of lady beetles in each treatment at vegetative stage. **d)** the different interaction effects of dragonfly abundance in single-cultivar, two-mixture and three-mixture at three different plant stages. **e)** the predicted number of dragonflies in single-cultivar, two-mixture, and three-mixture at the vegetative stage. **f)** the predicted number of dragonflies in seven treatments at the vegetative stage. SC: Santa Cruz, 81: 98C81, SB: Sbung, SC-81: Santa Cruz-98C81, SC-SB: Santa Cruz-Sbung, 81-SB: 98C81-Sbung, SC-81-SB: Santa Cruz-98C81-Sbung. Error lines represent confidence intervals from the models and the black dots are the predicted numbers. The letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

cultivars can have ecological effects in the field by attracting specific natural enemies at different plant stages. However, the mixing cultivars provide inconsistent effects on insect pest abundance and do not alter disease severity and grain yield. This study also highlights substantial differences between soybean cultivars in their effects on pests and natural enemies, disease sensitivity, and yield. Our results also demonstrate trade-offs among various characteristics of individual cultivars, and such knowledge could be utilized to adapt selection of cultivars to local conditions particularly with regards to specific pest and disease pressures.

4.1. The effects of increasing cultivar mixtures on natural enemies and insect pests

The observed significantly higher abundance of lady beetles and dragonflies in three-cultivar mixture support the hypotheses that increasing genetic diversity, through cultivar mixtures, can increase natural enemy abundance in the field (Grettenberger and Tooker, 2017; Ninkovic et al., 2011; Tooker and Frank, 2012). While the overall effects of cultivar mixtures on natural enemies were unclear in this study, including the temporal variation analyses allowed us to uncover some important effects. Interestingly, we only observed the higher abundance of two groups of natural enemies at vegetative stage, suggesting that certain natural enemies can be more attractive to higher genetic diversity at a specific plant stage. Since the increasing cultivar mixtures of soybean vary in phenotypic characteristics, they could be able to produce more diverse microhabitats in the three-mixture, leading to attract more natural enemies. Similarly, lady beetles preferred a mixtures of specific cultivar of barley (*Hordeum vulgare*) over monoculture before aphids arrived in the field and again after they left (Ninkovic et al., 2011). Therefore, it seems that certain natural enemies move into the mixture plots at certain plant stages in the field and leave later due to the lack of the food or pray during the late plant growth stage. Although higher plant diversity can increase attraction of certain natural enemies to the field at specific plant stages, it would be beneficial for suppressing pests at certain levels.

Natural enemies use plant volatiles to search for their habitats and preys. Increasing plant diversity in cultivar mixtures may increase the release of plant volatiles, if the cultivar differs in their volatile profiles. It is likely that the observed increased abundance of lady beetles and dragonflies in the three-cultivar mixture in our study could be due to the diverse plant volatile released by the three different cultivars. A preference of lady beetles for a specific combination of barley cultivars over individual cultivars has been observed in both laboratory and field

