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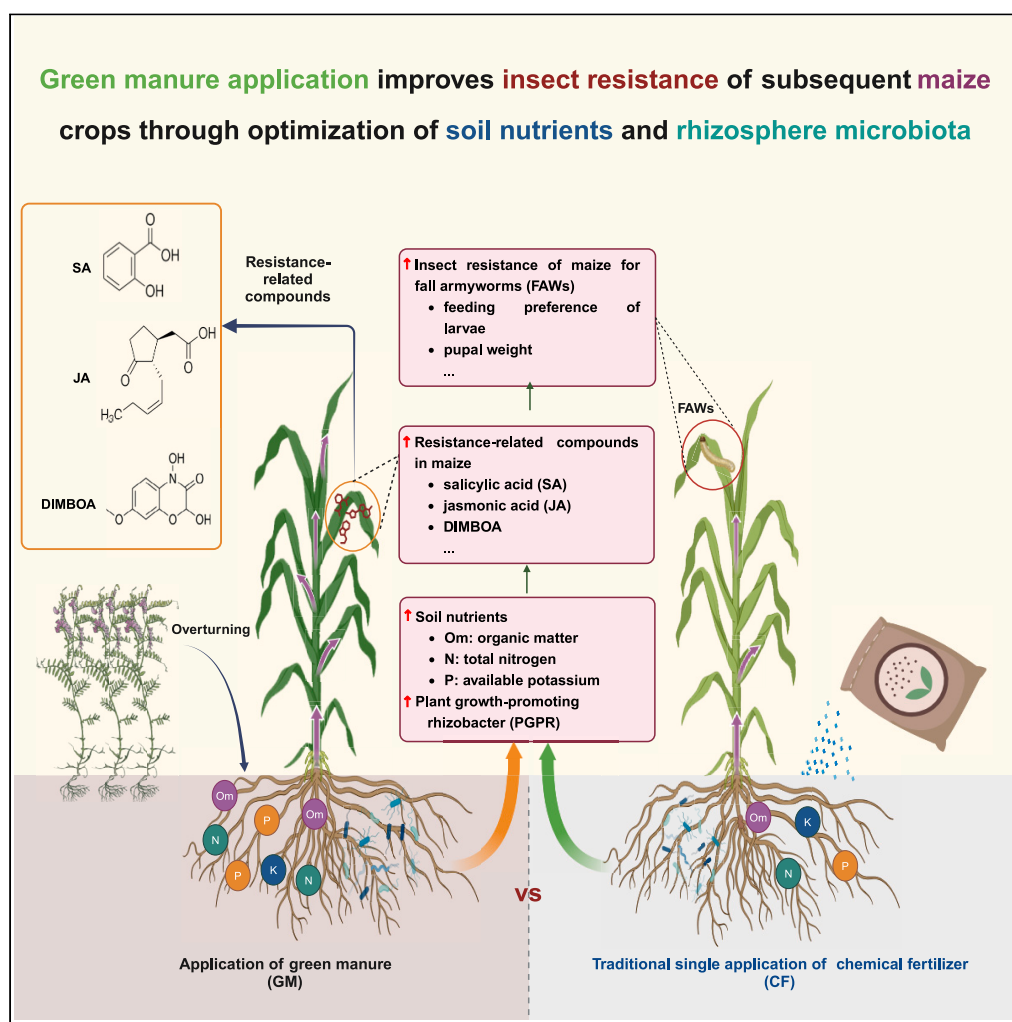**Article**

Green manure application improves insect resistance of subsequent crops through the optimization of soil nutrients and rhizosphere microbiota

Green manure application improves insect resistance of subsequent maize crops through optimization of soil nutrients and rhizosphere microbiota

Lin Ma, Daotong Wang, Lei Zhang, Yue Ge, Yueqiu Liu, Yunxia Cheng, Xingfu Jiang

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Highlights

We studied *V. villosa*–maize rotation system for resistance to fall armyworm (FAW)

V. villosa application improved insect resistance in the subsequent maize crop

Soil nutrient, rhizobacteria, and plant-resistance-compound status was improved

GM-amended soils and microbiota improve host plant resistances to pests

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Green manure application improves insect resistance of subsequent crops through the optimization of soil nutrients and rhizosphere microbiota

Lin Ma,[1,3] Daotong Wang,[1,3] Lei Zhang,[1,3] Yue Ge,[1,2] Yueqiu Liu,[2] Yunxia Cheng,[1] and Xingfu Jiang[1,4,*]

SUMMARY

Green manure (GM) enhances organic agriculture by improving soil quality and microbiota, yet its effects on plant resistance are unclear. Investigating the GM crop hairy vetch-maize rotation system, a widely adopted GM practice in China, we aimed to determine maize resistance to fall armyworm (FAW), *Spodoptera frugiperda* (Smith), a major pest. Greenhouse experiments with three fertilization treatments (chemical fertilizer, GM, and a combination) revealed that GM applications significantly improved maize resistance to FAW, evidenced by reduced larval feeding preference and pupal weight. GM also enriched soil nutrients, beneficial rhizobacteria, and resistance-related compounds, such as salicylic acid, jasmonic acid, and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), in maize. The results suggest that GM-amended soils and microbial communities may have an underestimated role in regulating host plant adaptation to pests by increasing plant resistance. This study can provide information for developing and implementing environmentally friendly and sustainable cropping systems with enhanced resistance to pests and diseases.

INTRODUCTION

Fertilizer application is frequently used to sustain crop quality and yield. However, in intensive farming, chemical fertilizer application typically exceeds than that required by the crops. Long-term excessive use of chemical fertilizers has become a major concern, as it has led to the degradation of soil quality, affected the soil nutrient conversion rate and growth of crops, and caused soil, water, and air pollution.[1–3] Effective methods to reduce chemical fertilizer application and increase fertilizer efficiency are constantly being researched and practiced to promote environmental protection, ecological improvement, and sustainable agricultural production and development.[4,5]

Green manure (GM) application is considered a good management practice and effective technical support for any agricultural production system.[6] It can improve soil nutrients and microbial community structure and, consequently, crop growth and yield.[7,8] In crop (e.g., maize, rice, wheat, and potato) rotation systems, it has been shown to play an active role in soil improvement and crop stability.[9–11] In rice–rice–GM rotational systems, the long-term planting of milk vetch (*Astragalus sinicus* L.) and winter rape (*Brassica napus* L.) as GM increased the content of dissolved organic matter in red paddy soils.[11] The incorporation of GM significantly increased the macro- and micronutrient contents of postharvest soil and avoided the adverse effects on sustainable rice production caused by continuous use of chemical fertilizers.[12] Compared with the more commonly used chemical fertilizers, the application of hairy vetch GM resulted in higher red pepper quality (T-N, P, and Mg content in plants) and yield, higher soil organic matter and available P, K, and Ca contents, and increased microbial populations that favor cultivation.[13]

A maize–GM crop rotation system has also been developed and is continuously promoted and practiced in the field.[14,15] The use of milk vetch GM altered soil properties (pH, alkali solution nitrogen, and available potassium) and soil microbial communities, and increased maize yield by 31.3%.[16] The combination of *Arachis pintoi* GM and natural phosphate improved maize growth, soil microbial community structure, and enzymatic activity.[17] Even in a low-fertility farmland rotation system, the application of GM has been shown to improve maize productivity, increase soil fertility and nutrients, and regulate soil microbial communities.[18] Compared to natural fallows, GM legume fallows can significantly increase maize grain yield and suppress weed populations.[19] Compared to monocropped maize, the maize–GM (soybean) rotational system can achieve low carbon emissions without compromising economic benefits.[15] Overall, GM can be used to reduce the application of chemical fertilizers and aid in sustainable agriculture.

Despite the emergence of research on the potential of GM to prevent and control subsequent crop diseases, pests, and weeds,[20,21] the prevention and control of GM and main crop rotation systems have been seriously neglected. GM can directly enhance the growth and health
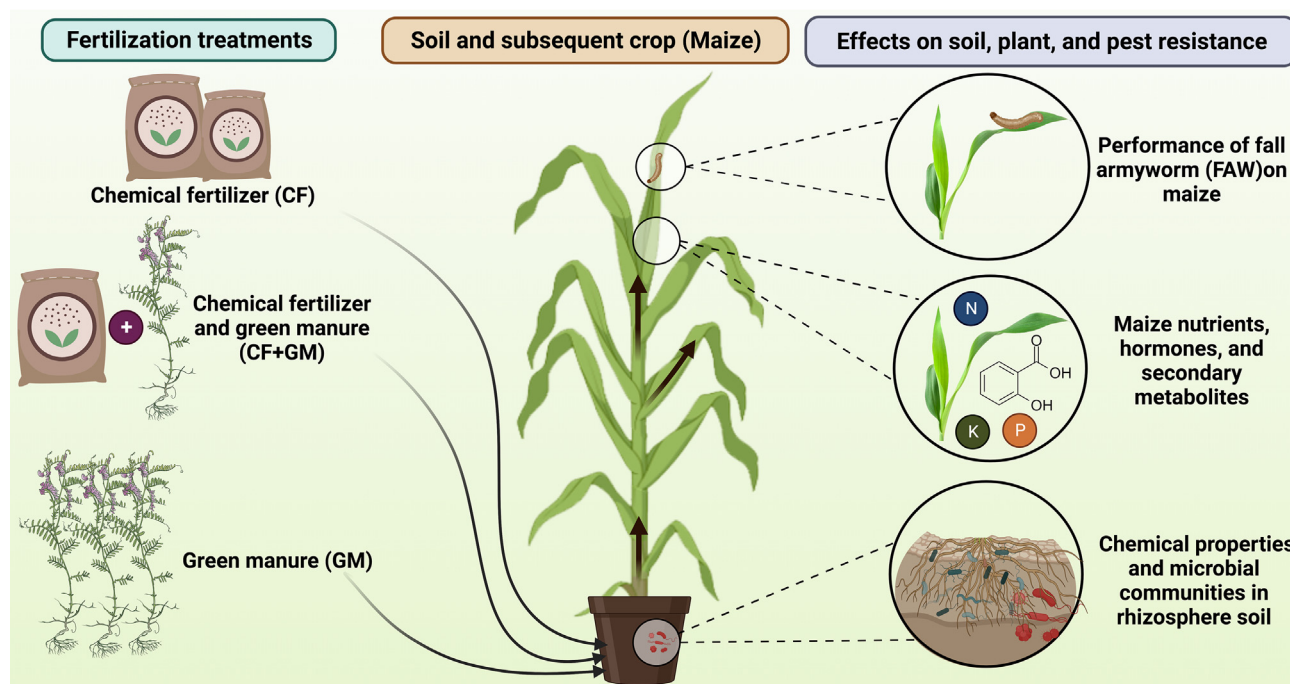
Figure 1. Experimental design
Effects of various fertilization treatments on rhizosphere soil, plant, and pest resistance of subsequent crops. Created with BioRender.com.

