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ORIGINAL CONTRIBUTION

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Differential effects of the rhizobacterium *Pseudomonas simiae* on above- and belowground chewing insect herbivores

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Abstract

Plant growth-promoting rhizobacteria (PGPR) can enhance plant growth and defence. Via plant-mediated effects, PGPR have been reported to impact the performance of generalist leaf-chewing insects either negatively or positively. However, only a few insect species, mainly feeding on aboveground tissues, have thus far been investigated. Here, we investigated how addition of rhizobacteria to the soil in which cabbage plants are growing affects the performance of three chewing insect herbivores, two leaf chewers and one root feeder. In a greenhouse experiment, we grew white cabbage (*Brassica oleracea*) plants in soil supplemented with and without the rhizobacterium *Pseudomonas simiae* WCS417r. We investigated the consequences for three important insect pests of *Brassica* species, larvae of the cabbage moth *Mamestra brassicae*, the diamondback moth *Plutella xylostella*, or the cabbage root fly *Delia radicum* after 5 weeks of plant growth. We recorded aboveground plant biomass, insect biomass, plant defence marker gene expression levels and plant defence-related hormone concentrations. Rhizobacterial inoculation increased aboveground plant biomass in non-infested plants but not in infested plants. Rhizobacterial inoculation affected insect performance differently: on PGPR-inoculated plants biomass of *Plutella xylostella* was lower, while biomass of *Delia radicum* was higher than on control plants. However, no effect was found on *Mamestra brassicae* biomass. Rhizobacterial inoculation increased the expression of the defence marker gene *LOX2* in *P. xylostella*-infested plants and *MYC2* in *M. brassicae*-infested plants. Transcription levels of the plant defence marker gene *PAL1* showed upregulation between inoculated and non-inoculated insect-free plants. Levels of the phytohormones jasmonic acid, salicylic acid and abscisic acid were similar in inoculated and non-inoculated plants. We conclude that rhizobacterial inoculation has potential to be applied in the protection of cabbage crops against the diamondback moth whereas this does not apply to reducing damage caused by the cabbage moth or cabbage root fly.

KEYWORDS

cabbage moth (*Mamestra brassicae*), cabbage root fly (*Delia radicum*), diamondback moth (*Plutella xylostella*), plant resistance, rhizobacteria (*Pseudomonas simiae* WCS417r), white cabbage (*Brassica oleracea*)

1 | INTRODUCTION

Insect damage to crops is estimated to be five to twenty per cent of global crop production (Deutsch et al., 2018). Increasing agricultural output calls for effective insect pest control, which is at the same time environmentally sustainable. One of the innovative contributions to sustainable pest control is the use of beneficial soil microbes. Beneficial microbes can enhance plant growth and defence against a range of attackers (Kloepper et al., 2004; Pieterse et al., 2014; Pineda et al., 2010; Raaijmakers et al., 2009). A group of plant beneficial microbes are plant growth-promoting rhizobacteria (PGPR). Plants may gain benefits from the interaction with the bacteria, for example through increased photosynthesis (Zhang et al., 2008) or increased nutrient supply (Pii et al., 2015). There are also indirect effects on plant fitness, such as the exclusion of pathogenic bacteria (Massalha et al., 2017) or antibiotic production by rhizobacteria (de Souza et al., 2003). Moreover, PGPR may mediate induced systemic resistance towards attackers such as herbivorous insects and pathogenic microbes (Pieterse et al., 1998, 2014; Pineda et al., 2010).

Rhizobacterial colonization primes plant defence against future attack: defence responses to attack are induced faster and stronger as a result of root colonization by PGPR (Conrath et al., 2006; Pieterse et al., 2014). Rhizobacterially induced systemic resistance (ISR) involves phytohormonal regulation, such as the jasmonic acid (JA) signalling pathway (Pieterse et al., 2012). Generally, rhizobacteria-triggered ISR has adverse effects on insect herbivores, specifically on chewing insects that mainly trigger the JA defence signalling pathway (Berendsen et al., 2012; Erb et al., 2012; Pineda et al., 2010). Nevertheless, there are examples where rhizobacterial colonization does not influence insect attackers (Disi et al., 2018) or even favours insect performance (Boutard-Hunt et al., 2009; Megali et al., 2014; Pineda et al., 2012).

