Article

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Comparative Effects of *Glycine max* and *Glycine soja* Leaves on *Clanis bilineata tsingtauica* Rearing Performance

Ping Zhao [1,†], Chen Meng [2,†], Syeda Wajeeha Gillani [2], Xueli Lu [2], Xi Jia [3], Meng Wang [4], Yu Bai [4], Yiru Song [4], Hongyan Hou [3], Yiqiang Li [2], Lu Wang [3,*] and Zongchang Xu [2,*]

[1] Jilin Provincial Key Laboratory of Plant Resource Science and Green Production, Jilin Normal University, Siping, Jilin Province, 136000, China

[2] Qingdao Key Laboratory of Coastal Saline-alkali Land Resources Mining and Biological Breeding, National Center of Technology Innovation for Comprehensive Utilization of Saline-Alkali Land, Marine Agriculture Research Center, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266100, China

[3] Yellow River Delta Modern Agriculture Research Institute, Shandong Academy of Agricultural Sciences, Dongying 257091, China

[4] College of Agronomy, Qingdao Agricultural University, Qingdao, 266109, China

* Correspondence: 18660191518@163.com (L.W.); xuzc1110@163.com (Z.X.)

Abstract

In China, the enormous gap between domestic soybean supply and increasing consumption necessitates large-scale soybean imports. The use of cultivated soybean (*Glycine max*) leaves as feed for the edible insect *Clanis bilineata tsingtauica* further reduces crop yields, posing a threat to national soybean production security. To address this issue, this study evaluated wild soybean (*Glycine soja*) as a potential alternative feed source. Comparative analyses examined the nutritional and anti-nutritional properties of *G. max* (cv. Qihuang34) and laboratory-preserved *G. soja* germplasm, together with their effects on larval growth performance, nutritional composition, and associated microbiota. *G. soja* leaves exhibited significantly higher crude fat (5.61% vs. 2.17%), ash (11.07% vs. 9.62%), neutral detergent fiber (23.75% vs. 21.00%), calcium (4.05 g/kg vs. 3.41 g/kg), and phosphorus (2.52 g/kg vs. 2.38 g/kg) than *G. max*, alongside lower trypsin inhibitor levels ($P<0.01$) despite higher phytic acid content ($P<0.05$). Fifth-instar larvae reared on *G. soja* leaves achieved a 12.9% increase in body weight (6.846 g vs. 6.066 g), higher crude protein (672.14 g/kg vs. 555.02 g/kg), total soluble sugar (21.27 mg/g vs. 8.96 mg/g), and soluble protein (26.35 mg/g vs. 24.71 mg/g), but lower crude fat (187.44 g/kg vs. 205.82 g/kg, $P<0.05$). 16S rRNA sequencing revealed distinct phyllosphere microbial communities, with *G. soja* enriched in diverse taxa (e.g., *Bacteroidota*, *Proteobacteria*) and *G. max* dominated by *Firmicutes*. Corresponding differences were observed in larval gut microbiota, with positive correlation analyses suggesting potential microbe transfer from *G. soja* leaves to larval guts. Overall, *G. soja* represents a promising alternative feed for *C. bilineata*, reducing competition with soybean grain production and supporting sustainable insect farming.

Keywords: wild soybean; nutritional ingredient; intestinal microflora; phyllosphere microbiota; edible insect

1. Introduction

Rapid population growth has intensified global demand for sustainable food sources to ensure long-term food security [1]. Edible insects have attracted increasing attention due to their short

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rearing cycle, efficient food conversion, and high protein content [2]. As a result, they are considered a promising alternative animal protein source capable of meeting rising global protein demand [3,4]. Consistent with this trend, the global market for edible insects continues to expand year after year [5].

Clanis bilineata tsingtauica, a member of the family Sphingidae within the order Lepidoptera, is a unique edible insect widely consumed in China [6,7]. The larvae, which feed on nutrient-rich soybean (*Glycine max*) leaves, are known for their high protein content, unsaturated fatty acids (UFAs), and vitamins [8], associated with brain development, protection against cellular degeneration, and endocrine regulation in humans. However, stable industrial production is compromised by challenges such as pest outbreaks, disease incidence, and photoperiod sensitivity, hindering constant supply.

Feeding behavior in phytophagous insects is strongly influenced by the nutritional composition of host plants [9]. Host nutrient profiles determine insect growth, development, and ultimately, their nutritional quality. Numerous species exhibit nutrition-driven host selection, such as the western flower thrip (*Frankliniella occidentalis*) shifting to nutrition-rich pollen [10] and *Bradysia spp.* preferring protein-rich chives (*Allium tuberosum*) and broad beans (*Vicia faba*) [11]. Nitrogen-rich plants are also favored by species such as gypsy moth (*Lymantria dispar*) [12,13].

