Generalist herbivorous insect modulates similar rhizosphere bacterial communities across different plant species

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Abstract

Plants and insects have developed an evolutionary relationship over time. It is known that aboveground insect damage influences molecular modifications in plant defense systems, leading to changes in root exudation patterns and carbon allocation. Mounting evidence demonstrated in specific crops shows that plants modulate their rhizosphere microbes in response to leaf-herbivory attack. However, the influence of a specific herbivore on rhizosphere microbiomes across different crop species and its communalities remains unknown. Here, we studied the relationships between aboveground insect herbivory (Trichoplusia ni) damage and rhizosphere microbiome effects across five plant species (Zea mays 'sh2,' Phaseolus vulgaris L. 'Seychelles,' Solanum lycopersicum 'Rutgers,' Beta vulgaris L. 'Burpee Bred,' and Arabidopsis thaliana Ecotype Col-0). We investigated whether insect damage may influence the recruitment of beneficial microbes across plant species to minimize the burden associated with the attack. Our results show a significant increase in the rhizosphere abundance of certain beneficial microbial taxa including Azospirillum, Burkholderia, and Arthrobacter, consistently across all plant species tested. Furthermore, a significant reduction in the biomass was observed among the second generation of plants grown under the conditioned soil without insect damage.

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ABSTRACT

Plants and insects have developed an evolutionary relationship over time. It is known that aboveground insect damage influences molecular modifications in plant defense systems, leading to changes in root exudation patterns and carbon allocation. Mounting evidence demonstrated in specific crops shows that plants modulate their rhizosphere microbes in response to leaf-herbivory attack. However, the influence of a specific herbivore on rhizosphere microbiomes across different crop species and its communalities remains unknown. Here, we studied the relationships between aboveground insect herbivory (*Trichoplusia ni*) damage and rhizosphere microbiome effects across five plant species (*Zea mays* 'sh2,' *Phaseolus vulgaris* L. 'Seychelles,'*Solanum lycopersicum* 'Rutgers,' *Beta vulgaris* L. 'Burpee Bred,' and *Arabidopsis thaliana* Ecotype Col-0). We investigated whether insect damage may influence the recruitment of beneficial microbes across plant species to minimize the burden associated with the attack. Our results show a significant increase in the rhizosphere abundance of certain beneficial microbial taxa including*Azospirillum* , *Burkholderia* , and *Arthrobacte* r, consistently across all plant species tested. Furthermore, a significant reduction in the biomass was observed among the second generation of plants grown under the conditioned soil without insect damage.

Keywords: 16S rRNA, herbivory, insects, plants, bacteria, rhizosphere, microbiota.

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INTRODUCTION

Plants are continually strengthening their mechanical and chemical defense processes against insects (Hahn et al., 2019; Willsey et al., 2017). Meanwhile, invertebrate herbivores have advanced their mechanisms of tolerance and resistance to overcome plant defense strategies (Huang et al., 2016; Kessler & Baldwin, 2002; Ryan, 1990;). This evolutionary interaction between plants and herbivores is an evidence of their interdependence and close mutualistic relationship (Mello & Silva-Filho, 2002; Sugio et al., 2015). However, the effect of plant-insect interactions on rhizosphere microbes is not fully understood.

Plants respond to insect attack by inducing internal resistance (IR) (Blaazer et al., 2018). Internal resistance can be divided into two categories: induced systemic resistance (ISR), and systemic acquired resistance (SAR) (Vallad et al., 2004). Both types of resistance entail a metabolic cost for the plant (Krattinger & Keller, 2016) where survival is achieved at the cost of growth (Morris et al., 2006).

ISR is potentiated by plant growth-promoting rhizobacteria (PGPR) such as species of *Pseudomonas* that cause no visible damage to the plant's root system (van Loon et al., 1998). ISR pathways can be regulated by jasmonate and ethylene (Knoester et al., 1999; Pieterse et al., 1998; Yan et al., 2002). Additionally, there is demonstrated specificity for plant genotypes to express ISR (van Wees et al., 1997; Yan et al., 2002).