of the main crops while also improving their resistance to diseases and pests.[22,23] The use of the GM *Fumaria parviflora* Lam. (Fumariaceae) significantly enhanced all plant growth parameters and provided protection against the southern root-knot nematode *Meloidogyne incognita* (Kofoid and White) chitwood in tomato cultivation.[24] GM from Indian mustard (*Brassica juncea*) and wild rocket (*Diplotaxis tenuifolia*) enhanced cucumber (*Cucumis sativus* L.) resistance to Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *Cucumerinum*.[25] GM intercropping can enhance plant diversity in various crop systems, including grain, economy, and orchard crops. Additionally, it can significantly increase the abundance and diversity of natural enemies that feed on or parasitize weeds, diseases, insects, and nematodes in the ecosystem by providing habitats,[26,27] which is indirectly beneficial for the biological control of crop pests.[28,29] The application of *Panicum* green manure significantly inhibits the invasion of major diseases and pests on maize, especially the Africa stem borer (*Busseola fusca* (Fuller)), maize streak virus, and gray leaf spot.[30] However, research on the effect and mechanism of GM application on subsequent crop insect resistance, as well as on whether the improvement in soil nutrients and microorganisms after GM application is related to such resistance, is lacking.

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is a major global pest that poses a serious threat to food and cash crops such as maize, wheat, and cotton because of its wide host range, strong migration ability, and competitive advantage over other pest species.[31,32] In the event of a severe infestation in fields, this pest can cause a reduction in maize production by 20–72% in America and Africa, posing a serious threat to the economic income of corn growers and global food security.[33–35] The alarming speed at which this pest invades and spreads was evident from its infestation in Myanmar in late 2018, followed by its rapid spread to 27 Chinese provinces within a year, where it established itself as the dominant pest.[36]

FAW has been known to damage GM crops.[37] It is a major pest threatening maize production. As mentioned above, the GM–maize rotation system has been widely promoted in China, but little is known about the insect resistance of subsequent crops when using GM. We hypothesized that GM–maize rotation can enhance the health and resistance of maize crops to FAW by regulating soil chemical properties and microbial structure in the rhizosphere. To test our hypothesis, we implemented three fertilization treatments and investigated the effect of various fertilization treatments on the insect resistance of subsequent maize crops to FAW (Figure 1). We also analyzed the comprehensive effects of these treatments on rhizosphere soil nutrients and microorganisms, as well as their correlation with insect resistance in subsequent maize crops (Figure 1). Our findings can serve as a foundation for implementing a novel approach for utilizing GM to manage pests and promote the sustainable development of agricultural ecosystems.

RESULTS

Effects of green manure application on subsequent maize resistance characteristics

The effects of various fertilization treatments on the resistance characteristics of maize crops were explored by measuring the levels of nutrients, hormones, and secondary metabolites in the leaves. There were no significant differences in the contents of major nutrient elements (nitrogen, potassium, phosphorus, and carbon) among subsequent maize crops under various fertilization treatments ($p > 0.05$) (Table S2).
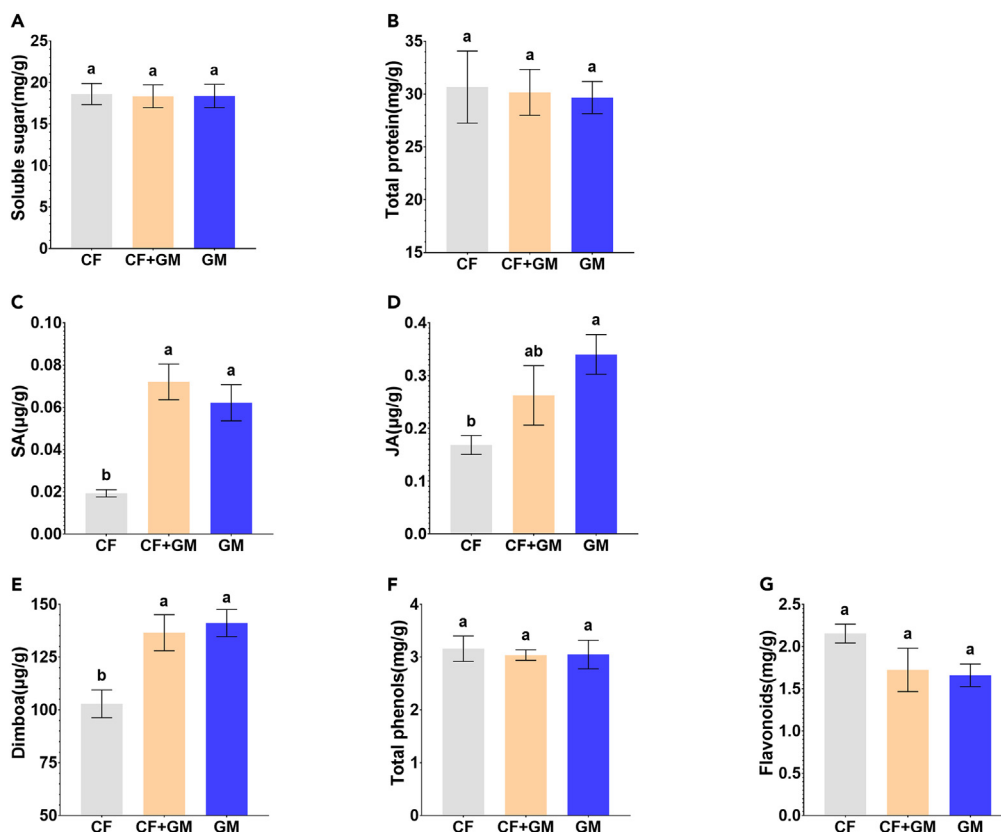
Figure 2. Levels of nutrients, hormones, and secondary metabolites in subsequent maize leaves under various fertilization treatments

Levels of nutrients (A, soluble sugar; B, total protein), hormones (C, SA; D, JA), and secondary metabolites (E, DIMBOA; F, total phenols; G, flavonoids) in subsequent maize leaves under various fertilization treatments. Lowercase letters indicate a significance difference ($p < 0.05$). SA: salicylic acid; JA: jasmonic acid; DIMBOA: 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; CF: chemical fertilizer; GM: green manure. Data are represented as mean $\pm$ SE.

Similarly, no significant differences were observed in the total protein and soluble sugar levels in the subsequent maize crops among the various treatments ($F_{2,14} = 0.012$, $p = 0.99$; $F_{2,14} = 0.040$, $p = 0.96$) (Figures 2A and 2B). However, the hormone levels in subsequent maize crops varied significantly across the different treatments. Compared with the CF treatment, both the CF + GM and GM treatments resulted in higher SA content in maize ($F_{2,14} = 16.024$, $p < 0.01$) (Figure 2C), whereas the GM treatment had the highest JA content in maize ($F_{2,14} = 4.569$, $p = 0.03$) (Figure 2D). Furthermore, the effects of the different treatments on the secondary metabolites of maize varied. The DIMBOA content in maize was significantly higher under the CF + GM and GM treatments than under the CF treatment ($F_{2,14} = 8.304$, $p < 0.01$) (Figure 2E). However, there were no significant differences in the total phenol and flavonoid content among the three fertilization treatments ($F_{2,14} = 0.100$, $p = 0.91$; for flavonoids, $F_{2,14} = 2.249$, $p = 0.15$) (Figures 2F and 2G).
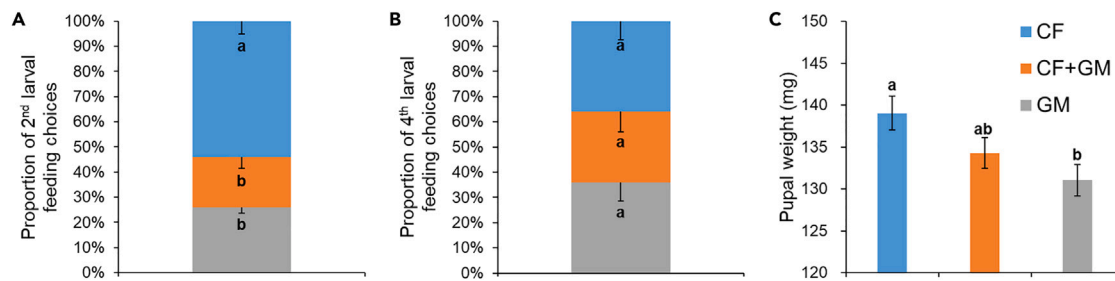
Performance of fall armyworm on subsequent maize crop under various fertilization treatments

The feeding preference of FAW larvae on subsequent maize crops under various fertilization treatments was measured. The results showed that the proportion of 2nd instar larvae in feeding choices on maize leaves was significantly lower under both the CF + GM and GM treatments than under the CF treatment ($F_{2,14} = 19.000$, $p < 0.01$) (Figure 3A). However, the 4th instar larvae did not show significant differences in their feeding choices of maize leaves under various fertilization treatments ($F_{2,14} = 0.364$, $p = 0.703$) (Figure 3B).