Microbial communities associated with phyllosphere and insect guts are integral for understanding nutrient metabolism, immune regulation, and host-microbe interactions [14,15]. The phyllosphere harbors diverse microbial taxa that modulate plant health and nutrient composition [16,17]. Once food enters the insect body, gut-colonizing microorganisms are closely linked to nutrient assimilation and physiological processes [18]. Gut bacteria have been shown to influence insects' host-plant preference, assisting them in identifying suitable plants for survival and reproduction [19]. Furthermore, the feeding characteristics of insects can significantly alter the structure of their gut microbiota, which in turn affects their growth and development [20]. Recent microbiological studies highlight the pivotal role of diet and environmental factors in the formation of the insect gut microbiota [21,22].

Wild soybean (*Glycine soja*), the progenitor of cultivated soybean (*Glycine max*) [23], is characterized by strong stress resistance, high adaptability, and a wide geographic distribution [24,25]. Its leaves contain higher concentrations of major free sugars, including glucose and sucrose, than those of cultivated soybean [26]. To evaluate the potential of *G. soja* as an alternative feed source for *C. bilineata*, this study compared the nutritional and anti-nutritional profiles of both soybean types and assessed their effects on *C. bilineata* larval performance and gut microbiota. Differences in phyllosphere microbial communities between the two soybean species were also examined, providing foundational insights for microbial regulation strategies aimed at enhancing *C. bilineata* production.

2. Results

2.1. Leaf Nutritional Components of G. soja and G. max

Leaf nutritional composition serves as a key indicator of forage quality. To compare wild (*G. soja*) and cultivated soybean (*G. max*), we quantified the nutritional components of their leaves. As summarized in Table 1, *G. max* leaves contained 2.17% crude fat, whereas *G. soja* leaves exhibited an approximately 2.58 times higher crude fat content than *G. max* ($P<0.01$). Ash, neutral detergent fiber, and Ca contents were also significantly higher in *G. soja* leaves ($P<0.01$). P content was also significantly higher in *G. soja* leaves than in *G. max* leaves ($P<0.05$). Conversely, crude fiber content was significantly higher in *G. max* leaves ($P<0.01$). No significant differences were observed in crude protein or acid detergent fiber between the two soybean types. Overall, these findings indicated that *G. soja* leaves possess a relatively stable and superior nutritional profile across several key indicators.

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Table 1. Nutritional components comparison of cultivated soybean and wild soybean leaves.

Indicators	Cultivated soybean leaf	Wild soybean leaf
Crude fat (%)	2.17±0.19	5.61±0.29**
Crude protein (g/kg)	242.51±0.73	240.73±1.35
Crude fiber (%)	9.68±0.29**	7.30±0.27
Ash content (%)	9.62±0.07	11.07±0.04**
Neutral detergent fiber (%)	21.00±0.38	23.75±0.39**
Acid detergent fiber (%)	13.21±2.78	14.24±1.80
P (g/kg)	2.38±0.05	2.52±0.02*
Ca（g/kg）	3.41±0.03	4.05±0.02**

Note: **, $P<0.01$; *, $P<0.05$.

2.2. Main Anti-Nutritional Components in Leaves of G. soja and G. max

Anti-nutritional factors can interfere with nutrient digestion and absorption in plants consumed by herbivorous insects such as *C. bilineata*. We quantified two key anti-nutritional factors, trypsin inhibitor and phytic acid, in leaves of both soybean types. Trypsin inhibitor levels was markedly lower in *G. soja* than in *G. max* ($P<0.01$, Figure 1A). In contrast, phytic acid content was significantly higher in *G. soja* leaves ($P<0.05$, Figure 1B). These findings suggest a distinct anti-nutritional profiles between the two species, which may differentially influence nutrients bioavailability when consumed by *C. bilineata*.