SAR also serves as systemic plant protection against subsequent invasion (Hammerschmidt, 2001; Sticher et al., 1997), which generally leads to the development of broad-spectrum and long-lasting responses against predators (Hammerschmidt, 2001). SAR is not restricted to one plant and can also be transmitted to neighboring plants to be used as an indirect form of defense through the use of organic compounds (Heil and Karban, 2010). Further, after wounding by the herbivore, specific plant tissues produce volatile organic compounds (VOCs) which play essential roles as alarm signals for undamaged neighbors (Franco et al., 2017). Root–root interactions, by transferring defense-related signaling compounds, also help boost defensive enzyme activities and defense-related gene expression in neighboring plants (Heil and Karban, 2010). At the root level, modulation effects of root-derived compounds, such as primary and secondary metabolites, in response to insect injury have been reported (Badri et al., 2013; De-la-Peña et al., 2010; Hubbard et al., 2019).

Microbial mediation of plant-herbivory interactions alters plant physiology, nutrition, and defensive chemistry (Barbosa et al, 1991). The systemic responses on aboveground organs following insect attack are well understood, but knowledge of the impacts on the associated microbial communities and specific microbial taxa after herbivory damage belowground remains scarce (Yi et al, 2011). Fewer reports have discussed the biological functions of the rhizosphere microbiota modulated after insect pest attack and how this relates to plant inducible resistance (Li-Li et al., 2018; Morgane et al., 2018). In a wide survey across different plant species, our results showed a significant relative abundance shift in the rhizosphere beneficial bacterial community after the aboveground attack of a specific insect. In addition, we suggest that bacterial recruitment initiated by the plant after aboveground insect attack can be outweighed by the specific cost in induced resistance in the subsequent generation.

MATERIALS AND METHODS

Soil substrate and plant material conditions

In the first experiment, five different plant species were sown in a soil substrate. The soil substrate was composed of $\frac{1}{4}$ cover crop soil which was collected at the Ag Research Development & Education Center (ARDEC) of Colorado State University located at 4616 NE Frontage Road, Fort Collins, CO 80524 (DMS 40° 38' 59.172" N and 104° 59' 44.34" W), where only experiments without agrochemical inputs are performed. Additionally, $\frac{1}{4}$ Thermorock® non-sterile horticultural vermiculite (#2 grosses 3.5 cubs. ft.), $\frac{1}{4}$ Promix Bx® peat moss, and $\frac{1}{4}$ QUIKRETE® Play Sand (Atlanta, GA) non-sterile sand were combined into the final substrate.

Super sweet hybrid corn (*Zea mays* 'Sh2'), bean (*Phaseolus vulgaris L.* 'Seychelles'), tomato (*Solanum lycopersicum*'Rutgers'), and red beet (*Beta vulgaris L* . 'Burpee bred') seeds were purchased from W. Atlee Burpee & Co. (Warminster, PA).*Arabidopsis thaliana* ecotype Col-0 seeds were obtained from Lehle (Round Rock, TX).

Seeds were sterilized in sodium hypochlorite solution and Milli-Q® water at 2% (V / V), subsequently rinsed four times with sterile distilled water, and planted directly into the soil substrate contained in 1L plastic pots.

Two seeds were placed in each pot to ensure the germination of at least one seedling per pot and incubated in growth chambers with a photoperiod of 16 h light / 8 h night at 25° C +/- 1° C for seven days. After seven days, the additional seedlings that germinated were thinned to leave only one plant per pot for all species. We used a completely randomized design with six replicates per treatment, maintaining one plant per pot. The seedlings of plants grew for four weeks in growth chambers. The plants were watered every two days with 50 mL sterilized water per pot.

Experimental design: herbivory experiment using *Trichoplusia ni*(Hübner)

In the fifth week of plant growth, each one of the different plant species was divided into two experimental groups: (1) Test Group: Plants with the herbivorous attack (n = 6); (2) Control Group: Plants without an herbivorous attack (n = 6). A population of *Trichoplusia ni*larvae in the third instar stage was obtained from Frontier Agricultural Sciences (Newark, DE). Four *T. ni* larvae were placed by each plant in this study.

Herbivory was introduced for three days, and then removed for days four and five. Then, new *Trichoplusia ni* larvae were introduced again for days six and seven. The insect removal allowed us to establish the effect of herbivory as well as keep enough biomass available for further testing after seven experimental days.