The pupal weight of FAW that fed on subsequent maize crop under various fertilization treatments showed no significant difference between the CF and GM treatments when compared with that in the CF + GM treatment; however, the GM treatment significantly reduced the pupal weight compared to that in the CF treatment ($F_{2,14} = 4.508$, $p = 0.014$) (Figure 3C).

We also measured the life-table parameters of FAWs fed on subsequent maize crops under various fertilization treatments; no significant difference in R_0, r, λ, and T was observed ($p > 0.05$) (Table S3).

The nutrition utilization indices of the 5th instar FAW larvae fed on subsequent maize leaves with various fertilization treatments were measured. The results showed no significant differences among the three treatments for all five nutritional utilization indices (RGR: $F_{2,29} = 0.157$, $p = 0.855$; RCR: $F_{2,29} = 0.034$, $p = 0.966$; ECI: $F_{2,29} = 0.907$, $p = 0.416$; ECD: $F_{2,29} = 1.145$, $p = 0.333$; and AD: $F_{2,29} = 0.925$, $p = 0.409$) (Table S4).

Figure 3. Performance of FAW on subsequent maize crop under various fertilization treatments

Feeding preference of 2nd (A) and 4th (B) instar larvae for subsequent maize leaves crop and pupal weight of FAW fed on subsequent maize crop (C) under various fertilization treatments. CF: chemical fertilizer; GM: green manure; FAW: fall armyworm. Lowercase letters indicate a significance difference ($p < 0.05$). Data are represented as mean ± SE.

Effects of green manure application on rhizosphere soil chemical properties

The chemical properties of the rhizosphere soil in the subsequent crops were significantly influenced by the various fertilization treatments. The contents of organic matter ($F_{2,12} = 59.77$, $p < 0.001$) (Figure 4A), total nitrogen ($F_{2,12} = 55.79$, $p < 0.001$) (Figure 4B), and available potassium ($F_{2,12} = 59.39$, $p < 0.001$) (Figure 4E) were the highest in the GM treatment, followed by the CF + GM treatment, while the CF treatment had the lowest levels. There was no significant difference between the GM and CF + GM treatments in terms of alkali-hydrolyzed nitrogen content; however, both treatments were significantly higher than the CF treatment ($F_{2,12} = 12.77$, $p < 0.01$) (Figure 4C). The available phosphorus content in the CF + GM treatment was the highest, and there was no significant difference between CF and GM ($F_{2,12} = 9.61$, $p < 0.01$) (Figure 4D). Furthermore, the pH value in the GM treatment was significantly higher than that in the CF and CF + GM treatments ($F_{2,12} = 6.111$, $p < 0.05$) (Figure 4F). Collectively, the application of GM promoted the accumulation of nutrients in the rhizosphere soil.

Effects of green manure application on rhizosphere soil microbial community composition

After performing metagenomic sequencing analysis on 12 rhizosphere soil samples from the three different treatments, 798,497 unigenes were obtained through CDS gene prediction. The average length of the unigenes was 508.22 bp. Among the three treatments, 786,394 shared unigenes, 2,436 unique unigenes in the CF treatment, 3,719 unique unigenes in the CF + GM treatment, and 5,948 unique unigenes in the GM treatment were observed.

The α-diversity of samples at the unigene level was estimated using Chao1, good coverage, and Shannon indices, among others (Figure S1). There was no difference in the good coverage index among the three treatments, and all were close to 1. This indicated that the probability of new species not being detected in the samples was low, suggesting that the sequencing results could accurately represent the real situation of the samples (Wilcoxon test, $p = 1.00$). The Chao1, Shannon, and Simpson indices showed no significant differences among the three treatments (Kruskal-Wallis test for Chao1, $p = 0.67$; Kruskal-Wallis test for Shannon, $p = 0.17$; Kruskal-Wallis test for Simpson, $p = 0.12$), indicating similar levels of species richness and evenness among the three treatments.

The β-diversity of samples at the unigene level illustrated similarities and differences in species composition and community structure. According to the principal coordinate analysis (PCoA), our results showed significant differences in microbial composition among the different treatments (adonis, $R^2 = 0.43$, $p = 0.01$) (Figure 5). The microbial community compositions between the GM and CF + GM treatments were similar (anosim for GM vs. CF + GM, $R = 0.14$, $p = 0.20$), but significantly different from that in the CF treatment (anosim for CF vs. CF + GM vs. GM, $R = 0.49$, $p < 0.01$).

All unigenes were annotated into 166 phyla, 299 classes, 519 orders, 1013 families, 3081 genera, and 16210 species using the NR database in BLAST. At the genus level, the top 20 genera with the highest relative abundance were detected and annotated across the three treatments (Figure 6A). *Bacteria_unclassified* occupied a dominant position in all treatments, with an average relative abundance of >18%. Among the top 20 genera with the greatest abundance, 5 showed significantly different abundances among the three treatments (Kruskal–Wallis test; $p < 0.05$). *Arthrobacter* ($p = 0.02$), *Variovorax* ($p = 0.01$), *Pseudoxanthomonas* ($p = 0.02$), *Glycomyces* ($p = 0.02$), and *Promicromonospora* ($p = 0.01$) were more abundant in the GM and CF + GM groups than in the CF group. Linear discriminant analysis (LDA) Effect Size (LefSe) analysis was performed to further explore the biomarkers with significant differences in relative abundance across treatments (Figures 6B–6D). There was little difference between the GM and CF + GM treatments, and only the genus *Variovorax* was found to be a biomarker with a higher abundance in the GM treatment (Figure 6B). Compared to the GM and CF + GM treatments, the CF treatment showed significant differences (Figures 6C and 6D). The genera *Glycomyces*, *Aeromicrobium*, and *Arthrobacter* were identified as biomarkers with higher abundances in both the GM and CF + GM treatments (LDA scores >3), whereas the genera *Promicromonospora* and *Variovorax* were identified as biomarkers with higher abundances, specifically in the GM treatment (LDA scores >3). Additionally, *Sphingobium* was found to be a biomarker with higher abundance in the CF treatment (LDA scores >3).

Furthermore, co-occurrence networks of rhizosphere microbial communities were constructed using Spearman's correlation analysis based on the 100 most abundant species (Figures 7A–7C). The topological features of the network, including the number of nodes, edges,
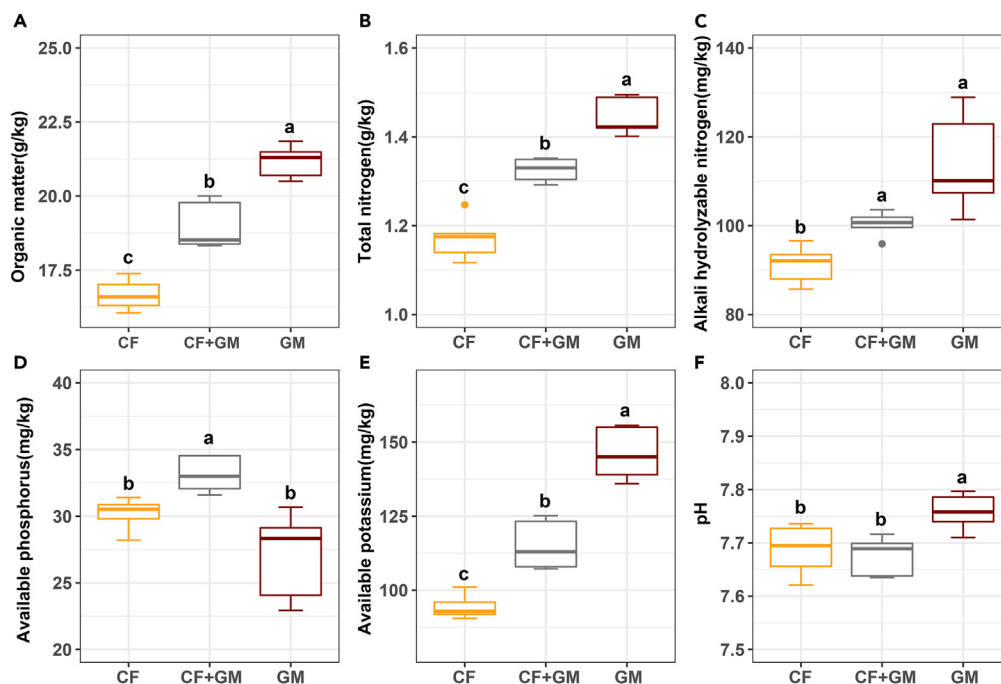
Figure 4. Rhizosphere soil chemical properties of subsequent crops under various fertilization treatments

Rhizosphere soil chemical properties of subsequent crops were influenced by various fertilization treatments: (A) organic matter (g/kg), (B) total nitrogen (g/kg), (C) alkali hydrolyzable nitrogen (mg/kg), (D) available phosphorus (mg/kg), (E) available potassium (mg/kg), and (F) pH. Lowercase letters indicate statistically significant differences between the various treatments ($p < 0.05$). CF: chemical fertilizer; GM: green manure. Data are represented as mean ± SE.

density, diameter, and average degree, were greater in the CF + GM treatment than in the other two treatments (Figures 7D and 7E, Table S5), indicating a higher complexity in the topological structure of the microbial community co-occurrence network in the CF + GM treatment.

