

Endophytic entomopathogenic fungi remodel phyllosphere microbiome and metabolome to suppress *Spodoptera frugiperda* and enhance parasitoid biocontrol

Zhengkai Yang

Guizhou University

Ziyu Han

Guizhou University

Xiao Zou

Guizhou University

Xiuxian Shen

Guizhou Tea Research Institute

Xiangyu Ji

Guizhou University

Dongsheng Li

Guizhou University

Yao Huang

Guizhou University

Yuanchan Yu

Guizhou University

Shimin Chen

Guizhou University

Feiran Wang

Guizhou University

Xiaofei Yu

Guizhou University

Mao-fa Yang

`mfyang@gzu.edu.cn`

Guizhou University

Keywords: Metabolome, Microbiome, Enzyme activity, *Microplitis manilae*

Posted Date: September 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10765463/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Spodoptera frugiperda severely damages maize and causes massive agricultural economic losses. Combining endophytic entomopathogenic fungi with natural enemies is a sustainable pest management approach. However, how these fungi modify plant physiology to affect *S. frugiperda* and regulate parasitoid performance remains unclear. Two endophytic entomopathogenic fungal strains (GL-5 and H8) were used. We detected fungal colonization effects on maize growth, leaf antioxidant enzyme activity, phyllosphere microbiota, and metabolome. We further investigated fungal-mediated changes in the development, reproduction, and detoxification metabolism of *S. frugiperda* and analyzed the tritrophic interaction with the parasitoid *Microplitis manilae*. Both strains significantly promoted maize seedling growth and disrupted *S. frugiperda* pupal development, prolonging development and suppressing pest population growth. GL-5 increased the parasitism rate and cocoon weight of *M. manilae*. Fungal colonization inhibited maize superoxide dismutase (SOD) and peroxidase (POD) activities. GL-5 activated larval glutathione S-transferase (GST), carboxylesterase (CarE), and cytochrome P450 (CYP450) detoxification pathways, while H8 enhanced acetylcholinesterase (AChE) activity and suppressed core detoxification systems. GL-5 enriched *Enterobacter* and induced flavonoid and phenolic acid accumulation; H8 recruited *Chryseobacterium* and regulated carbohydrate metabolism and alkaloid biosynthesis. Endophytic entomopathogenic fungi remodel maize phyllosphere microecology and metabolome, impairing pest detoxification and development and synergizing with natural enemies to suppress *S. frugiperda*. This study provides mechanistic support for eco-friendly fall armyworm management.

key message

λ Fungal colonization remodels the phyllosphere microbiome and metabolome, thereby impairing pest development and reproduction.

λ GL-5 increases the parasitism rate and cocoon weight of the parasitoid , enabling synergistic biological control.

λ Multi-omics analyses reveal strain-specific reprogramming of plant defense, offering eco-friendly IPM strategies against fall armyworm.

Introduction

Maize (*Zea mays* L.) is the second most widely cultivated crop globally, grown in over 170 countries, and ranks among the most important staple crops (Desika et al. 2024). Furthermore, maize is utilized for starch production and animal feed, rendering it industrially significant (Olaf et al. 2022). Fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797), is a major pest of maize. Native to the Americas, it spreads at an extremely rapid rate and has now invaded over 100 countries. As a highly destructive polyphagous pest, *S. frugiperda* poses a severe threat to maize production (Ganiger et al. 2018; Togola et al. 2025). To control maize pests, large quantities of chemical pesticides have been widely applied. However, their

overuse may trigger sustainability risks and public health hazards (Saroop and Tamchos 2024). At present, pest management strategies have shifted toward the use of biocontrol agents instead of hazardous chemical pesticides (Batool et al. 2022).

Since the discovery of endophytic colonization by entomopathogenic fungi (EPF), numerous studies have investigated their use in controlling plant pests and diseases (González-Mas et al. 2019; Russo et al. 2019). Endophytic colonization by entomopathogenic fungi has been shown to suppress insect pests across multiple crops, including sugar beet (Abdel-Raheem, 2005), maize (Garrido-Jurado et al. 2017), and potato (Zhang et al. 2023). The strategy of pest and disease control based on endophytic EPF provides an innovative perspective for integrated pest management (Quesada-Moraga, 2020).

Plant metabolites serve as defensive weapons against herbivores, interfering with insect survival and nutrient absorption (Guo et al. 2019; Qi et al. 2018). In response, herbivores deploy detoxification enzymes to eliminate plant-derived resistant compounds (Batool et al. 2020). However, plants are not passive targets, their defense mechanisms can be actively modulated by beneficial microbes. Indeed, entomopathogenic fungi have been shown to boost plant growth and enhance chemical defenses (Michereff et al. 2026; Imtiaz et al. 2020). For example, endophytic colonization by entomopathogenic fungi markedly elevates the levels of jasmonic acid, total flavonoids, tannins, and other metabolites in wheat, reducing the feeding efficiency and population fitness of *Rhopalosiphum padi* (Song et al. 2024). Such metabolic shifts also cover volatile organic compounds. Maize plants inoculated with *Beauveria bassiana* accumulate higher amounts of 2-ethyl-1-hexanol and 3-hexen-1-ol, altering oviposition preference in female *Ostrinia furnacalis* and decreasing the number of surviving larvae, pupae, and adults of this pest (Zhu et al. 2023).

Beyond growth promotion, endophytic microbes also modulate plant immune function by altering the composition of the plant microbiome (Wang et al. 2022; Qiao et al. 2023). For instance, maize inoculated with *Colletotrichum tofieldiae* exhibits reduced mycotoxin accumulation (Díaz-González et al. 2025). Furthermore, colonization by *Beauveria bassiana* can promote crop growth (Dash et al. 2018; Sánchez-Rodríguez et al. 2018).

Combined control against *Spodoptera littoralis* larvae using endophytically colonized *Metarhizium brunneum* together with the parasitoid *Hyposoter didymator* exhibited optimal efficacy relative to single-control treatments (Miranda-Fuentes et al. 2021). Endophytic colonization by *Beauveria bassiana* integrated with the parasitoid *Diglyphus isaea* achieved satisfactory control of pea leafminers (Akutse et al. 2014). Moreover, endophytic fungi modify plant volatile blends to improve the attractiveness of host plants to natural enemies (Pec et al. 2024; Pec et al. 2025). For example, sweet pepper plants inoculated with *Lecanicillium lecanii* emit indole, nerolidol and TMTT upon infestation by *Myzus persicae*, which strongly attracts aphid parasitoids (Wilberts et al. 2024). However, potential negative effects of endophytic fungi on insect natural enemies should be taken into consideration (Panwar et al. 2024). As an illustration, populations of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) were larger in plots

lacking endophytes compared with plots colonized by the endophyte *Neotyphodium* (Omacini et al. 2001).

We hypothesized that endophytic entomopathogenic fungi (GL-5 and H8) suppress *S. frugiperda* fitness and enhance *M. manila* parasitoid performance by modulating leaf metabolite profiles and phyllosphere microbiomes. As outlined in Fig. 1, our experimental framework sequentially evaluated fungal effects on maize growth, leaf metabolites, enzyme activities, and microbial communities; subsequently on pest growth, development, and reproduction; and finally on parasitoid parasitism rate, cocoon weight, emergence rate, and sex ratio. Multi-omics correlation analyses were then employed to link these observed changes. Overall, our findings support the use of fungal endophytes in integrated pest management against fall armyworm.

Materials and Methods

Study organisms

Maize cultivar Jinyu 579, *S. frugiperda*, parasitoid *M. manilae*, and two entomopathogenic fungal strains GL-5 (*Beauveria bassiana*) and H8 (*Cordyceps cateniannulata*) were used. Detailed information on plant cultivation, insect colony establishment, parasitoid rearing, fungal strain preservation, coordinates of sampling sites, culture medium, climate parameters, and supplier information is provided in Supplementary Material S1 (Liu et al. 2022; Tian et al. 2024; Ren et al. 2025; Guan et al. 2025).

Preparation and inoculation of fungal inoculum

The fungal inoculant was prepared following the method described by Wilberts et al. (2023). Briefly, fungal strains were cultured on half-strength Sabouraud Dextrose Agar (SDAY, Oxoid, UK) at a constant temperature of 25°C for 7 d. Tween 80 was then added, and conidia were gently scraped from the colony surface using a sterile spatula. A hemocytometer was used to adjust the spore concentration to 1×10^8 conidia mL⁻¹. The spore germination rate was guaranteed to exceed 90%, defined as germ tube length greater than twice the diameter of the conidium (Wilberts et al. 2023).

Root drenching was performed on maize seedlings at 3 d after sowing with the prepared spore suspension. Each pot received 50 mL of suspension once every 3 d for three consecutive applications prior to subsequent experiments. Three treatments were established in this trial: GL-5 strain inoculation, H8 strain inoculation, and an untreated control (CK) (Yang et al. 2026).

Determination of growth parameters of maize seedlings

Maize seedlings were randomly harvested at 7, 14, and 21 days after fungal inoculation to quantify growth parameters, including plant height, root length, leaf length, leaf width, fresh weight, and dry weight. Samples were dehydrated using a drying oven (DZ-2BCIV, Tianjin Taisite Instrument Co., Ltd.).

The drying protocol consisted of an initial heating step at 105°C for 30 min to deactivate enzymes, followed by continuous drying at 80°C for 48 h. The samples were weighed every 12 h until the weight difference between two successive measurements was less than 0.01 g, at which point constant weight was achieved. Five biological replicates were included for each sampling time point (Liu et al. 2022).

***S. frugiperda* rearing and life history observation**

Three treatments were set up: blank control (CK), GL-5 inoculation, and H8 inoculation. For each treatment, 100 larvae were randomly selected. Bioassays were performed to record the life-history traits of *S. frugiperda*, including developmental duration, survival, pupal morphology, adult lifespan and reproductive indicators under the identical climatic conditions used for previous insect rearing. The age-stage two-sex life table program TWOSEX-MSChart was adopted to compute a full set of demographic parameters. Complete experimental setup, feeding protocols, measurement standards and detailed life-table calculation formulas are provided in Supplementary Material S2 (Zhang et al. 2023; Wang et al. 2025; Chi et al. 1985, 2023).

Enzyme extraction and activity determination

Enzyme extraction and activity determination

We extracted crude enzyme solutions from maize leaf tissues and microbial cell pellets, then measured the activities of plant antioxidant enzymes, including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and insect detoxification enzymes including glutathione S-transferase (GST), acetylcholinesterase (AChE), carboxylesterase (CarE), and cytochrome P450 (CYP450), following modified classic spectrophotometric protocols. Full extraction procedures, reaction systems, incubation parameters, centrifugation conditions, wavelength settings and sonication lysis parameters for all enzyme assays are provided in Supplementary Material S3 (Mahmood et al. 2021; Feng et al. 2024; Feng et al. 2025; Yin et al. 2026).