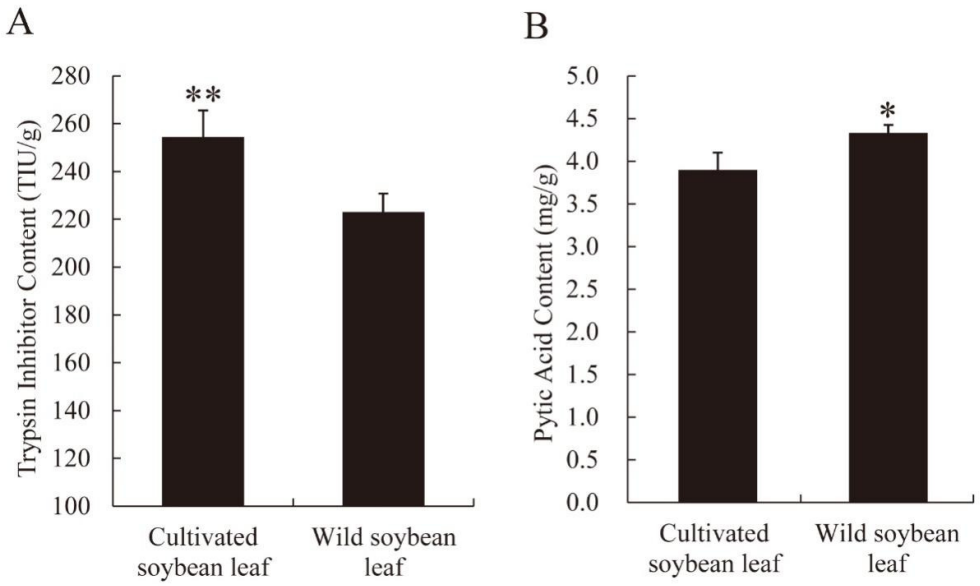

Figure 1. The main anti-nutritional factors of cultivated soybean leaves and wild soybean leaves. A, trypsin inhibitor content; B, phytic acid content. *, $P<0.05$; **, $P<0.01$.

2.3. Individual Weight and Nutritional Components of Fifth-Instar C.bilineata larvae

Dietary source significantly influenced the final body mass of *C. bilineata* larvae reared on *G. soja* reached a mean body mass of 6.846 g, which was significantly greater than the 6.066 g observed in larvae fed on *G. max*, representing a 12.9% increase (Figure 2A). The nutritional composition of the larvae was also significantly influenced by their dietary source. Larvae fed on *G. soja* leaves exhibited substantially higher crude protein content (672.14 g/kg) than those fed on *G. max* leaves (555.02 g/kg), corresponding to a 21.10% increase ($P<0.01$). Conversely, crude fat content was 9.81% lower in larvae fed on *G. soja* (187.44 g/kg) than in those fed on *G. max* (205.82 g/kg) ($P<0.05$). Total soluble sugar content was markedly higher in larvae reared on *G. soja* (21.27 mg/g) than in those reared on *G. max*

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(8.96 mg/g) (P<0.01). Total soluble protein was also slightly but significantly higher in the *G. soja*-fed group (26.35 mg/g) relative to the *G.* max-fed group (24.71 mg/g) (P<0.05). Furthermore, K content was significantly lower in larvae fed on *G. soja* (2.26 g/kg) than in those fed on *G. max* (2.44 g/kg) (P<0.01) (Table 2). Collectively, these results demonstrate that *G. soja* provides a more favorable nutritional foundation for larval development, reflected in enhanced protein assimilation and carbohydrate accumulation.

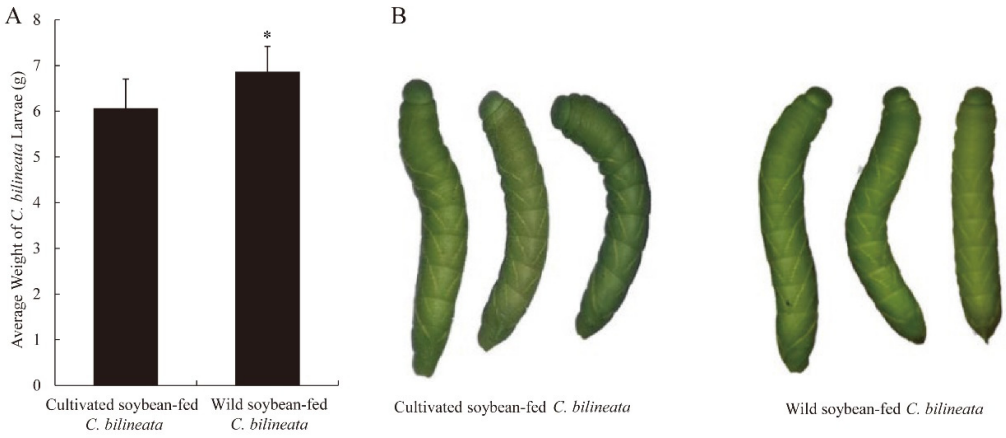

Figure 2. The average individual weight (A) and morphological comparison (B) of *C. bilineata* larvae fed by cultivated soybean and wild soybean leaves. Note: *, P＜0.05.

Table 2. The comparison of nutritional components on the fifth-instar of *C. bilineata* fed by cultivated soybean and wild soybean.