Correlation between microbial taxa and chemical properties of rhizosphere soil in subsequent crops, subsequent crop chemical characteristics, and insect resistance to fall armyworms

Various fertilization treatments, particularly the application of GM, significantly influenced the chemical properties and microorganisms of the rhizosphere soil in subsequent crops, as well as the chemical characteristics of these crops and their resistance to FAWs. Therefore, correlations were calculated between the microbiota taxa of the rhizosphere soil (the top four orders and top five genera based on the relative abundance differences among various fertilization treatments), subsequent crop chemical characteristics (such as JA, SA, and DIMBOA content), rhizosphere soil chemical properties (including the content of organic matter, total nitrogen, alkali-hydrolyzed nitrogen, available potassium, available phosphorus, and pH), and subsequent crop chemical characteristics. Additionally, correlations between crop chemical characteristics and FAWs performance (such as feeding preference and pupal weight) were examined.

For the top four orders in terms of relative abundance differences among the various fertilization treatments, the relative abundances of Corynebacteriales, Glycomycetales, and Micrococcales were significantly positively correlated, and that of Verrucomicrobiales was significantly negatively correlated with the contents of JA, SA, and DIMBOA in the subsequent maize crops (Figure 8A) ($p < 0.05$). For the top five genera in terms of relative abundance differences among various fertilization treatments, all genera, including *Variovorax*, *Glycomyces*, *Promicromonospora*, *Pseudoxanthomonas*, and *Arthrobacter* were significantly positively correlated with the contents of JA, SA, and DIMBOA in the subsequent maize crop (Figure 8A) ($p < 0.05$). For rhizosphere soil chemical properties, the contents of organic matter, total nitrogen, alkali-hydrolyzed nitrogen, and available potassium were significantly and positively correlated with the levels of JA, SA, and DIMBOA in the subsequent maize crops (Figure 8B) ($p < 0.05$). Regarding FAWs performance, the levels of hormones (JA and SA) and secondary substances (DIMBOA) in subsequent maize crops were significantly negatively correlated with the feeding preference of 2nd instar larvae toward subsequent maize crops (Figure 8C) ($p < 0.05$) but not with the feeding preference of 4th instar larvae toward subsequent maize crops or the pupal weight of FAWs on subsequent maize crops (Figure 8C) ($p > 0.05$).

DISCUSSION

Effects of green manure application on the plant resistance of subsequent crops

The choice of fertilization strategy can significantly affect soil nutrients, physical and chemical properties, and the microbiota present in the soil, ultimately influencing crop growth and development.[38,39] The resistance of a plant to biotic and abiotic stresses is influenced by its health
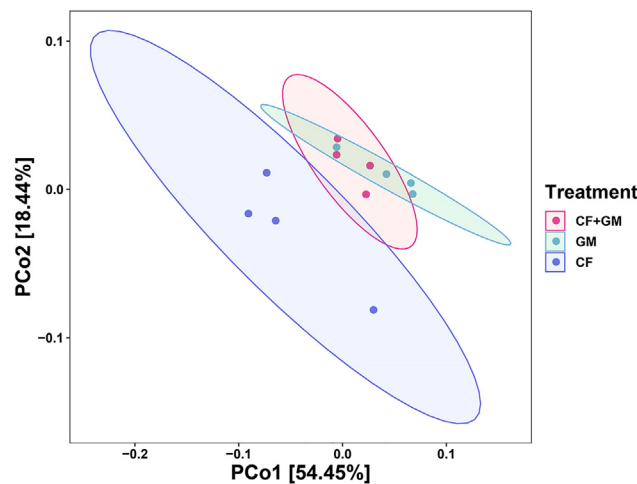
Figure 5. The principal coordinates analysis (PCoA) of rhizosphere microbial community among three different treatments based on Bray–Curtis distance
Different colors represent various groupings (adonis). CF: chemical fertilizer; GM: green manure.

status, which includes the levels of plant nutrition, hormones, and secondary metabolites.[39–41] In this study, the application of GM had no impact on plant nutrient content but significantly enhanced the levels of hormones and certain secondary metabolites in subsequent crops. The levels of SA, JA, and DIMBOA in maize in the GM treatment were notably higher than those in the CF treatment. Specific hormones or secondary metabolites present in host plants significantly affect the survival and growth of herbivorous pests.[42] SA and JA play crucial roles in regulating plant defense responses.[43] SA is commonly associated with pathogen infestation and damage caused by piercing-sucking pests, whereas the JA signaling pathway is typically activated by chewing insects.[44,45] DIMBOA, the primary cyclic hydroxamic acid found in maize, effectively inhibits insect feeding and growth by targeting key enzymes involved in nervous system, digestion, and detoxification processes.[46,47] The application of GM can significantly enhance crop resistance to pests and diseases by increasing the levels of hormones and secondary metabolites associated with plant defense.

Effects of green manure application on fall armyworm performance fed on subsequent maize crop

Advocates of organic agriculture generally contend that crops nourished by biological materials, such as GM and composted organic waste, exhibit greater resistance to pests than those grown using CFs.[21] For instance, organic management has been shown to plant resistance to insects by promoting SA accumulation in plants, which is facilitated by the rhizosphere microbial communities.[48] However, only a few studies support this perspective. While previous studies have already demonstrated the direct effects of GM crops on the growth potential of herbivorous insects,[37,49,50] our study focused on examining the effects of applying GM on the development, survival, and fecundity of herbivorous insects feeding on subsequent crops. In the present study, GM application had a significant effect on the performance of FAWs fed on subsequent maize crops. The 2nd instar FAW larvae showed a significantly lower feeding preference for the leaves of subsequent maize crops in the GM and CF + GM treatments than in the CF treatment. The pupal weight of FAWs fed subsequent maize crop leaves under the GM treatment was significantly lower than of those under the CF treatment. In other words, GM application resulted in a decrease in both feeding preference and the growth and development of FAWs in subsequent maize crops. However, the negative effects of GM application on FAW performance in subsequent crops appeared to be more pronounced in younger larvae and less pronounced in older larvae and adults. GM application had no significant effect on the feeding choice of 4th instar larvae, nutrient utilization index of 5th instar larvae, life-table parameters, or adult fecundity. This may be related to the increase in food intake and metabolism, as well as the resistance level of older larvae (from 4th instar to before-mature larvae),[51–53] enabling them to withstand changes in resistance levels induced by GM application on subsequent maize crops. However, a single application of GM may not sufficiently affect the subsequent crop resistance levels or the feeding and growth of older larvae. Further verification of the effects of multiple or long-term GM applications on subsequent crop resistance levels and phytophagous pest performance is required.[11,54]

Green manure application improved the chemical properties of rhizosphere soil

The rational application of GM can effectively reduce the reliance on chemical fertilizers in agricultural production by providing essential nutrients, which is a crucial technical method for achieving environmentally friendly and sustainable development of crops.[7] The incorporation of GM significantly enhances the biological properties of the soil, resulting in an increase in both the macro- and micronutrient contents.[12,55] Introducing various GM crops, including hairy vetch, during the summer fallow period enhanced the soil nutrient levels of available phosphorus, potassium, and hydrolyzable nitrogen, leading to an increase in winter wheat yield.[56] Compared
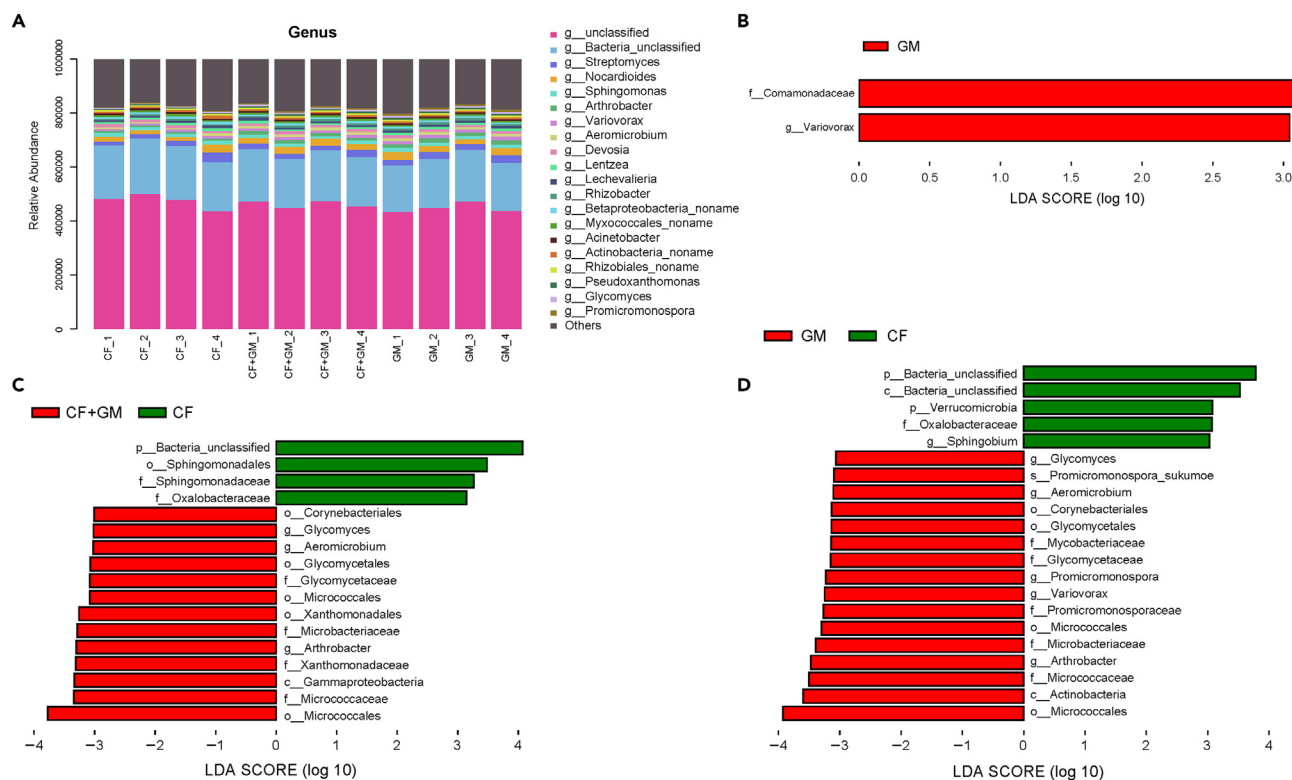
Figure 6. Relative abundance and LEFSe analysis of rhizosphere microbial community among three different treatments