The consequences for insect feeding on rhizobacteria-colonized plants may depend on several factors, such as the plant organ attacked. Therefore, the outcomes of rhizobacterial-treated plants on insect antagonists are potentially plant and/or insect specific. Plant defence can vary between belowground and aboveground tissues (Biere & Goverse, 2016). For example, in response to belowground herbivory, JA acid levels are enhanced in roots, but not as much as JA levels in leaves in response to aboveground herbivory. This suggests that hormonal sensitivity in roots is higher than in aboveground tissues (Erb et al., 2012). Aboveground and belowground environments differ distinctly in biological, physical and chemical properties as well as in the species of herbivorous insects that feed on them. Root herbivores have different traits compared to aboveground herbivores, such as longer life cycles and limited mobility (Johnson et al., 2016). So far, the effect of PGPR on plant defence against belowground-feeding insects has received little attention, which will be addressed in this present study.

Here, we investigated the effects of a PGPR on cabbage plants. We focus on host plant traits such as plant growth, plant carbon and nitrogen content and defence gene expression and biomass growth of three chewing insect herbivores, one root-feeding species and two leaf feeders. The cabbage root fly *Delia radicum* is considered a pest insect herbivore on roots of cruciferous crops, mainly in northern Europe. The females lay eggs near the stem of the plant, and after hatching the larvae mine into the plant's main root. The larvae pupate in the soil. The diamondback moth *Plutella xylostella* is the most important pest herbivore of cruciferous crops and has a global distribution (Zalucki et al., 2012). The first instar larvae are leaf miners, and the older larvae feed on the underside of the leaves with a preference for the younger leaves. The cabbage moth *Mamestra brassicae* feeds on several crop species and is a pest insect in Europe and Asia. The larvae feed first on the older leaves, but will later tunnel through the crop head, leaving behind frass that contributes to crop rotting.

We employed the rhizobacterium *Pseudomonas simiae* WCS417r, formerly *P. fluorescens* WCS417r, and the crucifer crop *Brassica oleracea* cv *capitata*. We addressed the following research questions: (a) How does *P. simiae* WCS417r inoculation affect the performance of *P. xylostella*, *M. brassicae* and *D. radicum* larvae? (b) How does *P. simiae* WCS417r colonization and insect feeding affect plant defence responses? Based on previous research on the effect of *P. simiae* soil inoculation on growth of *B. oleracea* plants and on performance of *P. xylostella* and *M. brassicae*, we hypothesized that rhizobacterial inoculation increases plant biomass and negatively affects performance of the three chewing insect herbivores, which would advocate support the use of these microbes in a sustainable agriculture.

2 | MATERIALS AND METHODS

2.1 | Rhizobacterium *Pseudomonas simiae* WCS417r and plant growth conditions

The non-pathogenic epiphytic rhizobacterium *Pseudomonas simiae* WCS417r, a rifampicin-resistant strain, was grown on King's B (KB) medium agar plates containing 25 μg/ml rifampicin during 48 hr at 28°C (Pieterse et al., 1996). Prior to soil inoculation, a bacterial solution was made with sterilized 10 mM $MgSO_4$ and adjusted to a cell density of 1×10^9 colony forming units CFU/ml ($OD_{660} = 1.0$).