Indicators	*G. max*-fed *C. bilineata*	*G.soja*-fed *C. bilineata*
Crude protein（g/kg）	555.02±3.16	672.14±3.88**
Crude fat content（g/kg）	205.82±4.62*	187.44±2.75
Total soluble sugar（mg/g）	8.96±0.36	21.27±0.45**
Total soluble protein (mg/g)	24.71±0.65	26.35±0.49*
K (g/kg)	2.44±0.01**	2.26±0.01

Note: **, P＜0.01; *, P＜0.05.

2.4. Phyllosphere and Gut Microbiota of Fifth-Instar C.bilineata larvae

Microbial community profiling was performed on leaves of both soybean types and on the excreta of fifth-instar *C.bilineata* larvae feeding on these hosts. Across all samples, a total of 423 OTUs were identified and classified at the genus level (Supplementary Table S1). PCA analysis demonstrated clear separation between the gut microbial communities of larvae fed on *G. max* versus. *G. soja* and among the phyllosphere microbiota of both plant types (Figure 3A), indicating distinct community structures. Heatmap analysis of the top 50 genera further revealed distinct these differences, showing pronounced divergence in microbial composition both between the two plant phyllospheres and between the corresponding larval gut microbiota(Figure 3B). These results indicate that the two host plants recruit distinct phyllosphere microbial communities, which are reflected in the differentiated the gut microbial community composition of the *C. bilineata* larvae feeding on each host plant. The divergence in host-associated microbial assemblages may contribute to the observed differences in larval nutritional profiles and growth performance.

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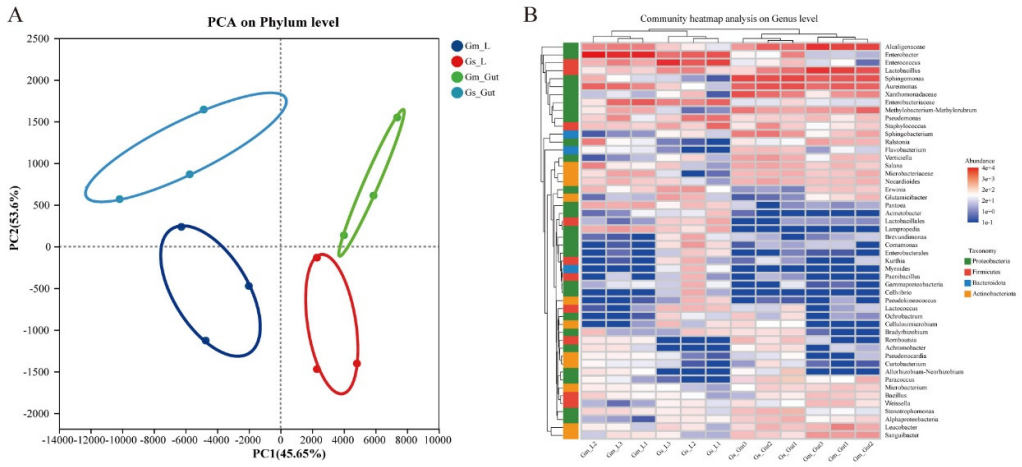

Figure 3. PCA Analysis (A) and Clustering Heatmap Analysis (B) of Phyllosphere Microbiota from Cultivated Soybean and Wild Soybean Leaves, and Gut Microbiota from *C. bilineata* Larvae Fed on These Two Plant Species. Note: Gm_L denotes cultivated soybean leaves; Gs_L denotes wild soybean leaves; Gm_Gut denotes the gut microbiota of *C. bilineata* larvae fed on cultivated soybean leaves; Gs_Gut denotes the gut microbiota of *C. bilineata* larvae fed on wild soybean leaves.

2.5. Comparative Analysis of Phyllosphere Microbiota of G. soja and G. max Leaves

Linear discriminant analysis effect size (LEfSe) with an LDA threshold > 2.0 was used to identify differentially abundant phyllosphere microorganisms between *G. soja* and *G. max* leaves (Figure 4).

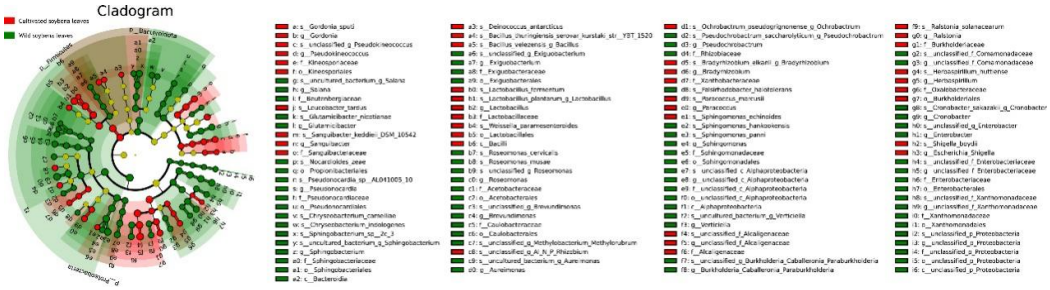

Figure 4. Phylogenetic Tree of Phyllosphere Microbiota Differences Between cultivated soybean and wild soybean.