Relative abundances of the top 20 bacterial genera with the highest abundances in various treatments (A) and LEFse analysis for pairwise comparisons between different treatments (LDA scores >3):

(B) GM vs. CF + GM; (C) CF vs. CF + GM; and (D) CF vs. GM. LEFse: LDA Effect Size; LDA: linear discriminant analysis; CF: chemical fertilizer; GM: green manure.

to mixed biochar and fertilizer treatments, soil microbial biomass C, mineral N, available P, and pH were generally higher in soils that received mixed GM and biochar applications.[55,57] The rhizosphere is a complex system wherein interactions occur between microorganisms and nutrient elements, both of which are influenced by plant species and the environment while also affecting biogeochemical cycling and plant growth.[58,59] In this study, we assessed the effects of various fertilization treatments on the chemical properties of rhizosphere soil in subsequent crops. The results revealed that compared with the application of only CF (CF treatment), the application of GM (for both GM and CF + GM treatments) significantly increased the content of nutrients (such as organic matter, total nitrogen, available potassium, and alkali-hydrolyzed) in the rhizosphere soil. The application of GM increased the levels of soil nutrient indicators from the initial fourth to the third class (moderate), effectively enhancing the soil quality to meet the requirements for subsequent crop growth and development. These results further emphasize the crucial ecological role of GM in enhancing soil nutrients within agricultural systems.

Effects of green manure application on subsequent maize crop rhizosphere microbial community composition

The composition of the rhizosphere microbial community is influenced by soil factors, agricultural practices, crop species, and environmental conditions.[60] Various fertilization strategies in agricultural production have significant impacts on the diversity and community structure of soil microbial populations.[61] The application of GM, as an ecofriendly management technique, has the potential to alter both soil microbial richness and community composition. Long-term rice–rice–GM rotation resulted in a decrease in microbial diversity and richness but also led to the enrichment of beneficial bacteria, such as *Acinetobacter* and *Pseudomonas* in the rice rhizosphere.[62] The application of GM, which consisted of a mixture of five cover crops (*Vicia sativa*, *Pisum sativum*, *Vicia faba*, *Secale cereale*, and *Brassica napus*), significantly increased the abundance of bacterial taxa involved in the soil nitrogen cycle, such as *Microvirga* sp., *Pontibacter* sp., and *Nitrospira* sp., in organic vineyards.[63] Incorporating hairy vetch as GM not only increased fungal biomass and diversity, but also stimulated the activity of soil fungi compared to that observed for chemical fertilizer and non-GM treatments, which led to an increased abundance of *Cladosporium* spp. and phosphatase activity in the soil.[64] In our current study, the application of GM had a significant effect on the rhizosphere soil microbiota. Among the top 20 most abundant genera, 5 genera (*Arthrobacter*, *Variovorax*, *Pseudoxanthomonas*, *Glycomyces*, and *Promicromonospora*) were more abundant in the GM and CF + GM treatments than in the CF treatment.
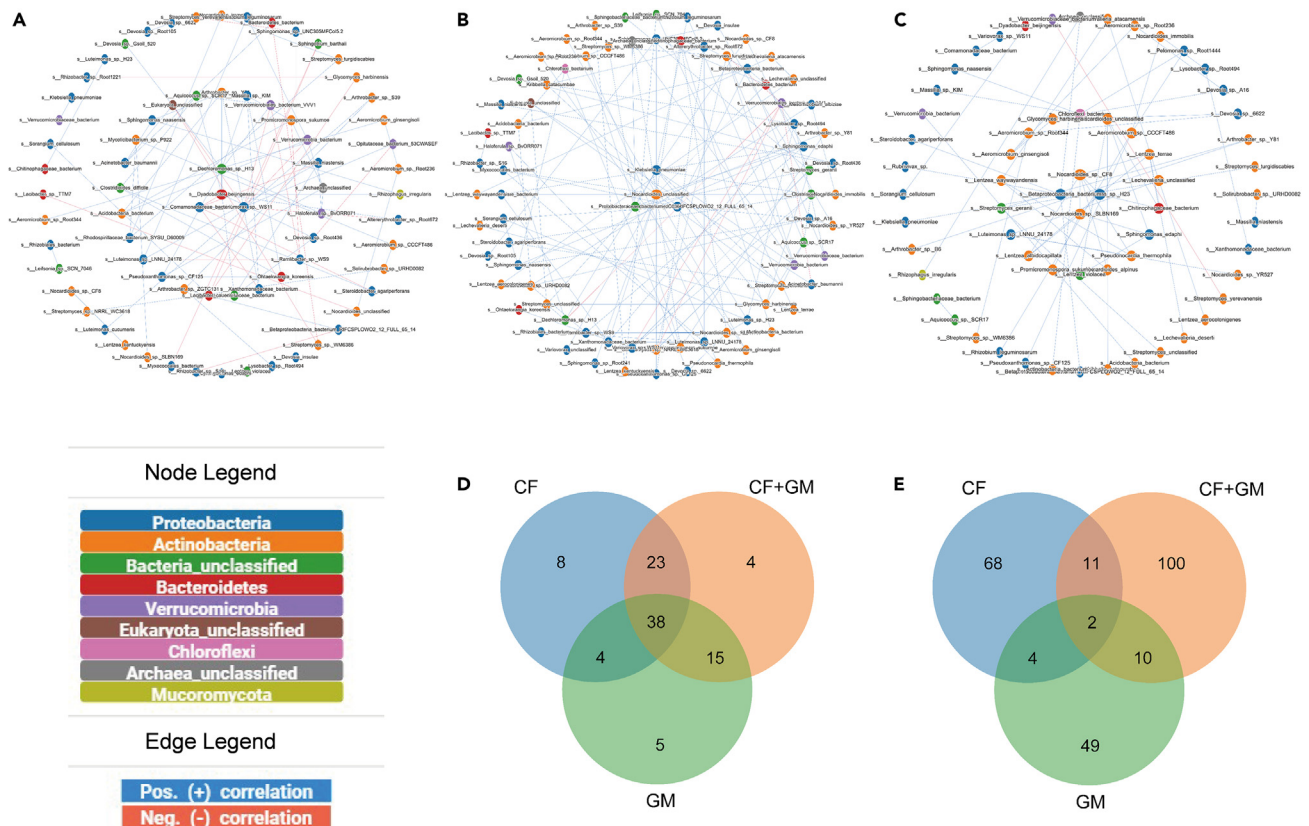
Figure 7. The co-occurrence network analysis of the top 100 species with various treatments based on the Spearman correlation algorithm (Spearman, p < 0.03, |r| > 0.8)

(A) CF treatment; (B) CF + GM treatment; (C) GM treatment; (D) Venn diagram showing edges with various treatments; (E) Venn diagram showing nodes with various treatments. CF: chemical fertilizer; GM: green manure.

The rhizosphere microbial community, also known as the "second genome" of plants, plays a crucial role in crop growth, development, and resistance by its interactions with crop roots and root exudates.[23,65,66] *Arthrobacter* has been reported to promote plant growth in rice and tobacco, as well as exhibit antibacterial activity against plant pathogens, such as *Ralstonia solanacearum* and human pathogens.[67,68] The inhibition of *Arabidopsis* root growth induced by a complex, ecologically realistic, synthetic root community can be reversed by a single bacterial genus, *Variovorax*.[69] *Pseudoxanthomonas* can enhance plant growth and facilitate bioremediation by degrading proteins and benzene compounds.[70] Ryegrass-rice rotation systems in Italian agriculture have been found to enhance the relative abundance of microorganisms involved in nutrient cycling, leading to improved soil fertility and enhanced rice growth.[71] Decomposition of cover crops alters the relative abundance of specific root-associated microbiomes, such as those comprising Bacillaceae and Mortierellomycetes, thereby enhancing the disease tolerance of cash crop seedlings.[23] Alterations in both the abundance and composition of rhizosphere soil microbial communities triggered by the application of GM, as demonstrated in this study, are expected to exert a significant effect on the growth, development, and resistance of subsequent crops.