The rhizosphere samples were collected from the immediate soil adjacent to the roots, 2 mm from the root surface, after plant herbivory for seven days. Substrate soil where the plants grew up was kept in a plant growth chamber (photoperiod of 16 h light / 8 h night at 25° C +/- 1° C) for 24 hours, and re-used for re-sowing plants in the following experiment (see next section). The fresh and dry biomass, root height, and root biomass information were collected from all repetitions and experimental groups. One-way ANOVA was used to perform biomass data analyses, and the means between control and insect group, for each one of the

plants studied, which presented differences compared by the T-Student test (Bonferroni) at 5% probability level.

Growth conditions of the second plant generation in re-used soil substrate

New seeds of sweet corn, *Arabidopsis* , beans, tomato, and red beet from the same batch of seeds previously used were sown in two types of substrate: 1) soil substrate from control plants and 2) soil substrate from the plants subjected to insect attack in the previous experiment. The seeds were sterilized and planted according to the protocol previously described. The plants were kept in a growth chamber in the same conditions as the first sowing. No additional fertilizer was applied. After four weeks, the plants were collected, and the dry biomass, fresh biomass, root height, and root biomass for all replicates were measured. The dry biomass data from the second experiment were used to perform data analyses. The means between control and insect group, for each one of the plants studied in the second generation, were compared by the T-Student test (Bonferroni) at 5% probability level. The free software *Sisvar 9.5* (Ferreira, 2019, http://www.dex.ufla.br/˜danielff/programas/sisvar.html) was used for the statistical analysis, and *OriginPro 8.5* software (OriginLab Corporation, MA, https://www.originlab.com/) was used for graphics visualization. The graphics represent the relative values data for each one of the plants studied.

DNA rhizosphere extraction and Illumina Miseq Sequencing

DNA of six replicates was extracted for each treatment (for five different plants) from the conditioned soil (soils from the first generation of plants after insect attack). Each sample was composed of 50 mg of rhizosphere soil. The total DNA was extracted using the*MoBio* kit (*PowerPlant* ® DNA Isolation Kit) according to the manufacturer's instructions. The DNA concentration was measured by a Nanodrop® spectrophotometer (*Thermo Fisher Scientific*). The extracted DNA was stored at -20° C until the samples were subjected to amplification and *Illumina Miseq* .

The sequencing was performed according to Illumina protocols. The 16S rRNA gene hypervariable region V3-V4 was targeted to estimate bacterial communities present in the rhizosphere soil samples.

The sequencing was performed by a two-step PCR: First qPCR was conducted to quantify the 16S rRNA V3-V4 region with Illumina adapter sequences against a standard curve to quantify bacterial biomass in rRNA copies per gram soil. The next round of PCR included the addition of Illumina barcode sequences for multiplexing of 94 samples, including positive (Zymo mock community) and negative (water) control.

In the first qPCR run the set primers 341F/785R were used with Illumina Miseq adapter sequences (Klindworth et al., 2012). The primer 341F is correspondent to sequence F5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
3'and the primer 785R is correspondent to sequence R5'-GTCTCGTGGGCTCGGAGATGTGTATAAGA

GACAGGACTACHVGGGTATCTAATCC-3'.

The quantitative polymerase chain reaction (qPCR) consisted of 20 µl reaction volumes containing 2 µl of template DNA (5ng/ µl) and 18 µl of the master mix. The master mix consisted of 10 µL of 2X Maxima SYBR Green (Thermo Scientific, Waltham, MA, USA), and 1 µL each (10 µM) of forward and reverse primers, with the addition of 6 µL of molecular grade water. The PCR cycling conditions were: 95º C for 5 minutes, 25 cycles of 95º C for 40 seconds, 55º C for 30 seconds, 72º C for 60 seconds, and final annealing at 72º C for 5 minutes. The amplicons from the last PCR cycling were purified using an in-house preparation of solid-phase reversible immobilization (SPRI) magnetic beads based on the methodology of Glenn (2011) with modifications and original protocol of Rohland and Reich (2012). For the standard curve, purified*Pseudomonas putida* KT2440 was used in the same cycle run with the amplicons' samples to quantify the starting rRNA copies g^{-1} soil fresh weight. A positive control of Zymo (ZymoBIOMICS, Irvine, CA) mock community DNA, and a negative control (water), were included in duplicate and carried through the rest of the protocol.