The analysis revealed that *G. soja* leaves exhibited enrichment across a broader range of microbial taxa at multiple taxonomic levels compared with *G. max* leaves. Specifically, *G. soja* leaves were enriched with 2 phyla (*Bacteroidota*, *Proteobacteria*), 2 classes (*Alphaproteobacteria*, *Bacteroidia*), 9 orders (e.g., *Acetobacterales*, *Enterobacterales*, *Sphingomonadales*), 10 families (e.g., *Acetobacteraceae*, *Enterobacteriaceae*, *Sphingomonadaceae*), 14 genera (e.g., *Aureimonas*, *Brevundimonas*, *Sphingomonas*), and 13 species (e.g., *Chryseobacterium indologenes*, *Cronobacter sakazakii*, *Sphingomonas hankookensis*).

In contrast, *G. max* leaves were enriched with a comparatively narrower range of taxa, including one phylum (*Firmicutes*), one class (*Bacilli*), three orders (*Burkholderiales*, *Kineosporiales*, *Lactobacillales*), seven families (e.g., *Burkholderiaceae*, *Lactobacillaceae*), nine genera (e.g., *Bradyrhizobium*, *Lactobacillus*, *Ralstonia*), and 16 species (e.g., *Bacillus velezensis*, *Lactobacillus plantarum*).

A notable enrichment of unclassified or uncultured microbial taxa was observed in the *G. soja* phyllosphere. At the class level, *G. soja* leaves harbored unclassified taxa such as *c_unclassified_p_Proteobacteria*, with this trend extending to 2 unclassified orders, 2 unclassified families, 5 unclassified genera, and 15 unclassified or uncultured species. Conversely, such

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unclassified taxa were scarce in *G. max* leaves, appearing only at the genus (1 taxon) and species levels (3 taxa).

2.6. Comparative Analysis of Gut Microbiota in C. bilineata Fed on G. soja and G. max Leaves

LEfSe with an LDA threshold > 2.0 was performed to identify differentially abundant intestinal microbes in *C. bilineata* fed *G. soja* versus *G. max* leaves (Figure 5). The gut microbiota of larvae fed *G. soja* leaves exhibited significant enrichment across a broader range of taxa, including 2 phyla (*Bacteroidota* and *Firmicutes*), 2 classes (*Bacilli* and *Bacteroidia*), 5 orders (*Cellvibrionales*, *Flavobacteriales*, *Lactobacillales*, *Sphingobacteriales*, and *Staphylococcales*), 7 families, 10 genera (including *Sphingobacterium*), and 8 species, several of which were also enriched in the *G. soja* phyllosphere. In contrast, the gut microbiota of larvae fed *G. max* leaves was enriched with a more limited set of taxa, including one phylum (*Proteobacteria*), one class (*Gammaproteobacteria*), one order (*Peptostreptococcales-Tissierellales*), 4 families (*Alcaligenaceae*, *Burkholderiaceae*, *Lactobacillaceae*, and *Streptococcaceae*), 5 genera (including *Lactobacillus* and *Ralstonia*), and 3 species (including *Ralstonia solanacearum*), several of which were also enriched in the *G. max* phyllosphere.

A notable enrichment of unclassified or uncultured taxa was observed in the gut of larval fed on *G. soja*, spanning all taxonomic levels, including 1 unclassified phylum (*p_unclassified_d_Bacteria*), 1 unclassified class, 2 unclassified orders, 3 unclassified families, 4 unclassified genera, and 10 unclassified or uncultured species. Conversely, the gut of larvae fed on G. max leaves contained substantially fewer unclassified taxa, limited to 2 genera and 3 species.

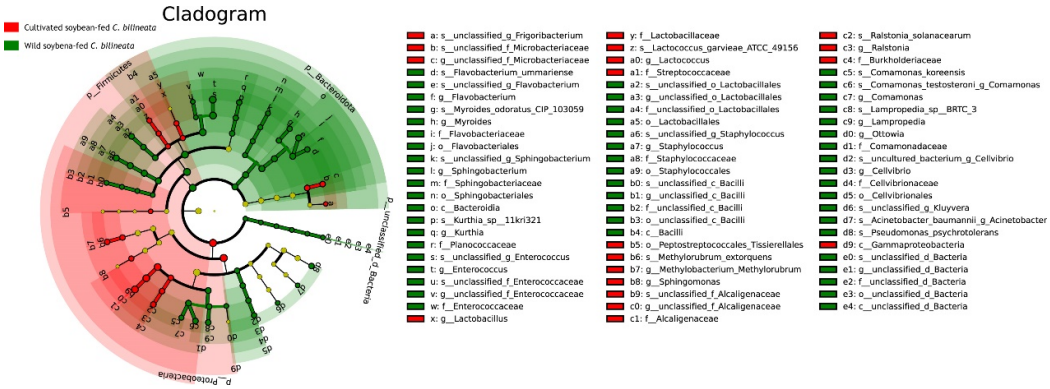

Figure 5. Phylogenetic Tree of Gut Microbiota Differences Between *C. bilineata* Larvae Fed on cultivated soybean and wild soybean.