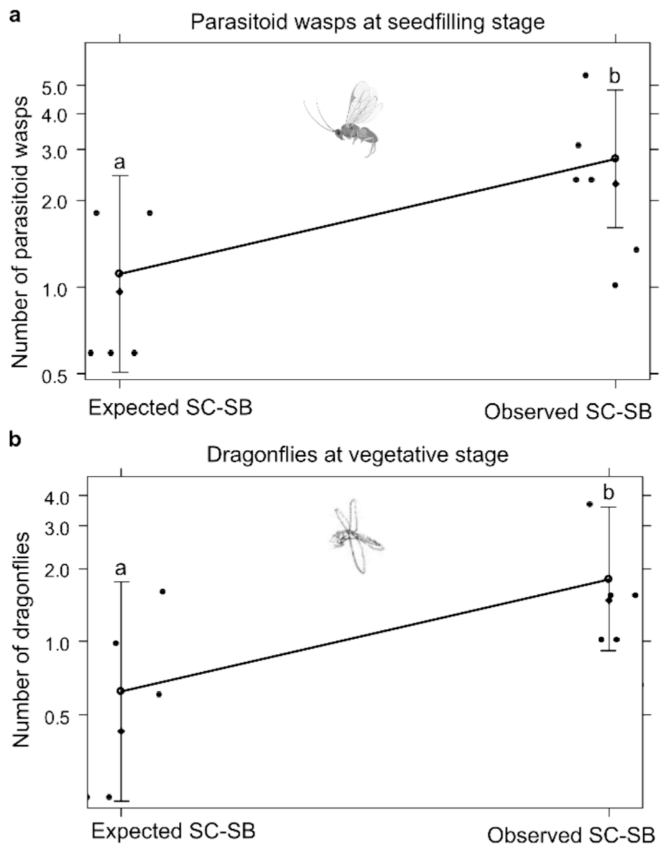

Fig. 6. The expected against observed values of parasitoid wasps and dragonflies in SC and SB. **a)** the comparison of predicted abundance of parasitoid wasps between expected value in SC and SB against the observed value in SC-SB in seedfilling stage. **b)** the comparison of predicted abundance of dragonflies between the expected value in SC and SB against the observed value in SC-SB in the vegetative stage. Error lines represent confidence intervals from the models and the black dots are the predicted numbers. The letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

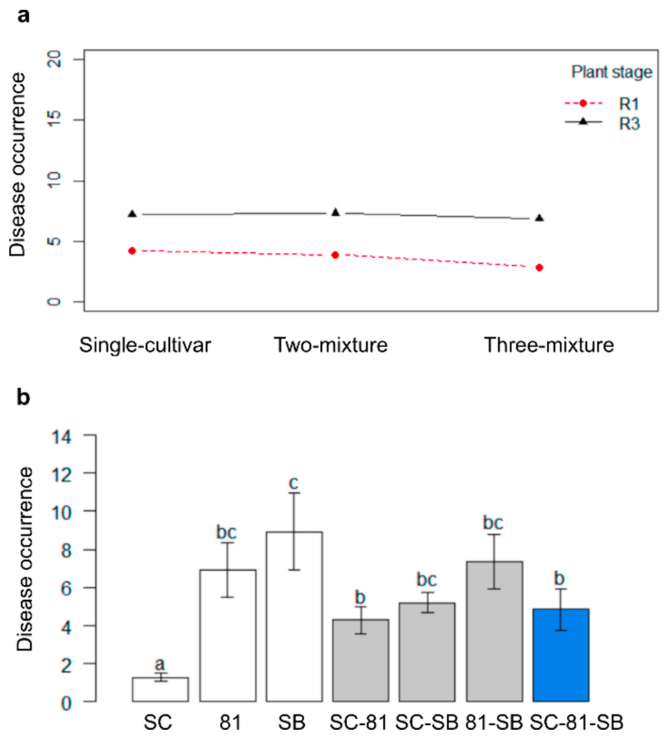

Fig. 7. Leaf disease occurrence in the field. **a)** The different interaction effects of disease occurrence in single-cultivar, two-mixture and three-mixture at reproductive stages (R1 and R3). **b)** Leaf disease occurrence in SC: Santa Cruz, 81: 98C81, SB: Sbung, SC-81: Santa Cruz-98C81, SC-SB: Santa Cruz-Sbung, 81-SB: 98C81-Sbung, SC-81-SB: Santa Cruz-98C81-Sbung. Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

settings in response to plant volatile chemical cues (Glinwood et al., 2009; Ninkovic et al., 2011). To the best of our knowledge, no previous study has demonstrated that different soybean cultivars may release distinct volatile profiles. Hence, future study should quantify the volatile profiles of individual cultivars and mixtures, and examine their potential effects on natural enemies. The observed significant increases of dragonfly and parasitoid wasp abundance in the mixtures of SC-SB over the individual cultivars suggest that these two cultivars hold a potential interactive effect in attracting natural enemies in the field. It is possible that plant-plant communication, via volatile interactions in cultivar mixtures, could be a mechanism that contribute to the increased abundance of natural enemies, confirming the bottom-up effects on natural enemy abundance (Ninkovic et al., 2006). Our study provides additional evidence that increasing plant diversity through cultivar mixtures can influence the abundance of certain natural enemy groups at different plant stages, contributing to the effective selection of cultivars for mixing to enhance natural enemy attraction. Therefore, the observed increased abundance of certain natural enemies could result in top-down effects on insect pests in more genetically diverse cultivar mixtures.