Seeds of white cabbage (*Brassica oleracea* cv. Christmas Drumhead, provided by the Centre for Genetic Resources, Wageningen, The Netherlands) were surface-sterilized with 80% ethanol for 1 min, followed by 15 min in 1% hypochlorite solution and washed three times with sterilized tap water. Seeds were incubated at 5°C for 3 days to synchronize germination and sown on twice autoclaved (121°C, 20 min, 24 hr in between treatments) soil (Horticoop b.v., Slingerland Potgrond) mixed 1:3 with Perlite (Agraperlite, grain size 3). Either rhizobacterial solution or 10 mM $MgSO_4$ solution was added to the soil at 50 ml/kg. After one week, plants were transplanted to $11 \times 11 \times 12$ cm pots with soil that was treated with *P. simiae* as previously described or with control soil treated with sterilized 10 mM $MgSO_4$. Plants were watered twice per week such that the soil stayed moist as visually judged from the soil surface, and 50-ml fertilizer Hyponex® was added once per week

after transplant. Plants were grown at 20 ± 2°C and 70% RH in a greenhouse. Photoperiod was maintained at 16:8 hr (light:dark) with additional lighting provided by halide bulbs (400 W) when photosynthetic active radiation (PAR) dropped below 400 μmol/s/m^2. The plants used in the experiments were 5 weeks old when they were infested with insects.

2.2 | Insect rearing

Cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae), was caught in 2013 near Zeewolde, The Netherlands, and reared at 22 ± 2°C, natural daylight, and fed on 1:1:1 mix of milk powder, sugar and yeast flakes. Larvae were reared on roots of Rutabaga, *Brassica napus*. Cabbage moth larvae *Mamestra brassicae* L. (Lepidoptera: Noctuidae) were reared on *Brassica oleracea* L. *gemmifera* cv. Cyrus at 22 ± 2°C, 40%–50% RH, 16L:8D photoperiod. Larvae of the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) were reared on *B. oleracea* L. *gemmifera* cv. Cyrus (22 ± 2°C, 40%–50% RH, 16L:8D photoperiod. Newly hatched larvae of all three species were used in the experiments.

2.3 | Insect performance, plant growth measurements and plant sampling

Plants were covered by a nylon mesh bag. On each plant either 5 neonate larvae of *M. brassicae* or *P. xylostella* were placed per plant (Kroes et al., 2016). For the root herbivore *D. radicum*, 20 neonate larvae were added carefully to the exposed top segment of the roots at the transition between root and shoot (Soler et al., 2010). Aboveground insects were weighed on day 4 and day 10 post-infestation (dpi) on a microbalance (CP2P, Sartorius AG, Germany) to the nearest 0.001 mg. Belowground insects emerging as adults from the soil in the pots were caught in the mesh bags and collected once every 24 hr. Flies were then frozen and weighed on a microbalance (CP2P, Sartorius AG) to the nearest 0.001 mg. After insect removal, plants were harvested and their fresh weight was determined. Subsequently, plants were dried at 70°C for 48 hr and weighed individually to the nearest 0.1 mg (Mettler Toledo). For nutrient analysis, the fifth leaf was collected, counted from the top. Leaves from four plants were pooled for infested plants, and from two plants for uninfested plants. The leaves were freeze-dried, ground in liquid nitrogen and weighed. Dried samples were stored at −20°C until analysis. Carbon and nitrogen content were assessed with a CHNS analyser (TruSpec CN determinator, LECO Corporation).

By the end of the experiment, the *B. oleracea* roots were inspected to confirm the presence of *Pseudomonas* bacteria. Root material (1 g fresh biomass) was collected and shaken vigorously for 1 min in 9 ml of 10 mM $MgSO_4$ containing 0.5 g of glass beads (425–600 μm, Sigma-Aldrich), and the solution was serially diluted. Dilutions were plated with 50 μl solution in duplicate onto KB agar medium supplemented with 25 μg/ml rifampicin, cyclohexamine 100 m/ml, chloramphenicol 13 mg/ml and ampicillin 50 mg/ml to select for fluorescent *Pseudomonas* spp. (Pieterse et al., 1996). The dilution plates were incubated for 48 hr at 28°C.