2.7. Potential Transmission and Synergistic Interactions Between Gut and Phyllosphere Microbiota of C. bilineata

Furthermore, correlation network analysis revealed distinct correlations between the gut microbiota of *C. bilineata* and the phyllosphere microbiota of the consumed leaves at the OTU level (Figure 6). The network comprised five modules, with Module 1 comprising 17 nodes (5 phyllosphere and 12 gut OTUs) and Module 2 containing 25 nodes (5 phyllosphere and 20 gut OTUs), while the remaining three modules each consisted of two nodes.

In contrast, negative correlations were mainly observed within Module 1 and primarily occurred between phyllosphere-phyllosphere or gut-gut OTUs, likely reflecting competitive interactions among microorganisms inhabiting same ecological niches. Overall, correlations between phyllosphere and gut microbiota were predominantly positive, indicating potential synergistic or co-enrichment effect between leaf-associated and gut-associated microbial communities (Figure 6).

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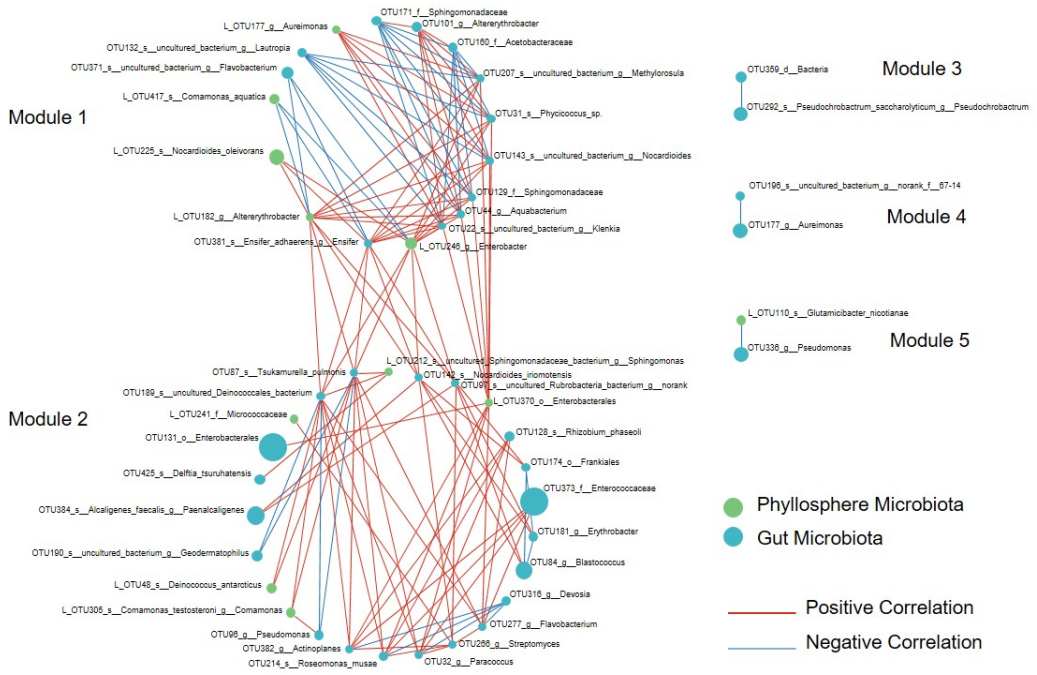

Figure 6. Correlation Network Analysis of Phyllosphere Microbiota and Psilogramma menephron Gut Microbiota. Predominant 2. Notably, strong positive correlations between *L_OTU370_o__Enterobacterales* and *OTU131_o__Enterobacterales*, as well as between *L_OTU48_s__Deinococcus_antarcticus* and *OTU189_s__uncultured_Deinococcales_bacterium*, suggest potential transmission of bacterial taxa from the diet to the larvae gut.