Cultivar mixtures have been reported to reduce insect pest pressure (Dahlin et al., 2018; Nboyine et al., 2021; Pan and Qin, 2014; Shoffner and Tooker, 2013; Snyder et al., 2020; Tooker and Frank, 2012), while our results do not show any consistent mixture effects on insect pests in soybean field. Similarly, cultivar mixtures provided inconsistent effects on herbivore abundance in soybean field (Grettenberger and Tooker, 2020), and no reduction of aphid populations in specific wheat field (Mansion-Vaquié et al., 2019) and barley field (Dahlin et al., 2018). The levels of biotic pressure have been recognized to affect herbivore control (Huang et al., 2012; Power, 1991). Our findings do not show a direct correlation between pests and overall abundance of natural enemies that would indicate a top-down control in our study system. The inconsistent mixture effects and the lack of top-down effects in cultivar mixtures on insect pests could be due to the low insect pest populations in this study. The observed low pest populations could be due to the agricultural landscapes (diversity of surrounding crops: cassava, bean, and maize), where the fields were surrounded by diverse crops, suggesting to affect herbivores (Bianchi, 2022; Kheirodin et al., 2023). The populations of insect pests could be altered, possibly due to the alterations in plant defensive response, pest movement, or predator attraction at diverse population levels (Grettenberger and Tooker, 2020). Alternatively, the observed inconsistent mixture effects on insect pests in this study could be due to the lack of interaction effects between selected cultivars. If the insect pest population were high, the effects of cultivar mixtures on pests could result in different outcomes. However, this is still uncertain due to the lack of research. Our study indicates the inconsistent effects of soybean cultivar mixtures on insect pest control, and outlines the need for further studies in higher insect pest pressure and in identifying cultivars with potential for the interaction effects of specific cultivar mixtures.

4.2. *The lacks of mixture effects on diseases and yield*

The diverse properties of individual cultivars contribute to the wide range of trade-off effects on insect pests, natural enemies, diseases and yield. While cultivar mixtures can reduce the severity of the disease (Vidal et al., 2020), we did not observe any such effects on the occurrence of disease symptoms in our study. It has been reported that the

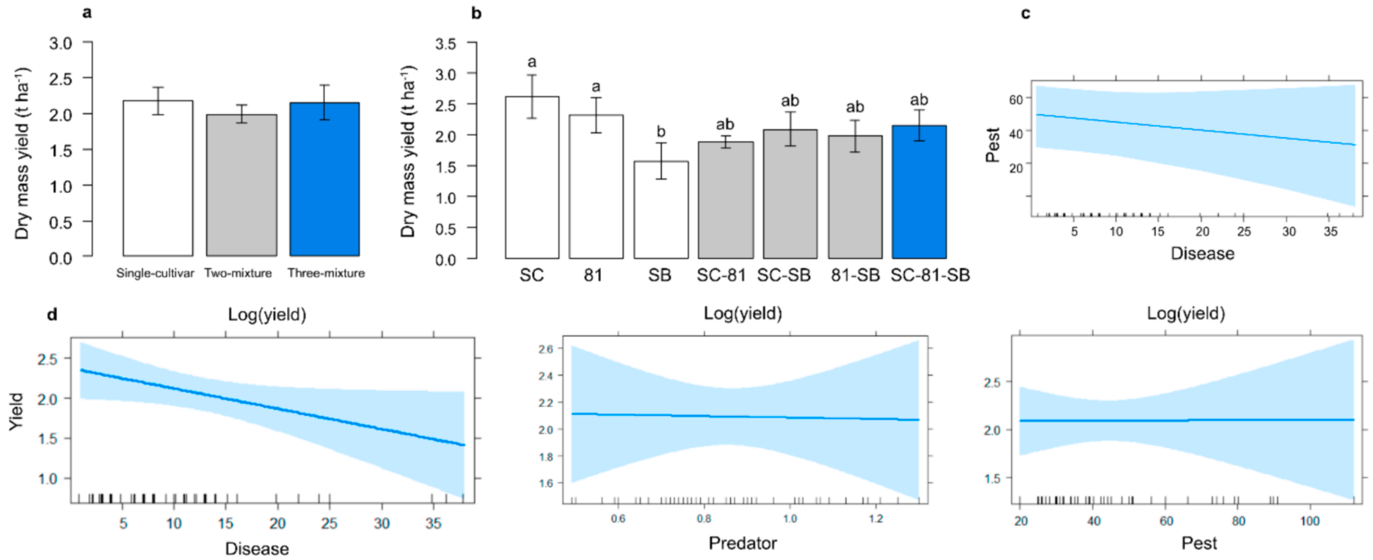

Fig. 8. Dry mass of soybean yield (in tons per hectare). **a)** Dry mass of soybean yield in single-cultivar, two-mixture and three-mixture. **b)** Dry mass of soybean yield in each of treatments. **c)** The correlation effects between insect pest and disease. **d)** The correlation effects between yield with disease, predator, and pest. SC: Santa Cruz, 81: 98C81, SB: Sbung, SC-81: Santa Cruz-98C81, SC-SB: Santa Cruz-Sbung, 81-SB: 98C81-Sbung, SC-81-SB: Santa Cruz-98C81-Sbung. Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses.