2.4 | Plant gene expression analysis

From the same batch of plants used for insect performance, we evaluated gene expression of the JA/ET associated *Lipoxygenase-2* (*LOX2*), transcription factor (*MYC2*) and SA associated genes *Phenylalanine ammonia-lyase-1* (*PAL1*) and *Pathogenesis-related protein-1* (*PR1*) in leaves. One leaf disc (1 cm diameter) was collected 24 hr after infestation. For *M. brassicae*- or *P. xylostella*-infested plants, the first fully expanded herbivore-damaged leaf was sampled. For *D. radicum*-infested plants and control plants, the fourth leaf from the top was sampled. Tissue was immediately frozen in liquid nitrogen and stored at −80°C for RNA extraction. Leaf discs from four plants were pooled for each insect treatment and from two plants for the control treatment without insects. Leaf samples were ground with pestle and mortar in liquid nitrogen.

Total RNA was isolated with an RNA extraction kit (Isolate II RNA Plant Kit, Bioline), according to the manufacturer's protocol. Synthesis of cDNA was carried out with a cDNA synthesis kit (SensiFAST, Bioline) following the manufacturer's instructions and diluted five times. Stock primers were diluted four times. Efficiency of each primer was determined before qRT-PCR analysis (CFX96™ Real-Time System, Bio-rad). For the full primer list, see Table S1. A Bio-rad 1000 machine was used to carry out qPCR. Reaction mixtures (25 μl) contained 10 μl of SYBR Green qPCR master mix (Bio-rad), cDNA and 5 μl of each primer. The thermocycle parameters were as following: initial polymerase activation, 10 min at 95°C, followed by 40 cycles of 30 s at 95°C, 60 s at 57°C and 30 s at 72°C. Conditions were determined by temperature gradient testing for all primers and a mixture of eight random samples cDNA from the experiment. From six reference genes (*Act-2*, *Btub*, *EF1a*, *GAPDH*, *PER4*, *SAR1a*), *SAR1a* and *Act-2* were selected as optimal reference genes. Relative gene expression was calculated from primer efficiency with the software qBase + 3.1 (Biogazelle), through the CNRQ (Calibrated Normalized Relative Quantity) method.

2.5 | Analysis of phytohormones jasmonic acid, salicylic acid and abscisic acid

From the same samples that were used for phytohormone analysis, a portion was lyophilized (Snijders type 2040 lyophylizer, Tilburg, The Netherlands) and extracted with methanol. Phytohormone analysis was performed on an Agilent 1200 series HPLC system (Agilent Technologies) coupled to a tandem mass spectrometer (Vadassery et al., 2012), with the modification that a tandem mass spectrometer QTRAP 6500 (SCIEX) was used. Total concentration of jasmonates (JAs) was calculated by summation of the concentrations of JA, JA-Ile, cis-OPDA, OH-JA, OH-JA-Ile and COOH-JA-Ile.

2.6 | Statistical analysis

To check normality and homogeneity of the data, we used Shapiro–Wilk's test, and inspecting residuals through visualization using qq-plots and Cullen and Frey graphs. Insect performance data were analysed with a linear mixed model or generalized linear mixed model, depending on normality and homogeneity of the data with plant and emergence day for *D. radicum*, fit by the REML method, as random factors. Plant biomass, gene expression, phytohormone and plant carbon and nitrogen data were analysed with Student's *t* test or generalized linear model depending on normality and homogeneity of the data, with $\alpha = 0.05$. All plants were included in analyses. If suitable, post hoc Tukey's tests were run with a maximum likelihood fit (Laplace Approximation). The statistical tests were carried out with RStudio version 1.1.423 (R Core Team, 2018) using packages car (Fox & Weisberg, 2019), lmtest (Zeileis & Hothorn, 2002), readxl, ggplot2, fitdistrplus and lme4 (Bates et al., 2015).