The *Illumina Nextera XT* index sequences were attached for each sample by a new qPCR run. The qPCR conditions were: 5 µL of first-round PCR product, 25 µL of 2X Maxima SYBR Green (Thermo Scientific,

Waltham, MA, USA), 10 µL water, and 5 µL each of forward and reverse indices, all combined for a total of 50µL and amplified at 95º C for 3 minutes, 8 cycles of 95º C for 30 seconds, 55º C for 30 seconds and 72º C for 30 seconds, followed by final annealing of 72º C for 5 minutes. The PCR product was bead-cleaned using SPRI beads and quantified using Qubit fluorometer (Thermo Scientific, Waltham, MA, USA) before normalization and pooling. The equality pool was run on a TapeStation system (Agilent Technologies, Santa Clara, CA, USA) to determine amplicon size and purity, and Kapa Biosystems (Sigma-Aldrich, St Louis, MO, USA). The last qPCR run was performed according to the manufacturers' instructions to determine the concentration, and MiSeq libraries were quantified. PhiX (15%) was used as an internal library control. The library was then subjected to 250 base pair paired-end multiplex sequencing (MiSeq V3-V4 reagent kit) on an Illumina MiSeq at CSU's Next Generation Sequencing Laboratory (Fort Collins, CO).

16S rRNA V3-V4 sequence analysis

De-multiplexed raw fastq files were processed with the R Bioconductor package (Callahan et al., 2016), and primers were removed from each sequence using the open-source Python program Cutadapt (Martin, 2011). The amplicon sequence variants (ASV) were inferred with the default pipeline in DADA2. Each ASV identified in DADA2 was classified to the closest reference sequence contained in the GreenGenes reference database (version 13_5_99) using the usearch_global option (minimum identify of 97%) contained in the open source program VSEARCH (Rognes et al., 2016). The analyses were conducted using the open source bioinformatic software myPhyloDB (https://nrrc.ars.usda.gov/myPhyloDB/home/), version 1.2.0 (Manter et al., 2016). A total of 55,484 high quality reads per sample were obtained. Samples with fewer than 3,000 reads were removed from the analysis. Four samples out of the 94 were removed. The average read length of bacteria for the 16S rRNA subunit was 601 bp.

Each taxonomic profile was used to determine bacterial phyla-specific abundances. The Shannon diversity index was used to assess initial bacterial alpha diversity. For beta diversity, microbial community composition was analyzed using principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity index. A complementary non-parametric multivariate statistical test, including permutational analysis of variance (PERMANOVA) and non-parametric univariate ANCOVA analyses, was used to test the differences in microbial communities with the Bray-Curtis distance and 999 permutations with myPhyloDB (Manter et al., 2016).

An ASV (Amplicon Sequence Variant) table was used as output from myPhyloDB at the family and genus level presented between each experimental group. The shared and unique ASVs among treatments were counted, and their distributions are shown by a Venn diagram from the packet 'jvenn', a plugin for the 'jQuery' (http://jvenn.toulouse.inra.fr/app/index.html) Javascript library (Bardou et al., 2014).

RESULTS

1. Insect herbivory preference experiment

Herbivory significantly reduced the biomass of all plants after feeding for a period of seven days ($p < 0.05$) (Figure 1). Sweet corn and beans were consumed by the pest insects at rates of 50% and 46%, respectively (Figure 1). *A. thaliana* biomass was also significantly consumed ($p < 0.05$) at a rate of 57%. In tomato and red beet, *T. ni* larvae consumption was around 79% and 86%, respectively. The *T. ni* insect demonstrated the lowest preference for beans and the highest preference for tomato and red beet, the most consumed plants in the experiment.

(Figure 1)

2. Bacterial community structure across plant species in response to insect attack

Microbial communities' dissimilarities were evaluated by Principal Coordinate Analysis (PCoA) across plant rhizospheres after *T. ni*attack (Figure 2A) and their controls (Figure 2B).