Processing of maize leaf samples and microbiome analysis

Maize leaf composite samples were collected at 12 days post-sowing, snap-frozen in liquid nitrogen, and preserved at – 80°C for multi-omics sequencing. Leaf microbial DNA was extracted, and the bacterial 16S rRNA V5–V7 region was amplified, purified, and sequenced on the Illumina NovaSeq-PE 250 platform. Raw amplicon data were quality-filtered, clustered into operational taxonomic units (OTUs), and taxonomically annotated using standard bioinformatic pipelines. Complete sampling protocols, DNA extraction kits, PCR reaction systems, thermal cycling parameters, library construction procedures and sequence analysis workflows are provided in Supplementary Material S4 (Zhang et al. 2022).

Metabolomic profiling of maize leaves

Metabolite extraction was performed on the prepared leaf samples for untargeted metabolomics analysis. Six plants were randomly selected from each treatment group for metabolite extraction and subsequent untargeted metabolomics profiling.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) detection was carried out using a Thermo UHPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column (Waters, USA). Raw LC-MS datasets were preprocessed with Progenesis Q1 software to generate a three-dimensional CSV matrix containing sample information, metabolite annotations and peak intensity values. Orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted to calculate variable importance in projection (VIP) values, and volcano plot analysis was also performed on the dataset. Metabolites satisfying the thresholds of $VIP > 1$ and $P < 0.05$ based on OPLS-DA and Student's t-test results were identified as differentially expressed metabolites (DEMs) (Jiang et al. 2022; Zhao et al. 2025).

Integrated analysis of microbiome and metabolome

Correlation networks between metabolites and microbial amplicon sequence variants (ASVs) were constructed according to the method described by Baker et al. (2024), with the correlation coefficient cutoff set to 0.8. Correlation calculation, network construction and topological analysis were performed using the igraph R package, and network visualization was achieved via Gephi version 0.10.1. In addition, correlation heatmap and chord diagram analyses were conducted to explore the associations between metabolites and microbial ASVs (Baker et al. 2024).

Measurement of parasitoid biological parameters on *S. frugiperda*

For each treatment, ten pairs of adult parasitoids and 1,500 second-instar *S. frugiperda* larvae were used. All insects were placed in square plastic containers (50 cm diameter, 30 cm height, 40 cm width). Fresh maize leaves from the respective treatments and cotton balls soaked in 20% honey solution were supplied inside the containers to ensure adequate nutrition and moisture. Parasitoid adults were transferred daily into new containers containing 300 2-day-old host larvae until female wasp death. Parasitoid parasitism rate, cocoon weight, eclosion rate, and offspring sex ratio were recorded (Tian et al. 2024).

Statistical analysis

The TWOSEX-MSChart software was adopted to calculate life table parameters. The bootstrap method with 100,000 replications was applied to estimate the mean values and standard errors (SE) of all life table indices. Pairwise bootstrap tests were performed to detect significant differences among treatments at a significance level of $P < 0.05$. One-way analysis of variance (ANOVA) was carried out via SPSS 25.0 (IBM, Armonk, New York, USA) to analyze pupal weight and pupation rate. Duncan's multiple range test was used for post-hoc multiple comparisons to identify significant differences between groups. All figures were plotted using OriginPro 2023 (OriginLab, Northampton, Massachusetts, USA).

Results

Effects of fungal inoculation on the growth of maize seedlings

Both fungal treatments significantly promoted the growth of maize seedlings (Table S1, Fig. 2D–I). At 14 and 21 days post-inoculation (dpi), the plant height of maize seedlings treated with either fungal strain was significantly greater than that of the control. Fungal inoculation exerted a more pronounced effect on root elongation. At 14 dpi, the root length of the GL-5 group (29.65 cm) was significantly higher than that of the H8 group (23.89 cm) and the control group (25.54 cm). At 14 dpi, the H8-treated seedlings had the longest leaves (31.47 cm), whereas the control group showed significantly shorter leaves (26.68 cm). Regarding leaf width, no significant differences were observed among treatments at any time point. Fungal inoculation persistently enhanced biomass accumulation in maize. At 14 dpi, fresh weights in the GL-5 group (6.33 g) and H8 group (5.88 g) were significantly higher than that of the control group (5.11 g). At 21 dpi, both fungal treatments maintained higher fresh weights than the control, although no significant differences were detected among treatments. The variation trend of dry weight was generally consistent with that of fresh weight.

Effects of fungal treatments on pupal size and pupal weight of *S. frugiperda*

Fungal treatments significantly inhibited pupal size and pupal weight of *S. frugiperda* (Table S2, Fig. 2A–C). The control group exhibited the longest pupal body length (15.19 mm), while the GL-5 treatment resulted in the shortest pupal length, representing a 10.1% reduction compared with the control. A similar trend was observed for pupal body width. Furthermore, both fungal treatments significantly reduced pupal weight: the control pupal weight was 0.21 g, and the GL-5 and H8 treatments decreased pupal weight by 28.6% and 23.8%, respectively. All pairwise comparisons among groups showed significant differences ($P < 0.05$).

Life-History responses of *S. frugiperda* to fungal inoculation

Fungal treatments significantly altered the developmental duration of each life stage of *S. frugiperda* (Table S3). Compared with the control, GL-5 treatment markedly prolonged the developmental duration of the 1st instar (3.58 d), 2nd instar (2.69 d), 6th instar (2.91 d), and prepupal stage (3.86 d), whereas H8 treatment mainly extended the 3rd instar larval duration (3.44 d) and pupal duration (10.49 d). In addition, the control group exhibited the longest adult longevity (11.90 d).

Obvious differences in mortality at each developmental stage of *S. frugiperda* were observed among treatments (Table S4, Fig. 3A–C). The total pre-adult mortality in the GL-5 group (64%) was significantly higher than that in the control group (50%), and correspondingly, the control group had the highest adult emergence rate (50%).

The two fungal strains exerted differential effects on the reproduction of *S. frugiperda* (Table S5, Fig. 3J–L). The adult pre-oviposition period (APOP) in the GL-5 group was significantly prolonged to 10.09 d, versus only 6.17 d in the control. Moreover, the total fecundity per female in the GL-5 group was only 99.00 eggs, representing a 69.9% reduction compared with the control, whereas the H8 group showed a higher fecundity (400.11 eggs per female) than the control. Both fungal treatments slightly extended female longevity, but GL-5 inoculation markedly shortened male longevity to 40.74 d.

Both fungal treatments significantly reduced the population growth potential of *S. frugiperda* (Table S6). The control had the highest intrinsic rate of increase ($r = 0.12 \text{ d}^{-1}$), finite rate of increase ($\lambda = 1.13 \text{ d}^{-1}$), and net reproductive rate ($R_0 = 88.84$). However, both fungal treatments significantly prolonged the mean generation time (T). The age-stage-specific reproductive value (v_{xj}) curves (Fig. 3D–F) showed that the peak reproductive value of females in the GL-5 treatment was greatly reduced compared with the control, while females in the H8 treatment exhibited a delayed and shortened reproductive window. Furthermore, the age-stage-specific life expectancy (e_{xj}) curves in all treatment groups declined gradually with increasing age (Fig. 3G–I). Newly hatched larvae in the control had the highest initial life expectancy (approximately 38 d), consistent with the elevated mortality observed across all developmental stages.

Effects of fungal treatments on *M. manilae*

Host larvae fed on fungus-inoculated maize exhibited differential susceptibility to parasitism by *M. manilae* (Table S7, Fig. 4). The parasitism rate reached its highest value (28.80%) when hosts fed on GL-5-treated maize, representing a 38.9% increase compared with the control group. The maize varieties treated with different fungi significantly affected cocoon weight. The cocoon weight in the GL-5 group was 10.9 mg, markedly higher than that in the control and H8 groups. The adult emergence rate of *M. manilae* was highest in the GL-5 group (90.05%), followed by the control (89.71%) and H8 (88.12%) groups. Host diet treatments significantly altered the proportion of female progeny of the parasitoid. The control group had the highest proportion of female offspring (0.251), and both fungal treatments reduced the percentage of female progeny.

Effects of fungal treatments on antioxidant and detoxifying enzyme activities

Inoculation with the two entomopathogenic fungal strains significantly altered the antioxidant enzyme profiles of maize leaves compared with the control (CK) (Table S8, Fig. 5A–D). Both GL-5 (256.38 U/g FW) and H8 (288.40 U/g FW) treatments markedly reduced leaf superoxide dismutase (SOD) activity. The CK group exhibited the highest peroxidase (POD) activity (779.15 U/g FW), while the GL-5 group had the lowest POD activity (627.91 U/g FW). CAT activity was significantly decreased only in the GL-5 treatment (40.66 U/g FW). No significant difference in glutathione S-transferase (GST) activity was observed among the three groups.

Feeding on fungus-treated maize significantly regulated the detoxification enzyme system of *S. frugiperda* larvae (Table S8, Fig. 5E–H). Larvae fed on GL-5-treated maize exhibited the highest activities of GST (4307.65 U/g FW), carboxylesterase (CarE) (9.71 U/g FW), and cytochrome P450 (5024.29 U/g FW). Acetylcholinesterase (AChE) activity showed the strongest induction in the H8 group, reaching 316.96 U/g FW, which was 162.6% higher than that of CK.

Microbial community diversity and composition in maize leaves

Significant differences in the richness and diversity of microbial communities in maize leaves were detected among the three treatments (Fig. S1A, B, E, F; Fig. S2A, B, E, F). Moreover, the rarefaction curves of all samples plateaued (Fig. S1D, Fig. S2D), confirming that sequencing depth was sufficient to capture the vast majority of bacterial taxa. Principal component analysis (PCA) (Fig. S1C, Fig. S2C) revealed that the bacterial and fungal communities of the BYM and HYM groups were clearly distinguished from each other.

Linear discriminant analysis effect size (LEfSe) and microbial community bar plots were used for further analysis (Fig. 6, Fig. S3). At the bacterial phylum level (Fig. S1G), the GL-5 treatment enriched Proteobacteria, while the control group enriched Actinobacteriota. At the bacterial genus level (Fig. 6A, Fig. 6C), the GL-5 treatment was dominated by *Enterobacter* (family Enterobacteriaceae), the H8 treatment enriched *Chryseobacterium*, and the control group enriched *Corynebacterium*. At the fungal phylum level (Fig. S2G), the H8 treatment enriched Basidiomycota, whereas Ascomycota served as the absolutely dominant phylum in the control and GL-5 groups. At the fungal genus level (Fig. S3A, Fig. S3C), the relative abundance of *Beauveria* was elevated in the GL-5 treatment, the H8 treatment enriched basidiomycetous yeasts including *Bullera*, and saprophytic fungi such as *Schizophyllum* accounted for a larger proportion in the control group.