Correlation between rhizosphere soil microbiome composition, chemical properties, and insect resistance of subsequent crops

The correlation between the rhizosphere soil microbiota and subsequent crop chemical characteristics can provide insights into the root-associated microbial groups that influence hormones and secondary substances in the subsequent crops. In our study, four of the top five rhizosphere soil microbial genera (in terms of differences in relative abundance induced by various fertilization strategies), *Variovorax*, *Pseudoxanthomonas*, *Glycomyces*, and *Promicromonospora*, were significantly and positively correlated with compounds (JA, SA, and DIMBOA) related to crop resistance. Plant growth-promoting rhizobacteria (PGPR), which are microorganisms that live in close association with plants, influence the synthesis and homeostasis of plant hormones, as well as various plant phenotypes, such as growth, health, and resistance.[65,72–74] Finkel et al.[69] demonstrated that *Variovorax* can manipulate plant hormone (auxin and ethylene) levels to maintain root growth. Two *Variovorax* strains (502[T] and T529) isolated from rhizosphere soil have shown the potential for plant growth promotion.[75] The
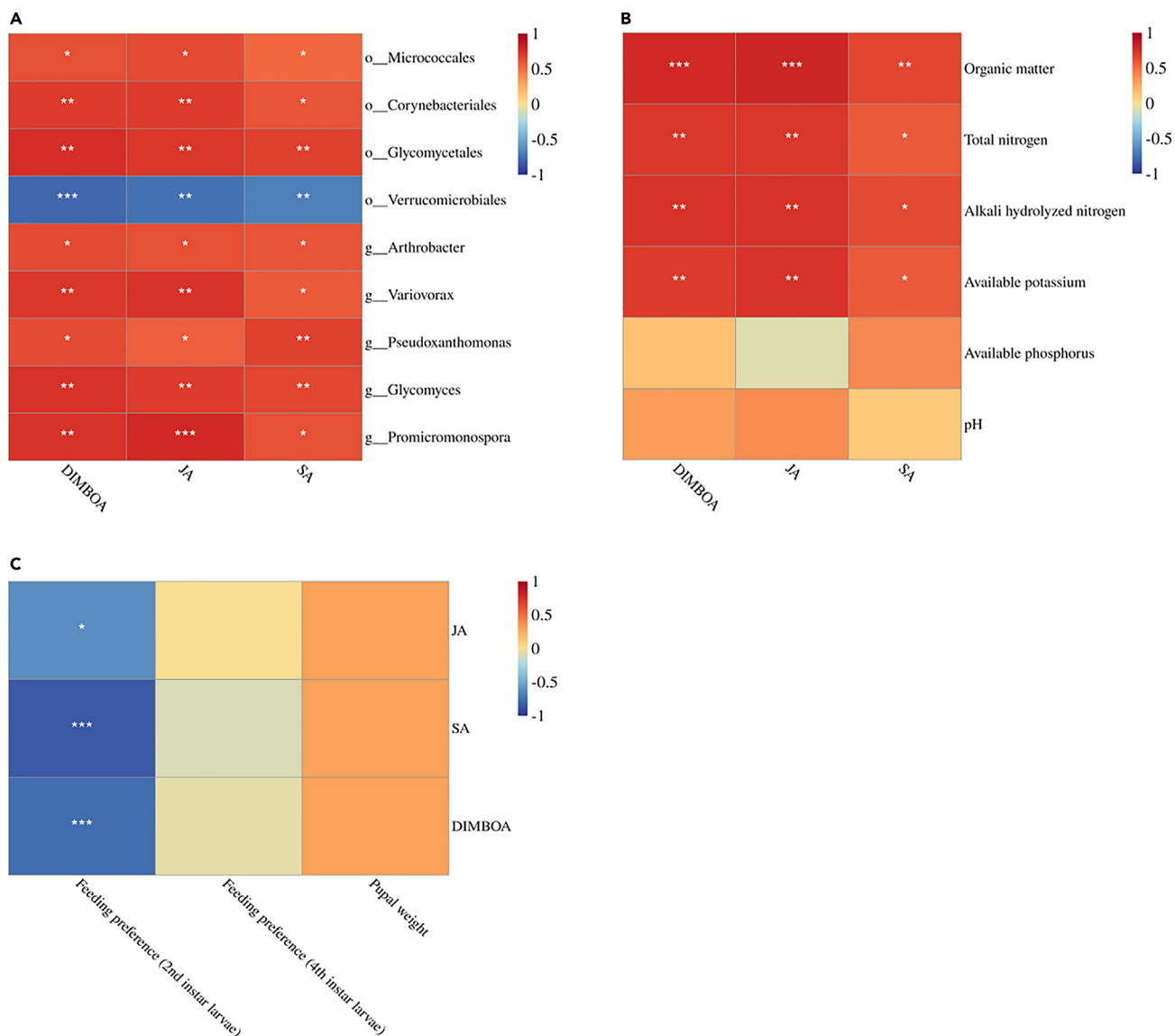
Figure 8. Spearman correlation analysis

(A) Correlations between microbiota taxa (order and genera) of rhizosphere soil and subsequent crop chemical characteristics.

(B) Correlations between rhizosphere soil chemical properties and subsequent crop chemical characteristics.

(C) Correlations between subsequent crop chemical characteristics and FAW performance. Colors represent correlation coefficients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. SA: salicylic acid; JA: jasmonic acid; DIMBOA: 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one.

application of a *Pseudoxanthomonas* strain (S254) isolated from agricultural soil may facilitate growth in As-contaminated lands.[76] Several endophytic PGPB synthesize JA and SA.[77] Interestingly, the application of a *Promicromonospora* strain (SE188) isolated from the rhizosphere improved *Solanum lycopersicum* plant growth and increased the level of SA in plant tissues.[78] de Vries et al.[79] reviewed how plants can positively influence their development and crop production by altering the rhizosphere or recruiting beneficial soil microbiomes. In this process, plant hormones such as abscisic acid, SA, and JA play roles in the interactions between plants and microorganisms under drought stress. Compared to untreated plants, soybean plants treated with *Bacillus aryabhattai* SRB02 exhibited greater levels of JA, showed significantly better heat stress tolerance and developed longer roots and shoots.[80] The application of the rhizobacterium *Rhodobacter sphaeroides* KE149 and biochar significantly increased the content of JA, and improved plant morphological attributes of soybean (*Glycine max* L.), such as root length, shoot length, and fresh biomass.[81] Under stressful circumstances, PGPR and SA integration can effectively promote tolerance and enhance plant growth.[82,83] The rhizosphere-associated microalga, *Micractinium rhizosphaerae* sp. nov., promotes plant growth and health by synthesizing a variety of plant growth-promoting compounds, including plant hormones such as indole-3-acetic

acid, SA, JA and abscisic acid.[84] Berendsen et al.[85] found that when *Arabidopsis thaliana* is attacked by the downy mildew pathogen *Hyaloperonospora arabidopsidis*, it accumulates specific beneficial bacteria in the rhizosphere, which can cooperate to help plants resist pathogens by regulating plant hormones SA and JA. Beneficial soil microorganisms can regulate hormone signaling, including the JA, ethylene, and SA pathways, thereby leading to gene expression, biosynthesis of secondary metabolites, plant defensive proteins, and different enzymes and volatile compounds that may induce defense against leaf-chewing and phloem-feeding insects.[86,87] Although we did not quantify this in our study, the combination of our findings with those of several existing studies demonstrate that various fertilization strategies, particularly the application of GM, can enhance hormone and secondary substance levels in subsequent crops by inducing changes in the rhizosphere soil microbial composition.

The correlation between the chemical properties of the rhizosphere soil and the chemical characteristics of subsequent crops can provide valuable insights into the soil nutrients that influence plant defense compounds in the subsequent crops. In our study, the organic matter, total nitrogen, alkali-hydrolyzed nitrogen, and available potassium contents in the rhizosphere soil were significantly positively correlated with the JA, SA, and DIMBOA contents in subsequent maize crops. A direct reduction in plant susceptibility to pests can be achieved through the improvement of plant health by implementing soil fertility management practices.[88] The increased availability of soil nutrients led to an increase in foliar nitrogen levels in the host plant (*Plantago* spp.) and a decrease in the female oviposition preference of the butterfly *Junonia coenia*.[41] Nitrogen deposition can change the concentrations of plant secondary metabolites,[89] high nitrogen or phosphorus levels in soil significantly reduced the caterpillar *Danaus plexippus* growth rate and the sequestration efficiency of cardenolides by *D. plexippus* feeding on *Asclepias curassavica*.[90]

Correspondingly, correlations between subsequent crop chemical characteristics and FAW performance can indicate how crop resistance-related compounds affect insects. Phytodefensive hormones (SA and JA) and secondary substances (DIMBOA) can help plants cope with pests and diseases and improve plant resistance.[44,91] Our results showed that changes in hormones and secondary substance levels in subsequent crops induced by GM application increased the resistance of the subsequent crop to FAW and inhibited the feeding preference of young FAW larvae for maize.

Conclusions

We studied the effects of GM application on resistance to FAW in subsequent maize crops and also determined the changes in plant nutrients, hormones, and secondary metabolites as well as alterations in soil microbial and chemical properties. Our findings indicated that compared to traditional single chemical fertilization strategies, GM application significantly enhances soil chemical properties, leading to increased levels of organic matter, total nitrogen, and available potassium in the rhizosphere soil. Additionally, it the composition of rhizosphere soil microbiota by increasing the relative abundance of several genera such as *Variovorax*, *Pseudoxanthomonas*, *Promicromonospora*, which have previously been identified as PGPR. The enhancement of soil nutrients and optimization of PGPR further facilitated plant growth, improved plant health, and increased the levels of resistance-related compounds such as SA, JA, and DIMBOA in subsequent crops. Ultimately, the adaptation (especially the host preference of young larvae) of FAW to maize in subsequent crops was reduced to some extent. Therefore, the application of GM enhanced insect resistance in subsequent crops. Our research enables the extension of the function of GM application, an important technical means of ecological management, by replacing chemical fertilizers and improving soil to enhance the resistance of subsequent crops to pests and diseases.

Limitations of the study

We conducted a series of greenhouse experiments to clarify the multidimensional effects of green manure application on soil-crop-pest interactions. Although the greenhouse and field conditions do indeed influence soil microbial communities, on one hand, greenhouse experiments allow for better control over experimental treatments such as fertilizer and green manure application rates, watering amounts, and agricultural practices, which help ensure consistency in the results. On the other hand, we used a glass greenhouse with screens on the top and sides for ventilation, aiming to maintain consistency in temperature, humidity, and other environmental factors between inside and outside of the greenhouse during the experiment. Furthermore, previous studies have shown that although there are differences between greenhouse and field environments, changes in microbial community structure from field to greenhouse are not significant enough to significantly alter overall structure and functionality.[92,93] Additionally, we plan to conduct field experiments in future research to further validate the effects of multiple or long-term GM applications on crop resistance to pests and diseases.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110320.