Bacterial community structure presented significant differences when comparing the rhizosphere microbiome samples after insect attack (Figure 2A) and control samples (Figure 2B). The rhizosphere microbiome cor-

responding to insect-attacked plants showed a clustering effect (Figure 2A). However, the control samples showed a separation of the rhizosphere bacterial communities influenced by plant genotype (Figure 2B).

(Figure 2)

3. Bacterial community composition and relative abundance among plant rhizospheres after insect attack

In order to understand the individual effect of insect attack per plant species, we made individual PCoA plots comparing microbial control communities versus microbial communities after insect attack. We found that four of the five plant species significantly modulated the bacterial communities' structure after the plants had been attacked by *T.ni* (Figures 3A, 3B, 3C, and 3E). Only tomato showed no significant effect (p=0.32) (Figure 3D).

In addition, sweet corn, beans, *Arabidopsis* , and red beet showed a significant difference ($p<0.05$) in bacteria phyla across plant species when comparing relative abundances of treatment versus control groups (Figures 3F 3G, 3H, and 3J, and Supplementary tables 1, 2, 3, and 5). The observed pattern did not hold true for tomato ($p >0.05$) (Figure 3I and Supplementary table 4).

Further, *Actinobacteria* and *Proteobacteria* were the most abundant phyla across all plant species (Figures 3F, 3G, 3H, 3I, and 3J). Relative abundance of the phyla *Actinobacteria* ,*Verrucomicrobia* , *Bacteroides* , *Firmicutes* , and*Acidobacteria* presented significant differences ($p<0.05$) in the control samples when compared with insect-attack rhizosphere samples in most of the plant species studied (Figures 3F, 3G, 3H, and 3J). The phylum *Proteobacteria* showed a significant difference ($p <0.05$) for insect damage samples for red beet only (Figure 3J).

(Figure 3)

4. Unique bacterial taxa (at genus level) by plant species after insect attack

After insect attack, seven unique genera were found in*Arabidopsis* (*Prauseria, Actinotalea, Pilimelia, Sporichthya, Nostoc, Truepera, Candidatus Amoebophilus*), five in maize (*Sedimentibacter, Caulobacter, Marinibacillus, DA101, Telmatospirillum*), four in tomato (*Denitrobacter, Chitinophaga, Roseococcus, Candidatus Koribacter*), three in red beet (*Klebsiella, Patulibacter, Methylocaldum*), and two in beans (*Methylotenera, Pelomonas*).

Those bacterial genera presented a low relative abundance in each of the plant rhizospheres studied after *T. ni* attack (Supplementary Table 7). However, in tomato, the genus *Denitrobacter,* and in red beet the genus *Klebsiella,* presented a high relative abundance among each of the plant rhizospheres studied (Supplementary Table 7).

5. Shared bacterial taxa (at the genus level) by plant species after insect attack

To determine the shared bacterial communities among plant species after insect attack, we assessed the taxonomic units at the genus level from all crop plants using a Venn diagram. We found forty-nine overlapping bacterial genera between the control and the insect-attacked groups for all crops (Figure 4). Supplementary table 8 shows the relative abundance of the genera that were overlapping between insect attack and control for sweet corn, beans, *Arabidopsis* , tomato, and red beet rhizospheres.

(Figure 4)

From the eleven genera present exclusively in *T. ni* attacked treatments (left side of Venn diagram in Figure 4 and Table 1), five genera including*Azospirillum* ,*Achromobacter* , *Arthrobacter* , *Hydrogenophaga,* and*Burkholderia* showed the highest relative abundance. When comparing bacteria genera within each crop, *Azospirillum* and*Burkholderia* were significantly higher in relative abundance in sweet corn crop ($p<0.05$) (Table 1A). In beans, tomato and reed beet (Table 1B, 1C, and 1D, respectively), the genus *Arthrobacter*presented a significant difference and a high relative abundance ($p<0.05$) when compared to other overlapping bacteria. No significant differences among those genera were observed within the*A. thaliana* rhizosphere (Table 1C) after *T. ni* attack.

(Table 1. A, B, C, D.)