Venn diagrams (Fig. 6D, Fig. S3D) showed that the H8 treatment, GL-5 treatment, and control group contained 68, 34, and 39 unique bacterial OTUs, respectively. For fungi, the H8 group, control group, and GL-5 group contained 44, 45, and 18 unique fungal OTUs, respectively. Heatmaps of predicted functional enzymes (Fig. 6B, Fig. S3B) revealed that enzymes associated with carbohydrate metabolism and xenobiotic degradation were generally more abundant in fungal treatment groups, indicating that fungal inoculation enhanced the metabolic potential of leaf microbial communities. Furthermore, the abundances of enzymes involved in carbohydrate degradation and secondary metabolism differed significantly between the two fungal treatment groups.

Metabolic profiling and KEGG pathway enrichment analysis

The variable importance in projection (VIP) plots (Fig. 7A, C) showed that the significantly up-regulated metabolites in the GL-5 treatment group included phytosterone A, diosmin, chlorogenic acid, 3-hydroxy-2-methylpyridine, and 7-hydroxy-3-methoxy-1-primeverosxanthone. The differential metabolites in the H8

treatment group were cryptocaryogenic acid, 5-(1-naphthyl)-4-(3-pyridyl)-4H-1,2,4-triazole-3-thiol, 2-hydroxymethyl-5-methoxy- γ -pyrone, and concaxin C19. Volcano plots (Fig. 7B, D) further confirmed that most differential metabolites were markedly up-regulated after fungal inoculation.

KEGG pathway enrichment analysis revealed that both fungal treatments co-enriched plant hormone signal transduction, galactose metabolism, and phenylpropanoid biosynthesis. In addition, the GL-5 strain exhibited higher enrichment in flavone and flavonol biosynthesis, ascorbate metabolism, and the TCA cycle. The H8 strain significantly enriched ABC transporters, starch, and sucrose metabolism, and alkaloid biosynthesis pathways (Fig. S4).

Correlation between phyllosphere microbes and differential leaf metabolites

Integrative Spearman correlation network and heatmap analyses were employed to reveal the linkage between dominant phyllosphere microorganisms and differential metabolites induced by entomopathogenic fungal inoculation.

The fungal genus *Aspergillus* exhibited a widespread positive correlation with most defense-related secondary metabolites, including flavonoids, phenolic acids, terpenoids, and amino acid derivatives (Fig. 8A–B, Fig. S5A). Only a small subset of lipid and alcohol metabolites showed significant negative correlations with *Aspergillus*. In contrast, the saprophytic fungus *Rhizopus* displayed an opposite correlation pattern: it was significantly negatively correlated with anti-herbivore secondary metabolites, while positively correlated with primary carbohydrate and fatty acid metabolites (Fig. S5B).

Core phyllosphere bacterial taxa showed distinct correlation signatures with leaf metabolites. *Novoosphingobium* and unclassified Enterobacteriaceae (Fig. 8C, Fig. S5C) were significantly positively correlated with phenylpropanoids, flavonoids, and alkaloids—key metabolites mediating plant anti-insect defense—while exhibiting negative correlations with soluble sugars and short-chain organic acids, suggesting that these bacteria specifically promote the accumulation of defensive secondary metabolites. The genus *Pseudomonas* possessed the most complex correlation network (Fig. 8D, Fig. S5D), with strong positive correlations with defense-related secondary metabolites and significant negative correlations with photosynthetic primary metabolites such as monosaccharides and fatty acids.

Discussion

Endophytic colonization by both entomopathogenic fungal strains promoted maize seedling growth, corroborating earlier findings (Yousef-Yousef et al. 2025). Strain GL-5 exerted a stronger promoting effect on root elongation, whereas strain H8 was more beneficial to leaf elongation. These results indicate divergence in symbiotic compatibility and regulatory pathways between different fungal strains and the host plant (Kumar et al. 2025).

Maize plants colonized by endophytic fungi exhibited significant resistance against *S. frugiperda*. Fungal colonization impeded normal pupal development of the pest, which agreed with most previous studies (Sánchez-Rodríguez et al. 2018; Kuzhuppillymyal-Prabhakarankutty et al. 2021). This may be attributable to the significant inhibition of SOD and POD activities in maize leaves by both strains. Endophytic colonization remodelled basal antioxidant homeostasis and activated specific defensive pathways, substituting the functions of constitutive antioxidant enzymes (Liu et al. 2025; Li et al. 2026).

Furthermore, the two strains exhibited distinct regulatory patterns on pest population dynamics. GL-5 suppressed population expansion mainly by prolonging the developmental duration of young larvae and prepupae, delaying the pre-oviposition period, increasing mortality of old-instar larvae, and reducing adult eclosion rate and fecundity per female. Enzyme activity assays showed that GL-5 significantly induced the activities of GST, CarE, and especially CYP450 in larvae. In addition, leaf microbiome sequencing confirmed that GL-5 specifically enriched beneficial taxa such as *Enterobacter*, which are positively correlated with phenylpropanoid and flavonoid biosynthesis and participate in plant anti-herbivore defence (Chung et al. 2013; Francioli et al. 2025). Metabolomic results revealed that GL-5 mainly activated flavonoid and flavonol biosynthesis, ascorbate metabolism and the TCA cycle, strengthening phenolic antioxidant and anti-herbivore secondary metabolism. In contrast, H8 mainly extended the developmental time of the 3rd-instar larvae and pupae. Although H8 failed to inhibit pest reproduction, it slowed population growth by prolonging generation time. Enzyme activity analysis indicated that H8 suppressed core detoxification enzymes while specifically increasing AChE activity, impairing larval performance. Leaf microbiome sequencing confirmed that H8 enriched beneficial taxa including *Chryseobacterium*, a genus reported to regulate primary metabolism such as carbohydrate and starch metabolism and facilitate alkaloid synthesis (Bashir et al. 2022). Metabolomic results revealed that H8 mainly modulated starch and sucrose metabolism, ABC transporters and alkaloid biosynthesis, focusing on the regulation of primary metabolism and nitrogen-containing defensive metabolites.

Both strains significantly decreased the population expansion potential, achieving sustained suppression of *S. frugiperda* (Altaf et al. 2023). This may represent an indirect defensive outcome derived from the reconfiguration of maize physiological metabolism and microecological structure triggered by fungal colonization (Yuan et al. 2026). Moreover, such divergent regulatory patterns on pest metabolism clarify the core physiological mechanisms underlying the distinct insect-resistant phenotypes between the two strains (Kshatriya and Gershenzon, 2024; Rafique et al. 2025). Functional prediction further indicated that fungal colonization may enrich microbial genes involved in carbohydrate metabolism and xenobiotic degradation in leaves. Colonization by both strains altered leaf metabolomes, and shared pathways including plant hormone signal transduction, galactose metabolism, and phenylpropanoid biosynthesis represent the core conserved defensive pathways of maize in response to endophytic fungi (Toppo et al. 2023; Yuan et al. 2026). Moreover, these metabolic characteristics were highly consistent with their growth-promoting effects, insect-resistant phenotypes and enzyme activity patterns.

Regarding parasitoid performance, GL-5 inoculation significantly increased the parasitism rate of *M. manilae* on *S. frugiperda* and enhanced parasitoid developmental fitness, indicating strong potential for

synergistic biological control. We propose that GL-5 colonization may reshape maize volatile and defensive metabolite profiles, thereby altering the physiological status of host larvae and enhancing host susceptibility to parasitism (Sonia et al. 2024), which is supported by the detoxification enzyme results obtained in this study. However, most previous reports suggested that fungal colonization exerts negligible influences on parasitoids (Panwar et al. 2024; Rizwan et al. 2021). Interestingly, both fungal treatments reduced the female ratio of parasitoid progeny, which may imply that endophyte-mediated shifts in plant defences indirectly modify parasitoid sex allocation. We speculate that this phenomenon arises from adaptive adjustments of *M. manilae* responding to physiological variations in *S. frugiperda* larvae (Jensen et al. 2020).

Correlation analyses between microbial taxa and metabolites revealed that endophytic fungi reinforce plant resistance by reshaping phyllosphere microbial communities and reprogramming leaf metabolomes. For instance, beneficial phyllosphere taxa including *Aspergillus*, *Sphingomonas*, and *Pseudomonas* formed synergistic defensive modules, exhibiting strong positive correlations with core anti-herbivore secondary metabolites such as phenylpropanoids, flavonoids, and terpenoids (Yang et al. 2024; Han et al. 2026; Baker et al. 2024).

Conclusion

Both endophytic strains GL-5 and H8 promoted maize seedling growth and elicited strain-specific resistance against *S. frugiperda*: GL-5 activated flavonoid and phenolic defensive pathways and improved parasitoid performance, whereas H8 modulated carbohydrate metabolism and alkaloid biosynthesis to disrupt larval neural homeostasis. These protective effects were achieved via remodelling of the phyllosphere microbial community and reprogramming of maize leaf metabolism. Collectively, our findings elucidate the tritrophic interaction mechanisms among maize, *S. frugiperda*, and parasitoids mediated by endophytic fungi, and demonstrate that both GL-5 and H8 can be deployed in concert with *M. manila* for integrated management of fall armyworm. This study provides a mechanistic basis for developing endophyte-based biocontrol strategies, and highlights the need for future field-based validation of strain-parasitoid interaction outcomes, as well as molecular-level investigations into how fungal endophytic colonization reshapes plant volatile emissions and orchestrates parasitoid behavioral regulation.

Declarations

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Author Contribution

ZKY: Conceptualization, Methodology, Data Curation, Writing - Original Draft, Writing - Review & Editing. ZYH: Data Curation, Writing - Original Draft, Formal analysis. XZ: Methodology, Writing - review and editing. XXS: Writing—review and editing. XYJ: Methodology. DSL: Visualization. YH: Methodology. YCY: Formal analysis. SMC: Formal analysis. FRW: Formal analysis. XFY: Visualization. MFY: Supervision, Conceptualization, Resources, Writing - Review & Editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgement

We thank Professor Hsin Chi (National Chung Hsing University, Taichung, Taiwan) for patient guidance using the TWOSEX-MSChart program. This study was supported by the Major Special Projects of the Guizhou Branch of the China National Tobacco Corporation (2023XM06, 2023520000240176).