3. Discussion

Plant nutrients play a crucial role in insect growth and development [36]. Among these nutrients, protein serves as a key determinant of performance in phytophagous insects, which tend to prefer host plants with higher protein content to maximize nutrient acquisition from limited food intake [37]. Consistent with this pattern, feeding on rice plants with higher nitrogen content enhances survival and shortens developmental duration in the brown planthopper (*Nilaparvata lugens*) [38]. In the present study, *G. soja* leaves contained significantly greater content of crude fat, crude fiber, ash, neutral detergent fiber, acid detergent fiber, P, and Ca than *G. max* leaves, whereas crude protein contents did not differ significantly between the two soybeans types (Table 1).

Proteolytic enzymes in phytophagous insects vary widely in prevalence and catalytic efficiency across taxa and feeding strategies [39]. Plant-derived trypsin inhibitors can irreversibly bind intestinal trypsin, forming inactive complexes that disrupts protein digestion, absorption, and utilization [40,41]. In addition, protease-inhibitor complexes may act as negative feedback signals that further suppress insect feeding [42]. Together, these effects impair dietary protein utilization and overall food intake, ultimately hindering insect development and leading to mortality due to protein deficiency. Phytic acid, naturally present in legumes, reduce the bioavailability of essential minerals, amino acids, and proteins through chelation and complex formation [43]. Its degradation can release phosphate that becomes available to the host or gut microbiota, while phytic acid also plays a defensive role against phytophagous insects [44]. Our results showed that *G. soja* leaves contained lower levels of trypsin inhibitors but higher phytic acid content than *G. max* leaves. Correspondingly, larvae feeding on *G. soja* leaves exhibited higher average body weight and elevated crude protein, total soluble sugar, and soluble protein contents, potentially reflecting the combined effects of enhanced nutrient availability and reduced protease inhibition.

Phyllosphere-associated microbial communities play critical roles in plant productivity and ecological interactions. In our study, the *G. soja* phyllosphere possessed a stronger capacity for

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microbial recruitment, sustaining a more diverse and taxonomically rich community, including a substantial proportion of rare or poorly characterized taxa. In contrast, the *G. max* phyllosphere was dominated by fewer, more readily identifiable microorganisms. This difference may reflect variation in leaf-derived metabolites and volatiles that shape microbial colonization niches. Bidirectional transfer of bacteria between plant phyllospheres and insects has been documented previously [45–47]. The plant phyllosphere bacteria ingested by insects can colonize insect guts to facilitate detoxification, nutrient acquisition, and survival [48]. In larvae fed on *G. soja* leaves, consistent microbial transfer from leaf to gut was observed, spanning taxonomical levels from the phylum *Bacteroidota* to the genus *Sphingobacterium*, both of which were enriched in the *G. soja* phyllosphere. A similar, though more limited, pattern was evident in larvae fed on *G. max* leaves. Our findings reflect the disparities observed in phyllosphere microbiota, indicating a parallel divergence in the gut microbiota of larvae feeding on these leaves.

Together, these findings indicate significant microbial transfer along the food chain from both *G. soja* and *G. max* leaves to the gut of *C. bilineata* larvae. The rich and diverse phyllospheric microbial community influences the compositional diversity of the intestinal microbiota in *C. bilineata* larvae, underscoring a direct dietary impact on gut microbial assembly.

4. Materials and Methods

4.1. Plant Material Preparation

The cultivated soybean (*G. max*) variety was Qihuang 34, while the wild soybean (*G. soja*) consisted of homozygous lines originally collected from Dongying and maintained in the laboratory [27]. The experiment was conducted beginning in early June 2024 at the Yellow River Delta Modern Agriculture Research Institute, Shandong Academy of Agricultural Sciences, Shandong, China. Both soybean types were grown under standard field management in plots of approximately 50 m^2, using 0.6 m row spacing and 0.15 m intra-row spacing. One week before releasing *C. bilineata* eggs, each plot was covered with insect-proof nets (Supplementary Figure S1) to prevent larval predation.

4.2. Inoculation of C. bilineata larvae

Forty days after sowing when plants had developed sufficient biomass), commercially purchased *C. bilineata* eggs were attached to the abaxial surface of *G. max* and *G. soja* leaves. One egg cluster (30-50 eggs) was placed per m^2, allowing natural hatching (Supplementary Figure S1).

4.3. Plant Material Sampling

The developmental progression of *C. bilineata* larvae in each plot was monitored regularly. When approximately 70% of larvae reached the fifth instar, fully expanded upper young leaves from *G. max* and *G. soja* were collected. Field observations indicated that *C. bilineata* predominantly feeds on young upper leaves; therefore, these leaves were used for nutritional component analysis and phyllosphere microbial sequencing. For both *G. max* and *G. soja* plots, samples were collected from three-density larval locations. Immediately after sampling, leaves were flash-frozen in liquid nitrogen and stored at -80 °C for subsequent analyses.