Insect attack affects plant performance in the following generation

In order to understand how plant biomass accumulation was affected in response to the observed shift in the microbiome upon insect attack, a second generation of plants was grown in the absence of herbivore insects reusing the soil of the first generation. There was a clear reduction in the biomass of all crops grown in the substrate that previously experienced insect attack when compared with the plants sown in the control substrate (Figure 5). The most significant reduction in biomass was observed in beans (78.49%) and red beet (70.58%). In addition, we observed a less significant reduction in the biomass of sweet corn (35.84%), whereas tomato plants did not show a significant difference in biomass production. Sweet corn, beans, *Arabidopsis,*and red beet were found to be statistically significant; ANOVA one-way ($p<0.05$) followed by the T-Student (Bonferroni) (Figure 5).

Interestingly, comparison of biomass changes between generation one and two across beans and *Arabidopsis* plants showed that the plant biomass of the second generation grown under the microbiome shifted soil (Figure 5) was more dramatically reduced than the reduction observed from the actual insect feeding during the first generation (Figure 1).

Discussion

Recent rhizosphere microbiome studies have shown that insect infestation reshapes the overall microbiome structure in single crops (Kong et al, 2016; Li-Li et al., 2018). However, there is a limited understanding of the rhizosphere microbiome modulation across a variety of plant crops sampled at the same time, and the potential recruitment of plan beneficial microbial taxa in response to insect attack.

The present study evaluates the magnitude of the legacy effects of aboveground insect damage in rhizosphere microbiomes across five plant species. Our results showed that plant species shifted the microbial community composition in response to the herbivorous insect attack, presumably for promoting the recruitment of several plant-beneficial bacterial groups. PGPR bacteria taxa such as *Azospirillum* ,*Burkholderia* , and *Arthrobacter* increased significantly after insect attack across plant species. Our finding agrees with Kong et al. (2016) which demonstrates that whitefly infestation in aboveground organs leads to the recruitment of specific bacterial groups (e.g. *Pseudomonas ssp* .) conferring beneficial traits to pepper plants.

Our study showed a core rhizobiome that was consistently responsive in changes of relative abundance between control and insect attack treatments and across plant species; these include the phyla*Actinobacteria, Verrucomicrobia, Bacteroides, Firmicutes* and*Acidobacteria* . These phyla significantly shifted in response to insect attack for most crops. At the genus level, every plant species held a specific microbiome comprised by unique bacteria (Supplementary table 8). Nine out of the 49 bacterial genera shared by conditioned soils across plant species became significantly more abundant. These genera include *Azospirillum* , *Achromobacter* ,*Arthrobacter* , *Hydrogenophaga,* and *Burkholderia* . In addition, every crop species showed at least one overabundant beneficial genus compared to the others. This observation may suggest that plant species select a specific group of microbes to exert a similar function due to difference in each plant species' root exudate-derived metabolome profile (Hubbard et al, 2019). Most of the shared microbial species that significantly shifted are known to be beneficial for the plant. For instance, *Azospirillum* and *Burkholderia* are two well-known free-living nitrogen-fixing bacteria, as well as taxa associated with soil disease suppression (Jing and Qingye, 2012; Mendes et al., 2011). Members of the *Achromobacter* genus are known to be endophytes and root plant growth promoters (Bertrand et al, 2000; Jha and Kumar, 2009), and *Arthrobacter* induces nutrient solubilization and growth promotion (Banerjee et al, 2010; Velazquez-Becerra et al, 2011). These taxa are also implicated in the induction of plant immunity. It is established that PGPR bacteria have an IRS-eliciting effect in certain plant species (Shouan et al., 2001; Zehnder et al., 2000). For instance,*Burkholderia* inoculation incremented the accumulation of resistance-related enzymes (chitinase and β-1,3-glucanase), and carbohydrate and lipid-based molecular patterns related to defense-differential gene expression in corn and wheat (Elya et al., 2010; Madala et al., 2012).