Data Availability

The microbiome data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) database under the accession number PRJNA1510338. The metabolomics data have been deposited in the MetaboLights repository and are accessible under the study identifier REQ20260811222596 (temporary accession; permanent accession will be assigned upon data publication). All other data and materials integral to this paper are available to readers upon reasonable request to the corresponding authors.

References

1. Abdel-Raheem (2005) Possibility of using the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* for controlling the sugar beet insects *Cassida vittata* Vill. and *Scrobipalpa ocellatella* Boh. in Egypt. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Cairo, Egypt.
2. Ahmad I, Jiménez-Gasco MM, Luthe DS, Shakeel SN, Barbercheck ME (2020) Endophytic *Metarhizium robertsii* promotes maize growth, suppresses insect growth, and alters plant defense gene expression. Biol Control 144:104167. <https://doi.org/10.1016/j.biocontrol.2019.104167>.
3. Akutse KS, Fiaboe KK, Van den Berg J, Ekesi S, Maniania NK (2014) Effects of endophyte colonization of *Vicia faba* (Fabaceae) plants on the life–history of leafminer parasitoids *Phaeditoma scabriventris* (Hymenoptera: Braconidae) and *Diglyphus isaea* (Hymenoptera: Eulophidae). PLoS One 9:e109965. <https://doi.org/10.1371/journal.pone.0109965>.
4. Altaf N, Ullah MI, Afzal M, Ali S, Rizwan M, Al-Shuraym LA, Alhelaify SS, Sayed S (2023) Endophytic colonization by *Beauveria bassiana* and *Metarhizium anisopliae* in maize plants affects the fitness of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Microorganisms 11:1067. <https://doi.org/10.3390/microorganisms11041067>.

5. Baker NR, Zhalnina K, Yuan M, Herman D, Ceja-Navarro JA, Sasse J, Jordan JS, Bowen BP, Wu L, Fossum C, Chew A, Fu Y, Saha M, Zhou J, Pett-Ridge J, Northen TR, Firestone MK (2024) Nutrient and moisture limitations reveal keystone metabolites linking rhizosphere metabolomes and microbiomes. *Proc Natl Acad Sci U S A* 121:e2303439121. <https://doi.org/10.1073/pnas.2303439121>.
6. Bashir I, War AF, Rafiq I, Reshi ZA, Rashid I, Shouche YS (2022) Phyllosphere microbiome: diversity and functions. *Microbiol Res* 254:126888. <https://doi.org/10.1016/j.micres.2021.126888>.
7. Batool R, Umer MJ, Wang Y, He K, Shabbir MZ, Zhang T, Bai S, Chen J, Wang Z (2022) Myco-synergism boosts herbivory-induced maize defense by triggering antioxidants and phytohormone signaling. *Front Plant Sci* 13:790504. <https://doi.org/10.3389/fpls.2022.790504>.
8. Batool R, Umer MJ, Wang Y, He K, Zhang T, Bai S, Zhi T, Chen J, Wang Z (2020) Synergistic effect of *Beauveria bassiana* and *Trichoderma asperellum* to induce maize (*Zea mays* L.) defense against the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera, Crambidae) and larval immune response. *Int J Mol Sci* 21:8215. <https://doi.org/10.3390/ijms21218215>.
9. Chung SH, Rosa C, Scully ED, Peiffer M, Tooker JF, Hoover K, Luthe DS, Felton GW (2013) Herbivore exploits orally secreted bacteria to suppress plant defenses. *Proc Natl Acad Sci U S A* 110:15728–15733. <https://doi.org/10.1073/pnas.1308867110>.
10. Dash CK, Bamisile BS, Keppanan R, Qasim M, Lin YW, Islam SU, Hussain M, Wang LD (2018) Endophytic entomopathogenic fungi enhance the growth of *Phaseolus vulgaris* L. (Fabaceae) and negatively affect the development and reproduction of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Microb Pathog* 125:385–392. <https://doi.org/10.1016/j.micpath.2018.09.034>.
11. Desika J, Yogendra K, Hepziba SJ, Patne N, Vivek BS, Ravikesavan R, Nair SK, Jaba J, Razak TA, Srinivasan S, Shettigar N (2024) Exploring metabolomics to innovate management approaches for fall armyworm (*Spodoptera frugiperda* [J.E. Smith]) infestation in maize (*Zea mays* L.). *Plants* 13:2451. <https://doi.org/10.3390/plants13172451>.
12. Díaz-González S, González-Sanz C, González-Bodí S, Marín P, Brunner F, Sacristán S (2025) Microbiome analysis reveals biocontrol of *Aspergillus* and mycotoxin mitigation in maize by the growth-promoting fungal endophyte *Colletotrichum tofieldiae* Ct0861. *Plants* 14:3236. <https://doi.org/10.3390/plants14213236>.
13. Feng Q, Yang J, Huang W, Jia J, Wang J, Pan F, Ji X (2025) Stage-Specific Toxicity of Novaluron to Second-Instar *Spodoptera frugiperda* and *Plutella xylostella* and Associated Enzyme Responses. *Insects* 16:1051. <https://doi.org/10.3390/insects16071051>.
14. Francioli D, Kampouris ID, Kuhl-Nagel T, Babin D, Sommermann L, Behr JH, Chowdhury SP, Zrenner R, Moradtalab N, Schlöter M, Geistlinger J, Ludewig U, Neumann G, Smalla K, Grosch R (2025) Microbial inoculants modulate the rhizosphere microbiome, alleviate plant stress responses, and enhance maize growth at field scale. *Genome Biol* 26:148. <https://doi.org/10.1186/s13059-025-03621-7>.

15. Ganiger P, Yeshwanth H, Muralimohan K, Vinay N, Kumar A, Chandrashekara K (2018) Occurrence of the new invasive pest, fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. *Curr Sci* 115:621–623. <https://doi.org/10.18520/cs/v115/i4/621-623>.
16. Garrido-Jurado I, Resquín-Romero G, Amarilla SP, Ríos-Moreno A, Carrasco L, Quesada-Moraga E (2017) Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *J Pest Sci* 90:319–330. <https://doi.org/10.1007/s10340-016-0770-0>.
17. González-Mas N, Cuenca-Medina M, Gutiérrez-Sánchez F, Quesada-Moraga E (2019) Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. *J Pest Sci* 92:1271–1281. <https://doi.org/10.1007/s10340-019-01120-1>.
18. Guan JQ, Liu J, Gou JY, Xu ZS, Zhou YM, Zou X (2025) Endophytic colonization via *Cordyceps cateniannulata* induces a growth-enhancing effect and increases the resistance of tomato plants against *Tetranychus urticae* (Koch). *Crop Prot* 193:107182. <https://doi.org/10.1016/j.cropro.2025.107182>.
19. Guo J, Qi J, He K, Wu J, Bai S, Zhang T, Zhao J, Wang Z (2019) The Asian corn borer *Ostrinia furnacalis* feeding increases the direct and indirect defence of mid-whorl stage commercial maize in the field. *Plant Biotechnol J* 17:88–102. <https://doi.org/10.1111/pbi.12949>.
20. Han Y, Li X, Zhou C, Xu T, Liu Y, Dou G, Hu J, Wang J (2026) Maize recruits beneficial microorganisms via rhizosphere metabolites as signals to construct a functional network for saline–alkaline stress resistance. *Front Plant Sci* 17:1843423. <https://doi.org/10.3389/fpls.2026.1843423>.
21. Hernández MMP (2025) *Metarhizium robertsii* alters sugarcane volatiles and their interactions with insects. Ph.D. Thesis, Universidade de São Paulo, Piracicaba, Brazil.
22. Jensen RE, Cabral C, Enkegaard A, Steenberg T (2020) Influence of the plant-interacting entomopathogenic fungus *Beauveria bassiana* on parasitoid host choice behavior, development, and plant defense pathways. *PLoS One* 15:e0238943. <https://doi.org/10.1371/journal.pone.0238943>.
23. Jiang L, Lin M, Wang H, Song H, Zhang L, Huang Q, Chen R, Song C, Li G, Cao Y (2022) Haplotype-resolved genome assembly of *Bletilla striata* (Thunb.) Reichb.f. to elucidate medicinal value. *Plant J* 111:1340–1353. <https://doi.org/10.1111/tpj.15898>.
24. Kshatriya K, Gershenzon J (2024) Disarming the defenses: insect detoxification of plant defense-related specialized metabolites. *Curr Opin Plant Biol* 81:102577. <https://doi.org/10.1016/j.pbi.2024.102577>.
25. Kumar S, Singh A, Verma RK (2025) Plant–entomopathogenic fungi interaction: recent progress and future prospects on endophytism-mediated growth promotion and biocontrol. *J Fungi* 11:245. <https://doi.org/10.3390/jof11030245>.
26. Kuzhuppillymyal-Prabhakarankutty L, Ferrara-Rivero FH, Tamez-Guerra P, Gomez-Flores R, Rodríguez-Padilla MC, Ek-Ramos MJ (2021) Effect of *Beauveria bassiana* seed treatment on *Zea mays* L.