disease severity is strongly related to the proportion of susceptible cultivars in the mixtures (Dai et al., 2012; Mundt, 2014; Vidal et al., 2020), which should optimally not exceed one-third to a quarter of susceptible plants (Gigot et al., 2014). Out of the tested cultivars, two were found to be significantly more susceptible than the third one. The proportion of susceptible cultivars in the cultivar mixtures exceeded a quarter, which could explain the absence of a mixture effect. The lack of observed effects may be attributed to the limited disease evaluation method employed in our study. We only surveyed the observed disease symptoms on the soybean leaves, which may have resulted in an incomplete assessment. Therefore, further investigation is required to assess specific types of soybean diseases and disease ratings specific for each disease.

Cultivar mixtures can improve yield production and stability (Reiss and Drinkwater, 2018; Tooker and Frank, 2012); however, our study do not show any mixture effects in soybean yield. Notably, the commercial cultivars have higher yield than the traditional one, which in combination with low disease susceptibility indicates that the use of traditional cultivars is likely to be less profitable. However, the different factors that may contribute to high disease occurrence on SB cultivar must be considered, probably due to the poor hygiene measures during seed harvesting, handling and storing. To better understand the pros and cons of using traditional cultivars, further investigation is needed to determine whether the high disease occurrence is due to the genetic properties of the cultivar or due to other factors. Our observed negative correlation between disease and yield indicates that it is crucial to critically assess soybean diseases in order to determine the effects of cultivar mixtures on diseases, thereby affecting yields. The effective selection of cultivars to be mixed could potentially uncover the potentials of cultivar mixture effects on biological pest and disease control in soybean cropping system. As this study only conducted in one-year field experiment and one location, we were not able to assess whether the mixture effect can influence over multiple years, locations and cropping systems. Therefore, future studies should be expanded to include multiple years, locations or other cropping systems to effectively determine the potential effects of cultivar mixtures.

5. Conclusion

This study indicates that cultivar mixtures enhance natural enemy abundance, inconsistently affect insect pests, and do not alter diseases and yields in a tropical soybean cropping system. Our study suggests that increasing crop diversity through three-cultivar mixtures and two specific cultivar mixtures can attract certain groups of natural enemies at specific plant stages. Our result further provides little support for using cultivar mixtures to control insect pests, including whiteflies in certain plant stage. The current study further contributes new knowledge on the trade-offs between pest and disease management and crop yield, when combining different soybean cultivars. The findings indicate that it could be challenging to control insect pests and diseases to enhance yields by using cultivar mixtures, and it is required significant scientific understandings and strategic development in developing cultivar mixtures as a feasible strategy for biological pest and disease control. To our best of knowledge, this is the first report on the effects of cultivar mixtures of soybean cropping system in Cambodia, and it also adds new literatures on the investigation of potential roles of cultivar mixtures in modern agriculture. This study provides a milestone to expand the scientific understanding of soybean cultivar mixtures for sustainable agriculture and crop protection in a tropical cropping system and beyond.

Declarations

Ethical approval

This study does not contain any studies with human participants or large animals performed by any of the authors. No approval of research ethics committees was required to accomplish the goals of this study because this study was conducted with insects and crop plants.

6. Authors' contributions

SK and VN designed the study. SK conducted the field experiment and collected all field data. SK, DR and VN analysed the data. SK wrote the first draft of manuscript. SK, DR, DM, SI, OU, SS, and VN edited the manuscript. All authors read, contributed to revisions and approved the manuscript.

7. Availability of data and materials

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

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CRediT authorship contribution statement

Sokha Kheam: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Diana Rubene:** Writing – review & editing, Supervision, Formal analysis. **Dimitrije Markovic:** Writing – review & editing, Supervision. **Saveng Ith:** Writing – review & editing, Supervision. **On Norong Uk:** Writing – review & editing. **Soth Soung:** Writing – review & editing. **Velemir Ninkovic:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105571.

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