It has been suggested that changes induced by aboveground herbivory in a present plant season can affect the performance of plants in subsequent growth seasons. This effect is known as the ecological soil legacy (Kardol et al, 2007; Van de Voorde et al, 2011; Wurst and Ohgushi, 2015). Soil legacy effects on plants are also linked to soil biota (Bezemer et al, 2013). For instance, Kostenco et al. (2012) demonstrated that feeding by aboveground insect herbivory on ragwort (*Jacobaea vulgaris*) induced changes in the composition of soil fungi. In our second-generation study, we observed a significant decrease in biomass accumulation from plants grown under the disturbed soil (soil from plants grown under insect herbivory) compared with the control (fresh soil). This finding suggests that even though a subset of beneficial microbes was significantly promoted by the plant in response to aboveground damage, the cost of induced systemic resistance may outweigh the potential benefits of the recruited bacterial taxa in the first generation. This tradeoff is known as the 'costs of resistance,' and implies plant fitness reduction in response to herbivory (Bergelson and Purrington, 1996; Strauss et al, 2002;). We hypothesized that the observed rise in beneficial members of bacteria communities can accumulate in the soil until they are capable of exerting a significant impact on plant fitness. It is worth noting that the observed bacteria may also be acting belowground by signaling plant hormone systems, which is not necessarily translated in an immediate gain in biomass in the next generation. This supposition warrants further testing.

In summary, aboveground herbivory impacts rhizosphere microbial communities across plant species. Plants modulate PGPRs (plant-growth promoting rhizobacteria), increasing their abundance under *Tricoplusia ni* attack. This increase in abundance of beneficial bacteria taxa is not reflected in biomass growth after the following generation of herbivory damage.

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CONFLICT OF INTEREST

We have no declaration of conflict of interest.

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FIGURES AND TABLE LIST

FIGURE 1. *Tricoplusia ni* plant consumption for five crops. Graph show differences between the control (CTRL) and insect (INSECT) groups for fresh biomass in the first sowing. The numbers in percent, above the bars correspond to the difference in percentage in plant consumption for the control group and the treatment for each cultivated plant. The numbers with two decimal places on each of the bars, corresponds to the average of six repetitions evaluated for the variable fresh biomass. The numerical averages are represented in grams. (*) Statistical significance was tested using ANOVA one-way test ($p<0.05$) followed by the test T-Student (Bonferroni) for each one of the crop plants separately.

FIGURE 2. Principal coordinate analysis (PCoA) of*pairwise* dissimilarities (Bray–Curtis index) for bacterial communities in *Arabidopsis* , tomato, red beet, sweet corn, and bean rhizospheres of (A) Insect attack group and (B) Control group.

FIGURE 3. Principal coordinates analysis (PCoA) of pairwise dissimilarities (Bray–Curtis index) for bacterial rhizosphere communities in taxonomic composition present in **(A)** sweet corn,**(B)** beans, **(C)** *A. thaliana* , **(D)**tomato, and **(E)** red beet. The PCoA compares rhizosphere in control groups and rhizosphere after the insect attack. **PERMANOVA* ($p<0.05$) and ns *PERMANOVA*($p<0.05$). **(E, F, G, H, I).** Taxonomical composition of bacteria by phylum level in **(F)** sweet corn, **(G)** beans,**(H)** *A. thaliana* , **(I)** tomato, and **(J)**red beet. The relative abundance of each phylum is shown in a scale from 0 to 100%. *ANOVA & Tukey's HSD post-hoc test ($p< 0.05$) and ns ANOVA & Tukey's HSD post-hoc test($p<0.05$).

FIGURE 4. Venn diagram showing the distribution of common ASVs assigned to the indicated genus among different plant species between the **(A)** insect attack group and **(B)** control group. The names from the genera in the Venn diagrams that are specific for each experimental group (eleven to insect group and nine to control group) and shared between the groups (forty-nine) are shown in supplementary table 6.

FIGURE 5. Difference in plant biomass between the control group (CTRL) and insect group (INSECT) in the second sowing. The numbers above the bars correspond to the difference in percentage between the two groups for each cultivated plant. The numbers with two decimal places on each of the bars, corresponding to the average of six repetitions evaluated for the variable fresh biomass. The numerical averages are represented in grams. (*) ANOVA one-way significant for$p< 0.05$ followed by the T- Student test (Bonferroni).

TABLE 1. Eleven bacterial genera and their respective relative abundance across crop rhizosphere samples under insect attack.**(A)** Corn, **(B)** Beans, **(C)** *A. thaliana* ,**(D)** Tomato, and **(E)** Red Beet. Eleven taxa

were analyzed based on their relative abundance values using analysis of variance (ANOVA) and Tukey's HSD post-hoc test. Significant difference ($p < 0.05$) are highlighted and labeled (*).