- response against *Spodoptera frugiperda*. Appl Sci 11:2887. <https://doi.org/10.3390/app11072887>.
27. Li W, Sun XY, Cui JL (2026) Research progress on endophytic fungi promoting seed germination: a potential breeding approach. Russ J Plant Physiol 73:3. <https://doi.org/10.1134/S1021443726600222>.
 28. Liu M, Xiang D, Hokkanen HMT, Niu T, Zhang J, Yang J, Wei Q, Chen H, Liu H, Li Y (2025) *Beauveria bassiana* induces strong defense and increases resistance in tomato to *Bemisia tabaci*. J Fungi 11:141. <https://doi.org/10.3390/jof11020141>.
 29. Liu Y, Yang Y, Wang B (2022) Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* play roles of maize (*Zea mays*) growth promoter. Sci Rep 12:15706. <https://doi.org/10.1038/s41598-022-19899-7>.
 30. Mahmood U, Hussain S, Hussain S, Ali B, Ashraf U, Zamir S, Al-Robai SA, Alzahrani FO, Hano C, El-Esawi MA (2021) Morpho-physio-biochemical and molecular responses of maize hybrids to salinity and waterlogging during stress and recovery phase. Plants 10:1345. <https://doi.org/10.3390/plants10071345>.
 31. Michereff MFF, Lopes RB, Quevedo IA, Rodrigues CM, Laumann RA, Borges M, Blassioli-Moraes MC (2026) Association of *Beauveria bassiana* with maize alters volatile organic compounds and enhances attraction of the egg parasitoid *Telenomus podisi*. J Pest Sci 99:88. <https://doi.org/10.1007/s10340-026-02057-7>.
 32. Miranda-Fuentes P, Yousef-Yousef M, Valverde-García P, Rodríguez-Gómez IM, Garrido-Jurado I, Quesada-Moraga E (2021) Entomopathogenic fungal endophyte-mediated tritrophic interactions between *Spodoptera littoralis* and its parasitoid *Hyposoter didymator*. J Pest Sci 94:933–945. <https://doi.org/10.1007/s10340-020-01306-7>.
 33. Omacini M, Chaneton EJ, Ghera CM, Müller CB (2001) Symbiotic fungal endophytes control insect host–parasite interaction webs. Nature 409:78–81. <https://doi.org/10.1038/35051066>.
 34. Olaf E, Jaleta M, Sonder K, Mottaleb K, Prasanna BM (2022) Global maize production, consumption and trade: trends and R&D implications. Food Secur 14:1295–1319. <https://doi.org/10.1007/s12571-022-01288-7>.
 35. Panwar N, Szczepaniec A (2024) Endophytic entomopathogenic fungi as biological control agents of insect pests. Pest Manag Sci 80:6033–6040. <https://doi.org/10.1002/ps.8322>.
 36. Pec M, Salazar-Mendoza P, Magalhães DM, Delalibera I, Bento JMS (2024) The endophytic entomopathogenic fungus *Metarhizium robertsii* alters sugarcane plant defenses, reducing *Diatraea saccharalis* oviposition and enhancing the attraction of *Cotesia flavipes*. Crop Prot 184:106872. <https://doi.org/10.1016/j.cropro.2024.106872>.
 37. Qi J, Malook SU, Shen G, Gao L, Zhang C, Li J, Zhang J, Wang L, Wu J (2018) Current understanding of maize and rice defense against insect herbivores. Plant Divers 40:189–195. <https://doi.org/10.1016/j.pld.2018.06.006>.
 38. Qiao L, Liu J, Zhou Z, Li Z, Zhou Y, Xu S, Yang Z, Qu J, Zou X (2023) Positive effects of *Cordyceps cateniannulata* colonization in tobacco: growth promotion and resistance to abiotic stress. Front

- Microbiol 14:1131184. <https://doi.org/10.3389/fmicb.2023.1131184>.
39. Quesada-Moraga E (2020) Entomopathogenic fungi as endophytes: their broader contribution to IPM and crop production. *Biocontrol Sci Technol* 30:864–877. <https://doi.org/10.1080/09583157.2020.1777999>.
40. Rafique S, Freed S, Sabir H, Azhar M, Naeem A, Ahmed R (2025) Transgenerational effect of *Cordyceps fumosorosea* (Wize) on demographic parameters and detoxifying enzymes of *Spodoptera frugiperda* (Smith). *Bull Entomol Res* 115:660–671. <https://doi.org/10.1017/S0007485325100242>.
41. Ren P, Di XY, Yan B, Shen XX, Tian S, Yang MF, Wu HZ (2025) Impact of short-term high temperature on *Microplitis manilae* (Hymenoptera: Braconidae) survival, fecundity and progeny development. *Ecol Evol* 15:e71626. <https://doi.org/10.1002/ece3.71626>.
42. Rizwan M, Atta B, Arshad M, Khan RR, Dageri A, Rizwan M, Ullah MI (2021) Non-detrimental impact of two concomitant entomopathogenic fungi on life-history parameters of a generalist predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Sci Rep* 11:20699. <https://doi.org/10.1038/s41598-021-00037-8>.
43. Russo ML, Scorsetti AC, Vianna MF, Allegrucci N, Ferreri NA, Cabello MN, Pelizza SA (2019) Effects of endophytic *Beauveria bassiana* (Ascomycota: Hypocreales) on biological, reproductive parameters and food preference of the soybean pest *Helicoverpa gelotopoeon*. *J King Saud Univ Sci* 31:1077–1082. <https://doi.org/10.1016/j.jksus.2018.07.013>.
44. Sánchez-Rodríguez AR, Raya-Díaz S, Zamarreño AM, García-Mina JM, Del Campillo MC, Quesada-Moraga E (2018) An endophytic *Beauveria bassiana* strain increases spike production in bread and durum wheat plants and effectively controls cotton leafworm (*Spodoptera littoralis*) larvae. *Biol Control* 116:90–102. <https://doi.org/10.1016/j.biocontrol.2017.07.012>.
45. Saroop S, Tamchos S (2024) Impact of pesticide application: Positive and negative side. In: Sharma A, Kumar V, Zheng B (Eds.), *Pesticides in the Environment*. Elsevier, 155–178. <https://doi.org/10.1016/B978-0-323-99427-9.00006-9>.
46. Song Y, Cui H, Guo W, Sindhu L, Lv S, Li L, Yu Y, Men X (2024) Endophytic fungi improved wheat resistance to *Rhopalosiphum padi* by decreasing its feeding efficiency and population fitness. *Ecotoxicol Environ Saf* 270:115865. <https://doi.org/10.1016/j.ecoenv.2023.115865>.
47. Tian S, Di XY, Yan B, Ren P, Wu HZ, Yang MF (2024) Parasitism success of *Microplitis manilae* (Hymenoptera: Braconidae) on different diet-fed *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. *J Appl Entomol* 148:199–204. <https://doi.org/10.1111/jen.13225>.
48. Togola A, Beyene Y, Bocco R, Tepa-Yotto G, Gowda M, Too A, Boddupalli P (2025) Fall armyworm (*Spodoptera frugiperda*) in Africa: insights into biology, ecology and impact on staple crops, food systems and management approaches. *Front Agron* 7:1538198. <https://doi.org/10.3389/fagro.2025.1538198>.
49. Toppo P, Kagatay LL, Gurung A, Singla P, Chakraborty R, Roy S, Mathur P (2023) Endophytic fungi mediates production of bioactive secondary metabolites via modulation of genes involved in key

- metabolic pathways and their contribution in different biotechnological sector. 3 Biotech 13:191. <https://doi.org/10.1007/s13205-023-03605-z>.
50. Wang Y, Pruitt RN, Nürnberger T, Wang Y (2022) Evasion of plant immunity by microbial pathogens. Nat Rev Microbiol 20:449–464. <https://doi.org/10.1038/s41579-022-00710-3>.
 51. Wang Z, Zhu H, Jin DC, Hou JT, Liu X (2025) Effects of three different host plants on two-sex life table parameters of the fall armyworm *Spodoptera frugiperda*. Sci Rep 15:141. <https://doi.org/10.1038/s41598-024-84103-x>.
 52. Waqar S, Bhat AA, Khan AA (2024) Endophytic fungi: unravelling plant–endophyte interaction and the multifaceted role of fungal endophytes in stress amelioration. Plant Physiol Biochem 206:108174. <https://doi.org/10.1016/j.plaphy.2023.108174>.
 53. Wilberts L, Van Hee S, Stockmans I, Meesters C, van Neerbos F, Gloder G, Geerinck MWJ, Wäckers F, Jacquemyn H, Lievens B (2023) Combining root inoculation with *Akanthomyces muscarius* (Hypocreales: Cordycipitaceae) and the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) to control aphids on sweet pepper. BioControl 68:579–589. <https://doi.org/10.1007/s10526-023-10211-8>.
 54. Wilberts L, Vuts J, Caulfield JC, Thomas G, Withall DM, Wäckers F, Birkett MA, Jacquemyn H, Lievens B (2024) Effects of root inoculation of entomopathogenic fungi on olfactory-mediated behavior and life-history traits of the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). Pest Manag Sci 80:307–316. <https://doi.org/10.1002/ps.7762>.
 55. Yang ZK, Zou X, Smagghe G, Shen XX, Zhang QQ, Ji XY, Bai XQ, Han ZY, Shen ZJ, Wang FR, Huang Y, Yang MF (2026) Associations between endophytic entomopathogenic fungi and *Myzus persicae*-tobacco-natural enemy interactions: links to volatile organic compound profiles and microbiome structure. Pest Manag Sci (in press). <https://doi.org/10.1002/ps.71134>.
 56. Yang Z, Liu T, Fan J, Chen Y, Wu S, Li J, Liu Z, Yang Z, Li L, Liu S, Yang H, Yin D, Meng Q, Tang Q (2024) Biocontrol agents modulate phyllosphere microbiota interactions against pathogen *Pseudomonas syringae*. Environ Sci Ecotechnol 21:100431. <https://doi.org/10.1016/j.ese.2024.100431>.
 57. Yao FL, Wu YY, Zhou SJ, Ding XL, Guan ZX, Lu XS, Zheng Y, Ramirez-Romero R, Desneux N, Weng QY, He YX (2024) Effects of continuous and transgenerational rearing in peanut leaves on the performance and enzyme activity of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Econ Entomol 117:2259–2268. <https://doi.org/10.1093/jee/toae204>.
 58. Yin ZJ, Zhao S, Li QM, Li Y, Li DY, Dai XY, Wang RJ, Wang CX, Zheng L, Tang B, Zhi JR (2026) Threats of multiple pesticides to a predator: Toxicity and sublethal impacts on *Orius nagaii*. Pestic Biochem Physiol 217:106836. <https://doi.org/10.1016/j.pestbp.2026.106836>.
 59. Yousef-Yousef M, García-Espinoza F, García del Rosal MJ, Quesada-Moraga E (2025) Guardians within: entomopathogenic fungi-driven antibiosis and compensatory growth combines to reduce the damage of herbivores to melon plants. J Pest Sci 98:291–308. <https://doi.org/10.1007/s10340-024-01812-y>.

60. Yuan Q, Wang X, Wang Z, Yan GW, Chen HL, Liu WJ, He LQ, Liu H, Wang ZY (2026) Genistein mediates *Beauveria bassiana*-induced resistance against *Bemisia tabaci* reproduction in tomato. Plant Cell Environ 49:4926–4939. <https://doi.org/10.1111/pce.70504>.

61. Zhang L, Zhang M, Huang S, Li L, Gao Q, Wang Y, Zhang S, Huang L, Yuan S, Wen Y, Liu K, Yu X, Li D, Zhang L, Xu H, Wei X, He P, Zhou W, Philippot L, Ai C (2022) A highly conserved core bacterial microbiota with nitrogen-fixation capacity inhabits the xylem sap in maize plants. Nat Commun 13:3361. <https://doi.org/10.1038/s41467-022-31113-w>.

62. Zhang MD, Wu SY, Yan JJ, Reitz S, Gao YL (2023) Establishment of *Beauveria bassiana* as a fungal endophyte in potato plants and its virulence against potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). Insect Sci 30:197–207. <https://doi.org/10.1111/1744-7917.13072>.