4.4. C. bilineata larvae and Feces Sampling

Sampling of *C. bilineata* larvae and their feces began three days after leaf collection. Fifth-instar larvae were collected from high-density areas in both *G. max* and *G. soja* plots. At each of three independent sampling sites per plot, 6-10 larvae were collected. Larvae were immediately flash-frozen in liquid nitrogen and stored at -80 °C. Fresh feces were collected from the same locations, flash-frozen in liquid nitrogen and stored at -80 °C for further experiments.

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4.5. Determination of Leaf and Larval Indicators

The determination of leaf nutritional components (crude fat, crude protein, crude fiber, ash, neutral detergent fiber, acid detergent fiber, phosphorus (P), and calcium (Ca) and larval nutritional indicators (crude protein, crude fat, soluble sugar, soluble protein, and potassium (K) followed the procedures described by Li et al. (2011) [28]. Phytic acid and trypsin inhibitor contents were determined following Liu et al. (2020) [29], with minor modifications. In brief, 5 g of leaf tissue was extracted with 40 mL of sodium sulfate-hydrochloric acid solution on a shaker for 4 h. The mixture was centrifuged at 8000 rpm for 5 min, and the supernatant was adjusted to 50 mL with the same solution and then filtered. For phytic acid purification, 2 mL of extract was mixed with 2 mL of 15% trichloroacetic acid (TCA) solution and incubated at 4 °C for 2 h. After centrifugation, 2 mL of the supernatant was adjusted to pH to 6.0-6.5 with 1 mol/L NaOH and diluted to 30 mL with distilled water. Standard curve preparation and quantification followed national standard procedures (GB5009.153-2016). For trypsin inhibitor activity, 1 g of leaf tissue was extracted with 50 mL of 0.01 mol/L NaOH solution, adjusted to pH to 9.5 ± 0.1 using 0.1 mol/L hydrochloric acid (HCl) solution, and incubated at 4 °C for 24 h. The extract was equilibrated at 25 °C, diluted to 100 mL with water, and analyzed using the national standard method (GB 5009.224-2016). For each analytical parameter, the measurements were performed in triplicate.

4.6. Microbial Sequencing and Related Analyses

Total microbial genomic DNA was extracted from leaf and fecal samples using *EasyPure®* Genomic DNA Kit (Transgen, EE101-01). The V5-V6 region of the bacterial 16S rRNA gene was amplified with primers 799F (5′-AACMGGATTAGATACCCKG -3′) and 1193R (5′-ACGTCATCCCCACCTTCC -3′) [30]. Amplified PCR products were pooled in equimolar amounts, and a DNA libraries were constructed using the SMRTbell prep kit 3.0 (Pacifc Biosciences, CA, USA). Sequencing was performed on the PacBio Sequel IIe System (Pacifc Biosciences, CA, USA) by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). High-fidelity (HiFi) reads were generated using circular consensus sequencing via SMRT Link v11.0. Optimized HiFi reads were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 [31,32] at a 97% sequence similarity threshold. The most abundant sequence in each OTU was selected as the representative sequence, and chloroplast sequences were removed during manual filtering. Taxonomic classification of each representative sequence was performed using the RDP Classifier version 2.2 [33] with a confidence threshold of 0.7. Metagenomic functions were predicted by PICRUSt2 [34] based on representative OTU sequences. Bioinformatic analyses of phyllosphere and gut microbiomes were carried out using the Majorbio Cloud platform (https://cloud.majorbio.com) [35].

4.7. Data Analysis

All data were subjected to one-way analysis of variance (ANOVA). Mean differences were compared using *Tukey's post hoc* test, with significant set at $P<0.05$ and $P<0.01$. Statistical analyses were performed using SPSS v 17.0 (SPSS Inc., Chicago, IL, USA).

5. Conclusions

This study evaluated the effects of nutrients, anti-nutritional factors, and phyllosphere microorganisms in wild (*G. soja*) and cultivated (*G. max*) soybean plants on larval nutrition and gut microbiota composition in *C. bilineata*. Compared with *G. max*, *G. soja* contained higher levels of nutrients, including crude protein and crude fat, as well as lower levels of plant trypsin inhibitors. Larvae fed on *G. soja* leaves exhibited higher biomass, protein content, and sugar content than those fed on *G. max* leaves. *G. soja* leaves exhibited a stronger capacity for microbial recruitment, supporting greater microbial diversity and abundance. Gut microbiota analysis revealed that, consistent with the differences in phyllosphere microorganisms between *G. soja* and *G. max*, larvae fed on the two leaf types also exhibited distinct gut microbial communities. Based on these results, *G. soja* plants may

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serve, to some extent, as a substitute for *G. max* in *C. bilineata* feeding. These results provide a scientific basis for the industrialization and commercialization of *C. bilineata* production.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

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