3 | RESULTS

3.1 | Effects of *P. simiae* soil inoculation on *P. xylostella* larval biomass and plant traits

The effects of rhizobacterial soil inoculation resulted in significant decrease of the body mass of *P. xylostella* larvae by 20% (LMM: $\chi^2 = 7.25$, $df = 1$, $p = .007$; Figure 1c) compared to the control after 10 days of insect feeding. For an overview of all experiments, measurements made and numbers of replicates, see Table 1. Shoot dry weight, leaf carbon and nitrogen content of plants infested by

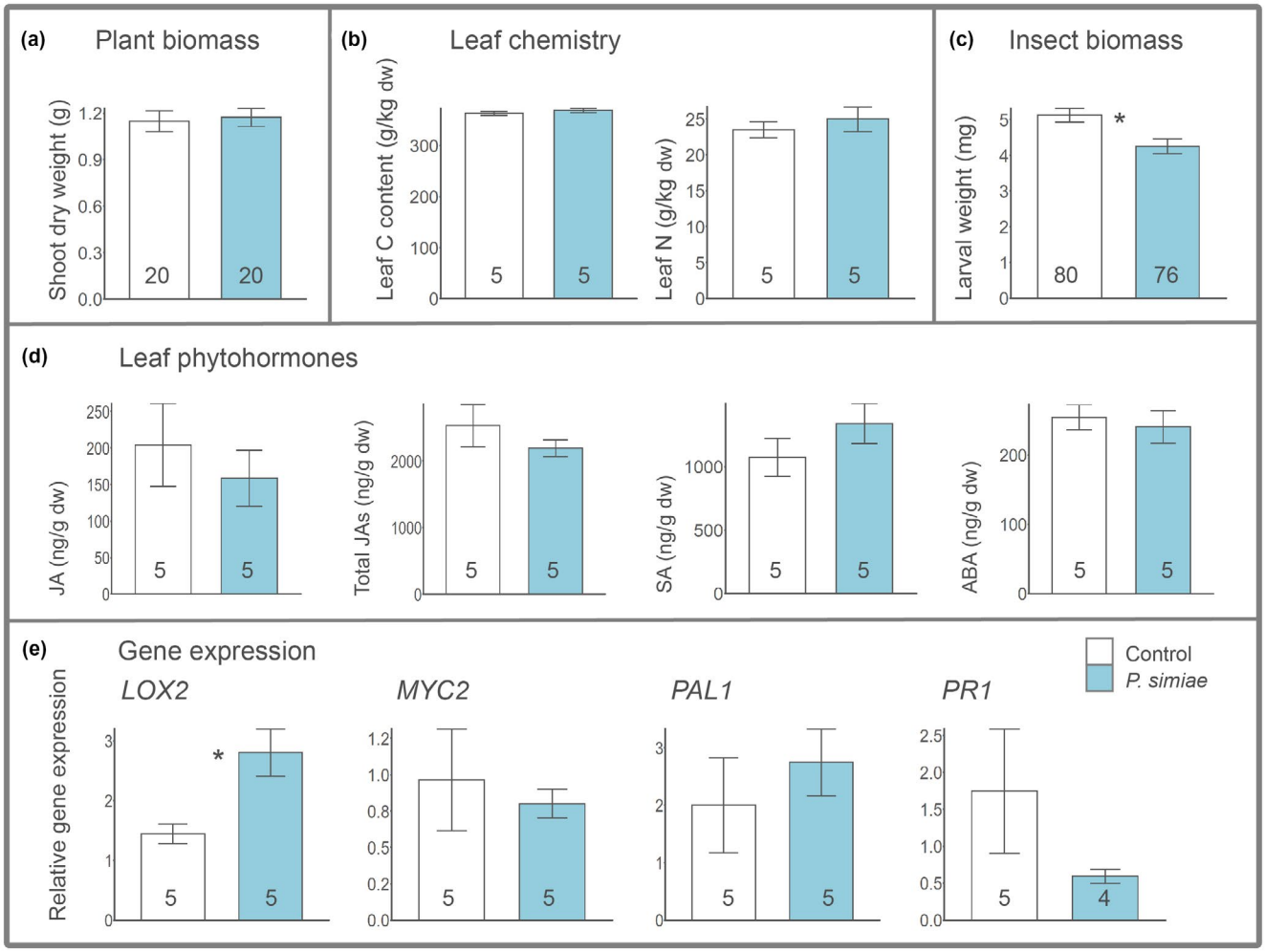

FIGURE 1 Effects of *Pseudomonas simiae* soil inoculation on *Plutella xylostella* growth performance and plant parameters. (a) Shoot dry weight of *P. xylostella*-infested cabbage plants growing in inoculated or non-inoculated soil. (b) Carbon and nitrogen content of leaves with *P. xylostella* feeding on inoculated or non-inoculated cabbage plants in g/kg dry weight. (c) *P. xylostella* larval biomass 10 days post-infestation feeding on inoculated or non-inoculated cabbage plants. (d) Concentrations of the plant hormones jasmonic acid (JA) and total jasmonates (JAs), salicylic acid (SA) and abscisic acid (ABA) in leaves of *P. xylostella*-infested plants growing in inoculated or non-inoculated soil in ng/g dry weight. (e) Plant defence marker gene expression in leaves of *P. xylostella*-infested plants growing in inoculated or non-inoculated soil relative to expression of housekeeping genes. Bars show mean ± *SE*; numbers in bars are number of replicates per treatment. Asterisk (*) indicates significant difference between control plants and plants grown on *P. simiae* inoculated soil [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Overview of experiments performed and the numbers of replicates for each of the plant and insect variables measured