63. Zhang QY, Zhang YL, Quandahor P, Gou YP, Li CC, Zhang KX, Liu CZ (2023) Oviposition preference and age-stage, two-sex life table analysis of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on different maize varieties. Insects 14:413. <https://doi.org/10.3390/insects14050413>.

64. Zhao X, Jiang WY, Liu JJ, Yan WJ, Zhang YH, Zhang Z, Zhang PD (2025) Unraveling the impact of PFOA toxicity on *Zostera marina* using a multi-omics approach: insights from growth, physiological, transcriptomic, and metabolomic signatures. J Hazard Mater 486:137024. <https://doi.org/10.1016/j.jhazmat.2024.137024>.

65. Zhu H, Fu J, Wang J, Bidochka MJ, Duan M, Xu W, Sui L, Ren B, Li Q, Zhang ZK (2023) Fitness consequences of oviposition choice by an herbivorous insect on a host plant colonized by an endophytic entomopathogenic fungus. J Pest Sci 96:745–758. <https://doi.org/10.1007/s10340-022-01527-y>.

Figures

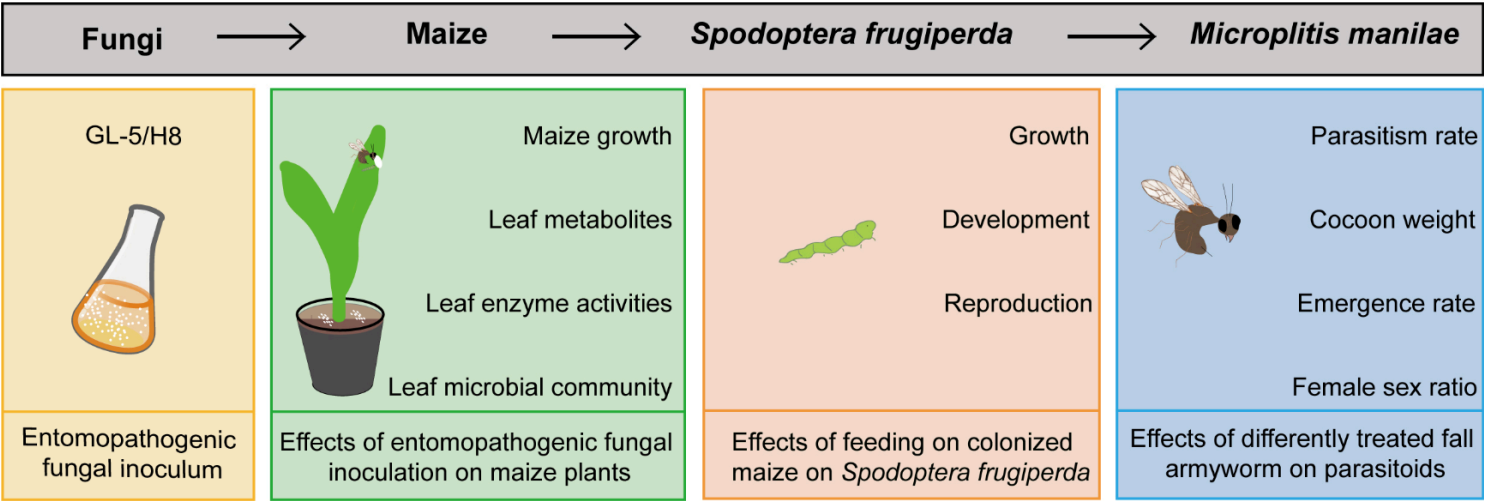


Figure 1

Schematic overview of the experimental design and measured variables across four trophic levels. Entomopathogenic fungal inocula (GL-5 and H8) were applied to maize plants, after which leaf growth,

metabolites, enzyme activities, and microbial communities were assessed. *S. frugiperda* fed on colonized maize were evaluated for growth, development, and reproduction. Subsequently, parasitoids (*Microplitis manilae*) reared on differently treated fall armyworm were examined for parasitism rate, cocoon weight, emergence rate, and female sex ratio. Arrows indicate the direction of trophic effects.

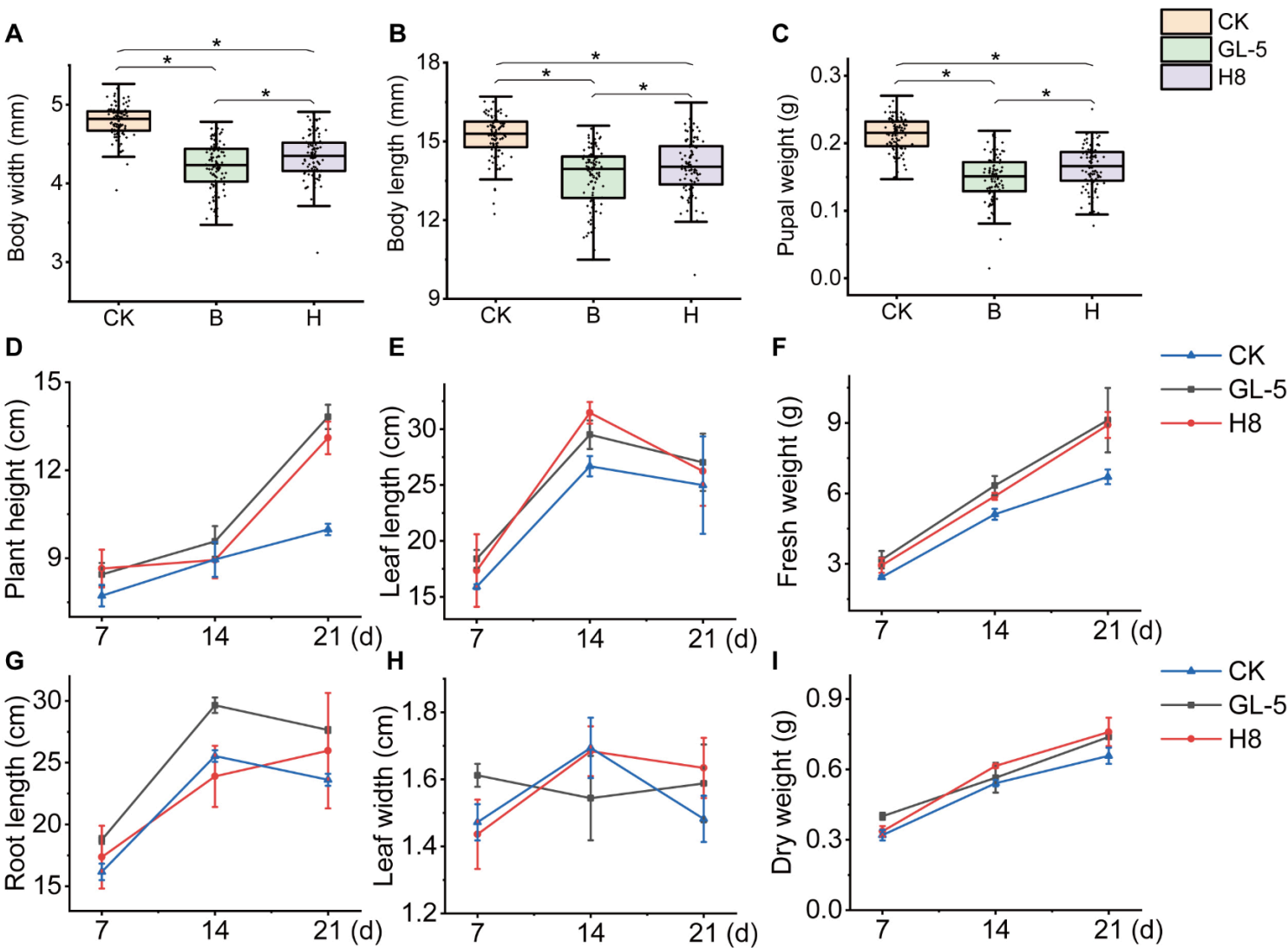


Figure 2

Growth performance of *S. frugiperda* larvae and maize seedlings under entomopathogenic fungal treatments. (A–C) Body width, body length and pupal weight of *S. frugiperda* larvae fed on maize inoculated with *B. bassiana* GL-5, *C. catenulata* H8 or sterile water (CK). (D–I) Temporal changes in plant height, leaf length, fresh weight, root length, leaf width and dry weight of maize seedlings at 7, 14 and 21 days post-inoculation (dpi). Asterisks indicate statistically significant differences between groups ($P < 0.05$).