AUTHOR CONTRIBUTIONS

Conceiving and designing the experiments, L.M., X.J., D.W., and L.Z.; performing the experiments, L.M., D.W., Y.G., Y.L., and Y.C.; analyzing the data, L.M., D.W., L.Z., and X.J.; writing the article, L.M., D.W., and X.J.; project administration, X.J., Y.C., and L.Z.; funding acquisition, X.J. and L.Z. All authors have read and agreed to the published version of the article.

DECLARATION OF INTERESTS

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this article.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
The fall armyworm (FAW), *Spodoptera frugiperda* (Smith)	Nanning, Guangxi Zhuang Autonomous Region of China	N/A
Hairy vetch, *Vicia villosa* Roth	Academy of Agricultural Sciences of Xinxiang City, Henan Province	Xushao 3
Maize, *Zea mays* L.	Institute of Crop Sciences, Chinese Academy of Agricultural Sciences	Zhengdan 958
Critical commercial assays		
Total Protein Assay Kit	Nanjing Jiancheng Bioengineering Institute	A045-4
Plant Soluble Sugar Content Test Kit	Nanjing Jiancheng Bioengineering Institute	A145-1-1
Plant Total Phenol Test Kit	Nanjing Jiancheng Bioengineering Institute	A143-1-1
Plant Flavonoids Test Kit	Nanjing Jiancheng Bioengineering Institute	A142-1-1
The E.Z.N.A.® Stool DNA Kit	Omega, Inc., USA	D4015-02
Deposited data		
Raw data	This paper	https://doi.org/10.7910/DVN/S3ZPZZ
Raw sequence reads	This paper	https://www.ncbi.nlm.nih.gov/bioproject/PRJNA869125
Software and algorithms		
TWO-SEX-MSChart (v2023)	Chi.[94]	http://140.120.197.173/ecology/prod02.htm
SPSS	International Business Machines Crop., Armonk, New York, USA	Version 25
R (v3.6.3)	Lucent Technologies	https://cran.r-project.org/
OmicStudio tools	LC-BIO	https://www.omicstudio.cn

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Xingfu Jiang (xfjiang@ippcaas.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data generated in the study, including raw data and raw sequence reads from metagenomic sequencing, have been deposited at Harvard Dataverse Repository and NCBI SRA database, respectively. They are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Insects, soil, and plants

The FAW, *S. frugiperda,* used in this study were initially collected from maize fields located in Nanning city, Guangxi Zhuang Autonomous Region of China (108.37°E, 22.82°N) during April 2019 and subsequently reared on maize seedlings to establish a population for multiple

generations under controlled conditions of $27\pm1°C$, 65% $\pm$ 5% relative humidity, and light:dark (L:D)=14 h:10 h photoperiod in a greenhouse.

The soil used in this study were collected in August 2020 from a field located in Shunyi, Beijing (40°13′N, 116°65′E). We assessed the initial nutrient index of the soil (Table S1) and found that, based on the geochemical grade of soil nutrients in the current standard DZ/T 029-2016,[95] available phosphorus was rated as second class (more abundant), whereas organic matter, total nitrogen, alkaline nitrogen, and available potassium were rated as fourth class (less deficient). The initial soil nutrients were insufficient to support the growth of subsequent crops, which made the impact of various fertilization treatments on subsequent crops more evident and significant.

Hairy vetch (*Vicia villosa* Roth; Conventional variety: Xushao 3, provided by the Academy of Agricultural Sciences of Xinxiang City, Henan Province), known for its ecological benefits of increasing soil organic matter and reducing soil erosion,[96] was used as the GM crop, followed by the cultivation of maize (*Zea mays* L.; Conventional variety: Zhengdan 958, provided by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences) as the subsequent crop.

Experimental design

Effects of GM application on rhizosphere soil nutrients and microbial communities were investigated. The contents of nutrients and resistance-related compounds of subsequent crops and insect resistance of the crops was further evaluated. Additionally, correlations between rhizosphere soil microbiota taxa (order and genera) and subsequent crop chemical characteristics, between rhizosphere soil chemical properties and subsequent crop chemical characteristics, and between subsequent crop chemical characteristics and FAW performance were determined. The overall concept is illustrated in Figure 1. Compared with the single application of chemical fertilizer, the current nitrogen (N) fertilizer reduction scheme, which involves reducing N fertilizer application by 20–40% while applying GM, can enhance yield stability and soil quality while maintaining crop yield. Additionally, soil organic matter content and the rate of GM application are positively correlated.[54,97] Based on these reports, we designed three fertilization treatments: a single application of chemical fertilizer (CF), GM, and a combination of chemical fertilizer and GM (CF + GM). For the CF treatment, only three chemical fertilizers were used: urea (N fertilizer), superphosphate (P fertilizer), and potassium chloride (K fertilizer). The amount of CF applied was determined based on local field usage and the current national standard (GB/T 37088-2018)[98] (based on yield >800 kg/667 m², fertilization was applied at a rate of 270 kg N ha⁻¹, 120 kg P ha⁻¹, and 240 kg K ha⁻¹).

For the GM treatment, only GM was used, with the overturning quantity of hairy vetch set at 30,000 kg ha⁻¹.[97] In contrast to CF, the CF + GM treatment reduced the amount of N fertilizer by 30% while increasing the overturning quantity of hairy vetch by 15,000 kg ha⁻¹. Under greenhouse conditions (22 $\pm$ 2°C), 50 flowerpots (diameter/height=27/26 cm) were set for each treatment. Each flowerpot was filled with soil weighing approximately 8–10 kg. Seeds of hairy vetch were sown in flowerpots in treatments CF + GM and GM treatments. During planting, no chemical fertilizer was added, and only water was provided. The amount of water in the 150 tested flower pots was consistent. At the full flowering stage of hairy vetch (four months after planting), the aboveground part of the hairy vetch was cut, chopped into 1–2 cm segments, and then overturned into flowerpots based on the different overturning capacities set for each treatment. According to the above description, in the GM treatment, the actual amount of GM turnover was 171.68 g per flowerpot; in the CF + GM treatment, the actual amount of GM turnover was 85.84 g per flowerpot. GM was turned over and decomposed for 20 d before maize planting.[99] Chemical fertilizer was applied as a base fertilizer one day before planting maize, which was the subsequent crop. For the CF treatment, 1.55 g of urea, 0.69 g of superphosphate, and 1.37 g of potassium chloride were applied to each flower pot. For the CF + GM treatment, 1.08 g of urea was applied to each pot, and the amounts of superphosphate and potassium chloride applied were the same as those for the CF treatment. Four maize seeds were planted per flower pot.

METHOD DETAILS

Determination of nutrients in maize

Under various fertilization treatments, nutrients, hormones, and secondary metabolites of subsequent maize crops at the 9–11 leaf stages were measured. This stage is a period of vigorous nutritional growth for maize plants, with a strong demand for nutrients. It can intuitively reflect the effects of different treatments, including GM application, on plants. Furthermore, this stage occurs before maize tasseling and is less affected by plant reproductive growth in terms of experimental results.[100] One maize plant was randomly selected from each pot, while the remaining maize plants were left intact and allowed to continue growing in their respective pots. The top 1–3 leaves of 10 plants were then combined to create a sample. Five samples were collected for each treatment and stored at -80°C before the measurements.

The nutritional indices measured included the levels of nutrient elements (total nitrogen, total phosphorus, total potassium, and total carbon) (Table S2) and nutrient compounds such as proteins and soluble sugars. Following the method described by Bao[101] and Zhang et al.,[102] 5 g of dry weight was taken from each sample, crushed, and analyzed for nutrient element content. Total nitrogen was determined using the Kjeldahl method,[103] whereas total phosphorus and total potassium were decomposed using H_2SO_4-H_2O_2 and then analyzed using molybdenum-antimony colorimetric and atomic absorption methods, respectively. The total carbon was measured using a total organic carbon analyzer. The total protein content of each sample (100 mg) was determined using a Total Protein Assay Kit (A045-4, Nanjing Jiancheng Bioengineering Institute), following the manufacturer's instructions, and the optical density (OD) value was measured using a microplate reader (Infinite M200, Tecan). The soluble sugar content of each sample (100 mg) was determined using a Plant Soluble Sugar Content Test Kit

(A145-1-1, Nanjing Jiancheng Bioengineering Institute), following the manufacturer's instructions, and the OD value was measured using an ultraviolet spectrophotometer (UV-2100: Unico (Shanghai) Instrument Co., Ltd., Shanghai, China).

Determination of hormones in maize

Salicylic acid (SA) and jasmonic acid (JA) levels in the maize leaves were determined for each treatment. Ethyl acetate was used to extract JA and SA from 150 mg of each sample following the method described by Wu et al.[104] and were quantified using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (AB SCIEX QTRAP 5500: AB Sciex Pte. Ltd., Framingham, US).

Determination of secondary metabolites in maize

The levels of secondary metabolites (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one [DIMBOA], total phenols, and flavonoids) in maize leaf samples from each treatment were determined. DIMBOA was extracted from 100 mg of each sample using a solvent mixture consisting of 50% methanol and 0.5% formic acid, as described by Qi et al.[105] Quantification was performed by HPLC-MS/MS (AB SCIEX QTRAP 5500: AB Sciex Pte. Ltd.). The total phenol content of each sample (100 mg) was determined using a Plant Total Phenol Test Kit (A143-1-1; Nanjing Jiancheng Bioengineering Institute), following the manufacturer's instructions. OD was measured using the ultraviolet spectrophotometer (UV-2100: Unico (Shanghai) Instrument Co., Ltd.). The flavonoid content of each sample (50 mg) was determined using a Plant Flavonoids Test Kit (A142-1-1; Nanjing Jiancheng Bioengineering Institute), following the manufacturer's instructions, and OD was measured using the ultraviolet spectrophotometer (UV-2100: Unico (Shanghai) Instrument Co., Ltd.).