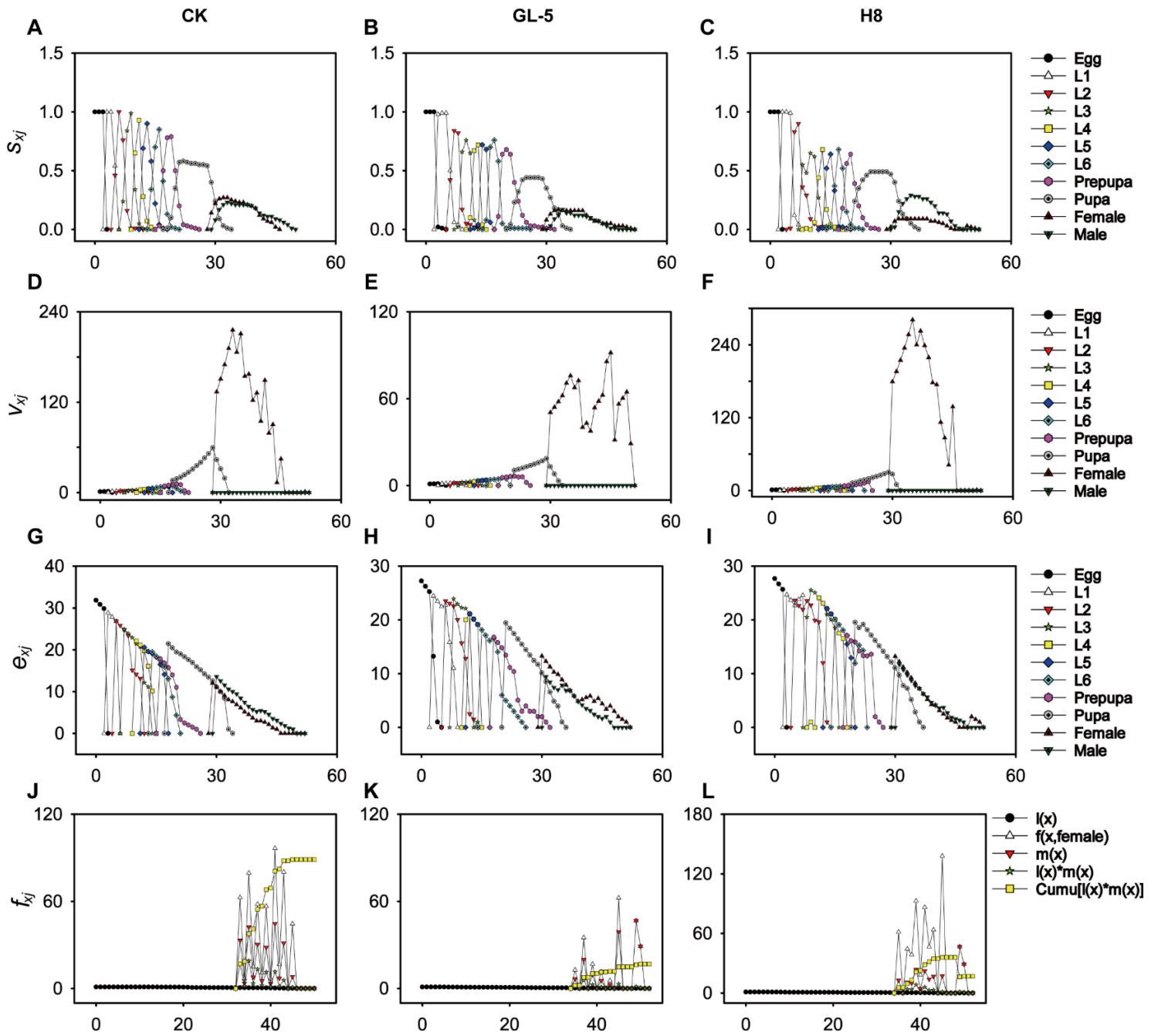


Figure 3

Age-stage, two-sex life table of *S. frugiperda* larvae fed on maize under different fungal treatments. (A–C) Age-stage specific survival rate (s_{xj}); (D–F) age-stage specific reproductive value (v_{xj}); (G–I) age-stage specific life expectancy (e_{xj}); (J–L) age-specific fecundity of female (f_{xj}), age-specific fecundity of the total population (m_x), age-specific net maternity ($l_x m_x$) and cumulative net reproductive rate. CK, control; GL-5, *Beauveria bassiana* GL-5 treatment; H8, *Cordyceps catenianulata* H8 treatment.

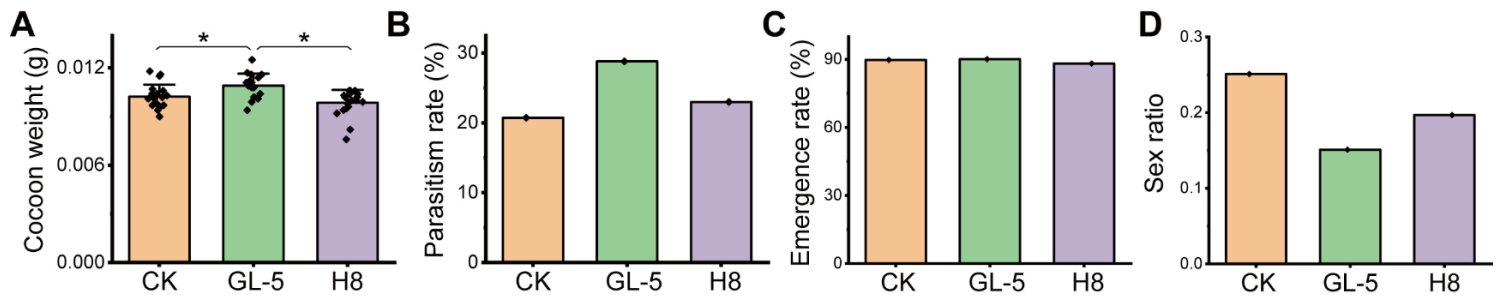


Figure 4

Fitness parameters of *M. manila* parasitizing *S. frugiperda* larvae fed on fungus-inoculated maize. (A) Parasitism rate. (B) Adult emergence rate. (C) Female sex ratio (proportion of female offspring). (D) Cocoon weight. CK, host larvae fed on control maize; GL-5, host larvae fed on *B. bassiana* GL-5 inoculated maize; H8, host larvae fed on *C. catenianulata* H8 inoculated maize. Data are presented as mean values (no significant differences were detected among treatments, $P > 0.05$).

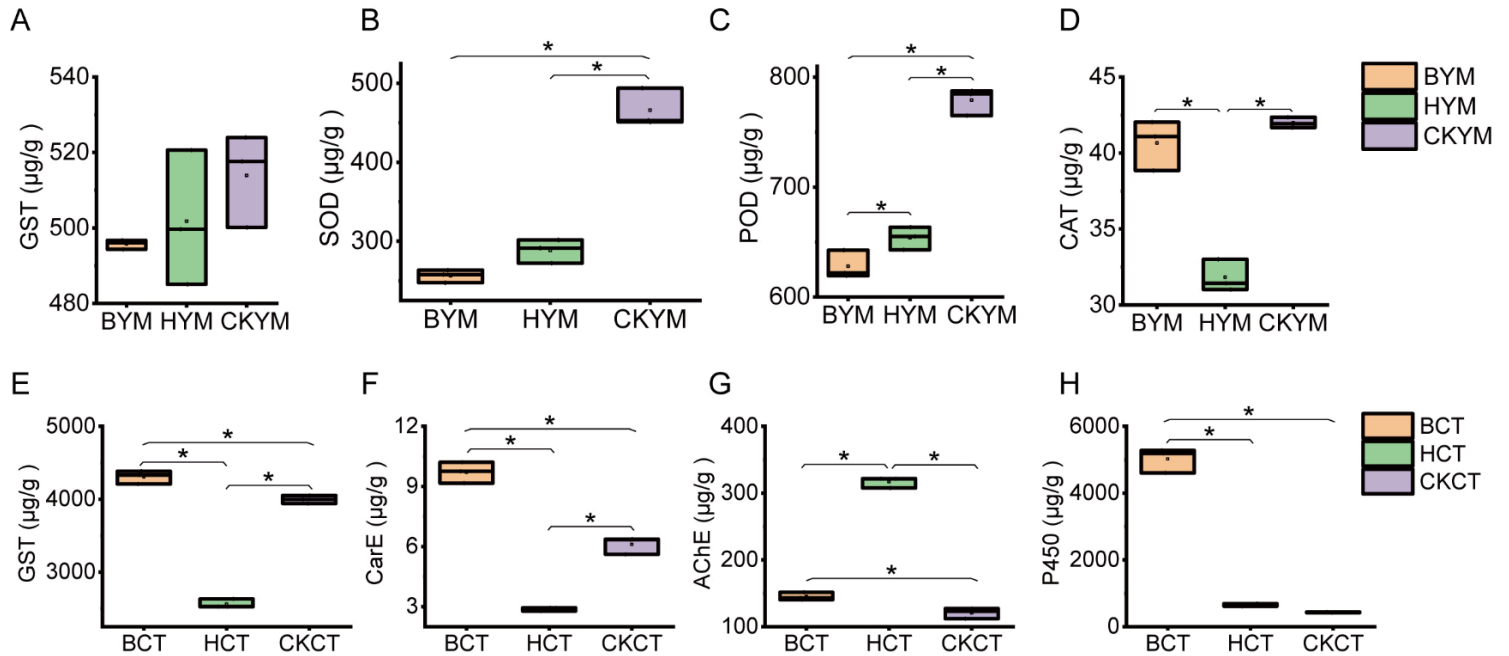


Figure 5

Activities of antioxidant enzymes in maize leaves and detoxifying enzymes in *S. frugiperda* larvae after entomopathogenic fungal inoculation. (A–D) Activities of glutathione S-transferase (GST), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in maize leaves inoculated with *Beauveria bassiana* GL-5 (BYM), *Cordyceps catenianulata* H8 (HYM) or sterile water control (CKYM). (E–H) Activities of GST, carboxylesterase (CarE), acetylcholinesterase (AChE) and cytochrome P450 (CYP450) in *S. frugiperda* larvae fed on maize from the corresponding treatments (BCT, HCT, CKCT). Asterisks indicate statistically significant differences between groups.

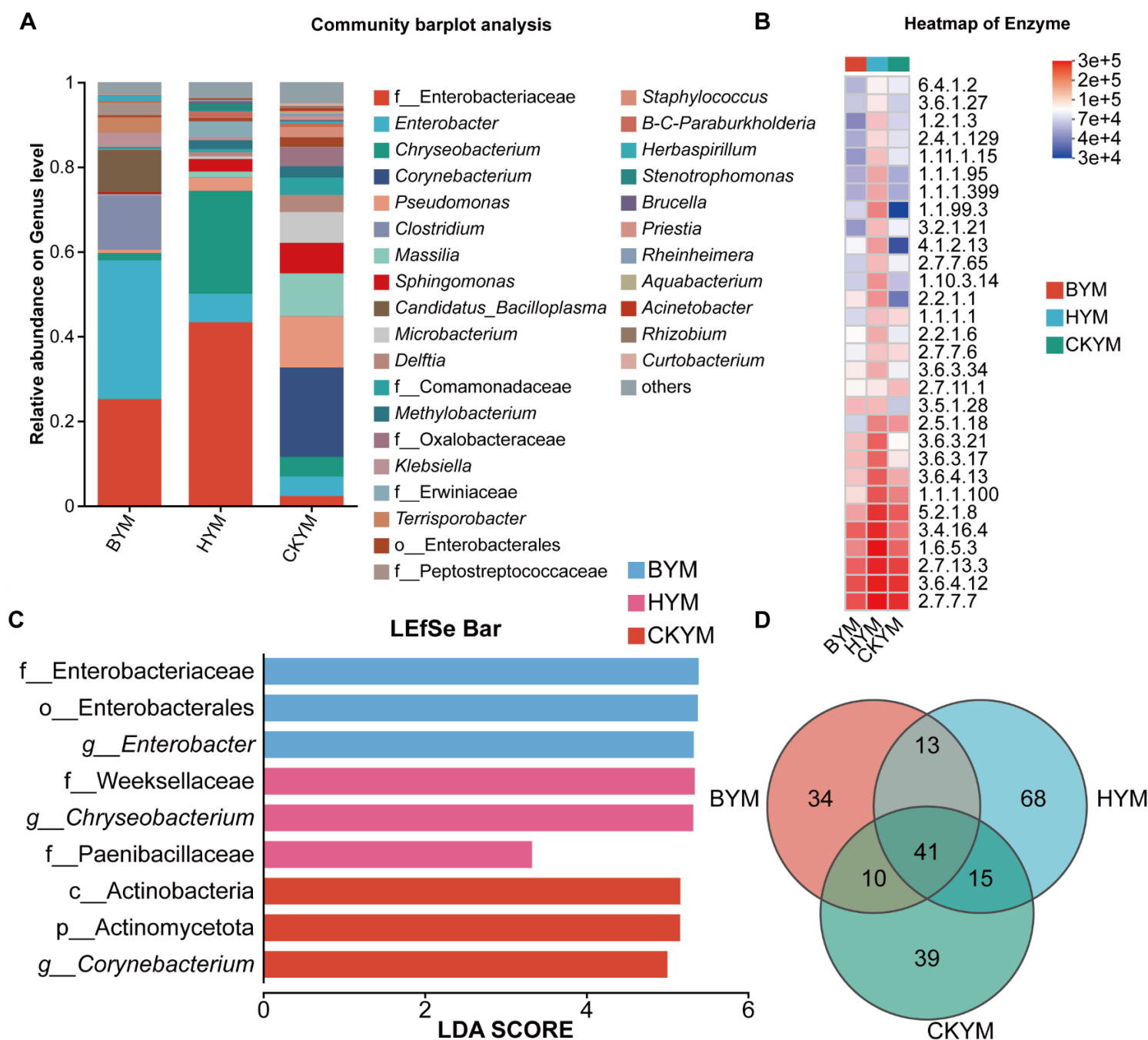


Figure 6

Bacterial community composition and differential taxa in maize leaves under different fungal treatments. (A) Relative abundance of bacterial communities at the genus level. (B) Heatmap of predicted functional enzyme profiles. (C) LEfSe bar plot showing differentially enriched bacterial taxa with LDA score > 4. (D) Venn diagram illustrating the number of shared and unique OTUs among the three groups. BYM, *Beauveria bassiana* GL-5-treated maize; HYM, *Cordyceps cateniannulata* H8-treated maize; CKYM, control maize.

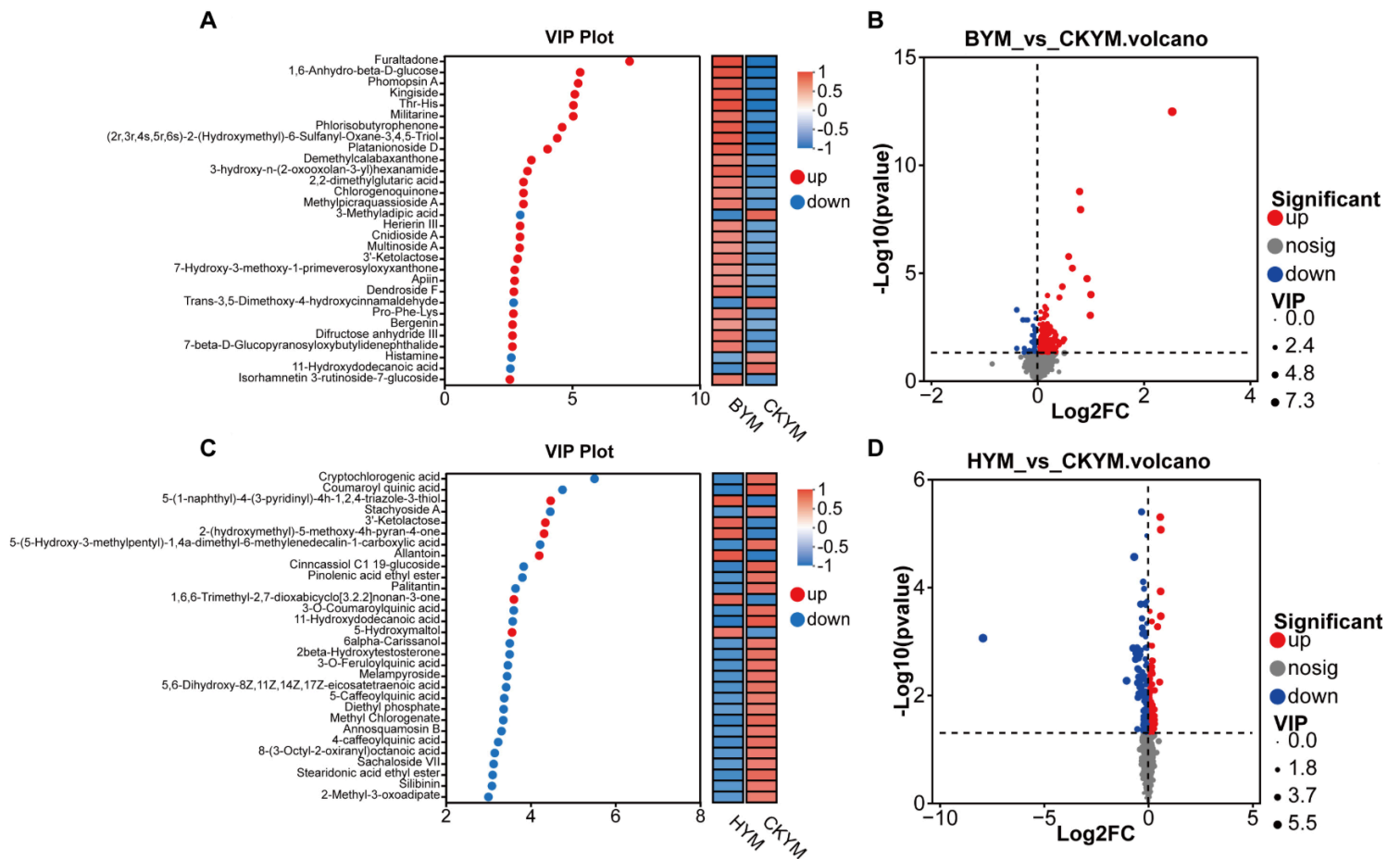


Figure 7

Screening of differential metabolites in maize leaves under entomopathogenic fungal treatments. (A, C) Variable importance in projection (VIP) plots from OPLS-DA analysis showing the top differential metabolites between (A) BYM and CKYM, and (C) HYM and CKYM. Red indicates up-regulated metabolites, blue indicates down-regulated metabolites. (B, D) Volcano plots illustrating the fold change and significance of differential metabolites between (B) BYM and CKYM, and (D) HYM and CKYM. Red dots represent significantly up-regulated metabolites, blue dots represent significantly down-regulated metabolites, and gray dots represent non-significant metabolites. Dot size corresponds to VIP value. BYM, *Beauveria bassiana* GL-5-treated maize; HYM, *Cordyceps cateniannulata*H8-treated maize; CKYM, control maize.

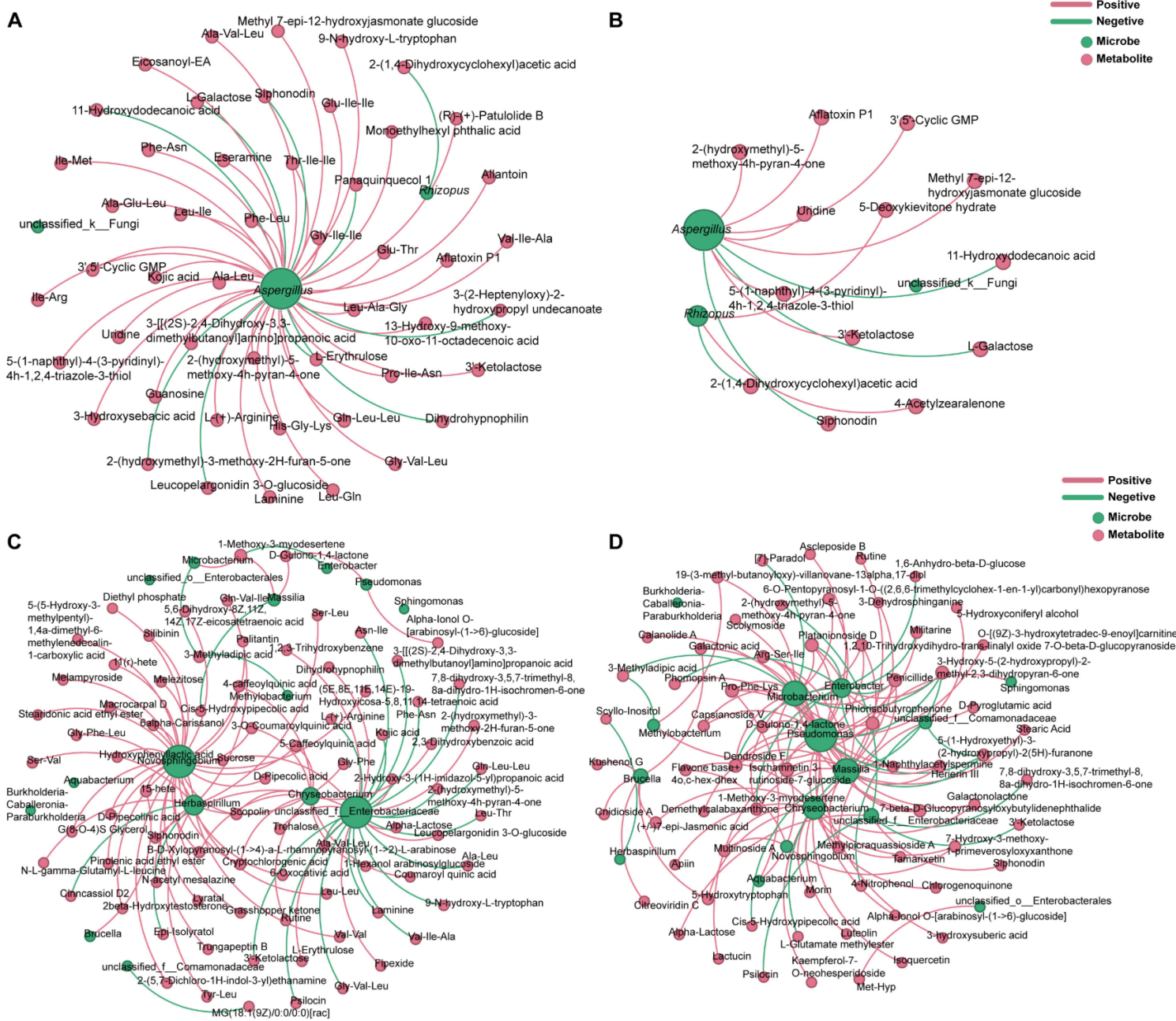


Figure 8

Correlation network between key microbial genera and differential metabolites in maize leaves based on Spearman correlation analysis. Green nodes represent dominant microbial taxa, pink nodes represent metabolites. Red lines indicate significant positive correlations, green lines indicate significant negative correlations. (A, B) Network of fungal genus *Aspergillus* and metabolites; (C) Network of core bacterial genera *Novosphingobium* and unclassified Enterobacteriaceae; (D) Network of bacterial genus *Pseudomonas* and metabolites.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable.xls](#)
- [SupplementaryInformation.docx](#)