Performance of FAW on subsequent maize crops

To investigate the effect of different fertilization strategies on pest resistance in subsequent maize crops, we measured the feeding preference, growth and development, fecundity, and nutrient utilization indices of FAW in subsequent maize crops under various fertilizer treatments. The top 1–3 leaves of maize plants at the 9–11 leaf stage, which were not used for collecting plant and rhizosphere soil samples from each pot of each treatment, were utilized for this part of the experiment.

The feeding preference of FAWs for subsequent maize leaves was determined under different treatments, following the method described by Tang and Wang.[106] Equally sized maize leaves from each treatment were placed at equal distances in the Petri dishes. Ten FAW larvae of the same instar were then introduced to the center of each dish after a 2-h starvation period, and the number of larvae on each type of leaf was observed and recorded after 6-h. Second- and fourth-instar larvae were measured separately with five replicates per instar.

Chi's[107] method was used to establish two-sex life tables for FAW on subsequent maize leaves under different treatments in order to determine various life-table parameters, such as the developmental time of each stage, net reproduction (R_0), intrinsic rate of increase (r), finite rate (λ), mean generation time (T) (Table S3), adult preoviposition period (APOP), and mean fecundity. For each treatment, 100 newly hatched larvae were individually placed in 6-well plates covered with toilet paper and a matching lid to prevent escape. Excised maize leaves were provided as food in the respective plates under different treatments. Each FAW individual, from the 1st to 6th instar larva and pupal stage, was housed with food in the wells of a 6-well plate. The larvae were provided with sufficient leaves, which were replenished or changed every alternate day. The survival and developmental times of FAW at the larval and pupal stages were recorded daily. Newly emerged females were individually paired with young males that emerged from the same treatment group in a glass tube (5 cm in diameter and 12 cm in height) covered with a piece of medical absorbent cotton gauze serving as the substrate for oviposition. Adult FAWs were fed a mixture of 10% (w/v) honey in sterile water, and their survival rates and egg-laying numbers were recorded daily until all adults died. Following the establishment of the life table, pupal weight on the fourth day was measured for 15 FAWs per treatment.

For each treatment, thirty 5th instar-2nd day FAW larvae that fed on subsequent maize leaves were used to determine the nutrient utilization indexes, as described by Waldbauer.[108] After 6 h of starvation, each larva was fed the corresponding food type for 24 h. The fresh weight and dry/fresh weight ratio of larvae before feeding, fresh weight and dry/fresh weight ratio of food before feeding, fresh weight and dry weight of larvae after feeding for 24 h, dry weight of uneaten food, and dry weight of feces were measured. The calculated indices included relative growth rate (RGR), approximate digestibility (AD), relative consumption rate (RCR), efficiency of conversion of digested food (ECD), and ingested food (ECI) (Table S4). The formulas for calculating each index are as follows:

$$RCR = \frac{W_{FI} - W_{UF}}{[(W_{IL} + W_{FL})/2] \times T} \times 100$$

$$RGR = \frac{W_{FL} - W_{IL}}{[(W_{IL} + W_{FL})/2] \times T} \times 100$$

$$AD = \frac{W_{FI} - W_{UF} - W_F}{W_{FI} - W_{UF}} \times 100$$

$$ECD = \frac{W_{FL} - W_{IL}}{W_{FI} - W_{UF} - W_F} \times 100$$

$$ECI = \frac{W_{FL} - W_{IL}}{W_{FI} - W_{UF}} \times 100$$

W_{FI} = dry weight of food introduced

W_{UF} = dry weight of uneaten food

W_{IL} = the initial dry weight of the larvae

W_{FL} = the final dry weight of the larvae

W_{F} = dry weight of feces.

Maize rhizosphere soil sample collection

Rhizosphere soil samples were collected from subsequent maize crops under different treatments when the maize plants reached the 9–11 leaf stage. One random maize plant was selected from each pot, which was the same plant selected to measure the nutrients, hormones, and secondary metabolites of subsequent crops. The rhizosphere soil of 10 maize plants from the same treatment was mixed as a sample. Five samples were collected from each treatment and stored at -80°C.

Measurement of soil chemical properties

The rhizosphere soil samples were air-dried, homogenized, and sieved through 0.15 mm and 0.85 mm meshes before being used to measure the chemical properties. Soil organic matter content was determined using the potassium dichromate volumetric method, as outlined in the current standard NY/T 1121.6-2006.[109] The alkali-hydrolyzable nitrogen content in the soil was determined using the alkali-hydrolyzable diffusion method outlined in the current standard LY/T 1228-2015.[110] Total nitrogen was determined using an automatic nitrogen determination apparatus as outlined in the current standard NY/T 1121.24-2012.[111] Available phosphorus was determined using the Mo-Sb Colorimetric Method, as described in the current standard NY/T 1121.7-2014,[112] with extraction performed using 0.5 mol/L NaHCO$_3$ (pH 8.5). Available potassium was extracted using 1 mol/L NH$_4$OAc (pH 7.0) and determined using the Flame Photometer Method outlined in the current standard NY/T 889-2004.[113] The soil pH was determined using the Potentiometry Method outlined in the current standard NY/T 1377-2007.[114]

Rhizosphere soil microbial communities

Four rhizosphere soil samples were used for each treatment to determine the rhizosphere microbial communities using metagenomic sequencing.[115]

DNA from different rhizosphere soil samples was extracted using The E.Z.N.A.® Stool DNA Kit (D4015-02, Omega, Inc., USA), according to the manufacturer's instructions. This reagent, designed to extract DNA from trace amounts of samples, has been shown to be effective in the isolation of DNA from most bacteria.[116,117] The sample blanks consisted of unused swabs processed through DNA extraction and were tested to contain no DNA amplicons. The total DNA was eluted in 50 μL of elution buffer by a modification of the procedure described by manufacturer (QIAGEN) and stored at -80°C until being subjected to polymerase chain reaction (PCR; Lc-Bio Technologies (Hangzhou) Co., Ltd., Hang Zhou, Zhejiang Province, China).

A DNA library was constructed using a TruSeq Nano DNA LT Library Preparation Kit (FC-121-4001).[117] DNA was fragmented by dsDNA Fragmentase (NEB, M0348S) by incubating at 37°C for 30 min. Library construction began with fragmented cDNA.[118] Blunt-end DNA fragments were generated using a combination of fill-in reactions and exonuclease activity, and size selection was performed using the provided sample purification beads. An A-base was then added to the blunt ends of each strand to prepare them for ligation to indexed adapters. Each adapter contained a T-base overhang for ligation to the A-tailed fragmented DNA. These adapters contained the full complement of the sequencing primer hybridization sites for single-, paired-end, and indexed reads. Single- or dual-index adapters were ligated to the fragments and the ligated products were amplified using PCR with the following set conditions: initial denaturation at 95°C for 3 min; eight cycles of denaturation at 98°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s; followed by a final extension at 72°C for 5 min.[119] Raw sequence reads from the Illumina sequencing are available in the NCBI SRA Database: PRJNA869125.

QUANTIFICATION AND STATISTICAL ANALYSIS

Sequencing data analysis

Raw sequencing reads were processed to obtain valid reads for further analysis of rhizosphere soil microbial communities. Sequencing adapters were removed from the sequencing reads using Cutadapt v1.9. Low-quality reads were trimmed using fqtrim v0.94, a sliding-window algorithm. Next, the reads were aligned to the host genome using bowtie2 v2.2.0 to remove host contamination.[120] Once quality-filtered reads were obtained, they were *de novo* assembled to construct the metagenome for each sample by IDBA-UD v1.1.1.[121] All coding sequences [CDS] of the metagenomic contigs were predicted using MetaGeneMark v3.26. The CDS of all samples were clustered using CD-HIT v4.6.1 to obtain the unigenes. The unigene abundance of each sample was estimated by Transcript per Kilobase per Million mapped

reads [TPM] based on the number of aligned reads using bowtie2 v2.2.0. The lowest common ancestor taxonomy of the unigenes was obtained by aligning them against the NCBI NR database using DIAMOND v 0.9.14.[122] Based on the taxonomic and abundance profile of unigenes, differential analysis was carried out at each taxonomic level using the Kruskal–Wallis test (Figure S1). The co-occurrence network analysis of the top 100 species with various treatments was constructed using the Spearman correlation algorithm (Spearman, $P < 0.03$, |r| > 0.8) in MetagenoNets (Table S5).[123]

Statistical analysis

All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) 25. Comparisons of nutrition, hormones, and secondary metabolites in subsequent maize leaves among different treatments were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc comparisons.

The raw life-table data were analyzed, and the life history parameters were estimated based on an age-stage, two-sex life table using the TWOSEX-MSChart software (Version2023).[94] The mean and standard error of the mean (SEM) of each life history parameter were calculated, and significant differences among FAWs fed on maize under different treatments were compared using the paired bootstrap test in the TWO-SEX-MSChart.[124] Differences were considered significant at a 95% confidence interval (CI) and $P < 0.05$. The feeding preference of 2nd or 4th instar larvae, nutrient utilization indices, and pupal weight of FAWs on subsequent maize crop leaves were compared among different treatments using ANOVA followed by Tukey's HSD post-hoc comparisons. The soil chemical properties were also compared among different treatments using ANOVA followed by Tukey HSD post-hoc comparisons.

Correlations between rhizosphere soil microbiota taxa (order and genera) and subsequent crop chemical characteristics, between rhizosphere soil chemical properties and subsequent crop chemical characteristics, and between subsequent crop chemical characteristics and FAW performance were calculated using the Spearman correlation test in R (v3.6.3). Clustering correlation heatmaps with signs were generated using the OmicStudio tools at https://www.omicstudio.cn.