Insect treatment	Inoculation	Number of plants	Plant growth		Number of insect individuals measured for biomass	Gene expression 24 hr PI	Carbon–nitrogen analysis and phytohormones
			6 weeks	12 weeks		Number of samples	Number of samples
Plutella xylostella	None	20	x		80	4	4
	Pseudomonas simiae	20	x		76	4	4
Mamestra brassicae	None	20	x		82	4	4
	Pseudomonas simiae	20	x		87	4	4
No insect	None	10	x			2	2
	Pseudomonas simiae	10	x			2	2
Delia radicum	None	21		x	122	4	4
	Pseudomonas simiae	20		x	89	4	4
No insect	None	10		x		2	2
	Pseudomonas simiae	10		x		2	2

P. xylostella were not affected by rhizobacterial soil inoculation (shoot dry weight: $\chi^2 = 0.08$, $df = 1$, $p = .78$; Figure 1a; carbon content: t test: $t = -1.01$, $df = 8$, $p = .34$; nitrogen content: t test: $t = -0.73$, $df = 8$, $p = .49$; Figure 1b). We explored possible phytochemical mechanisms for the reduced larval growth by examining defence-related plant traits. The concentrations of the phytohormones JA, SA and ABA in *P. xylostella*-infested leaves were not affected by soil inoculation with *P. simiae* (JA: t test: $t = 0.66$, $df = 8$, $p = .52$; total jasmonates: GLM: $\chi^2 = 1.07$, $df = 1$, $p = .30$; SA:GLM: $\chi^2 = 1.47$, $df = 1$, $p = .23$; ABA: t test: $t = 0.45$, $df = 8$, $p = .66$; Figure 1d). The transcript level of the plant defence marker gene *LOX2* was higher in leaves of plants grown in *P. simiae* inoculated soil compared to leaves of control plants after 24 hr of insect infestation; transcript levels for the other three genes were not affected by *P. simiae* inoculation (*LOX2*: GLM: $\chi^2 = 13.07$, $df = 1$, $p < .001$; GLM: *MYC2*: $\chi^2 = 0.24$, $df = 1$, $p = .63$; *PAL1*: GLM: $\chi^2 = 3.39$, $df = 1$, $p = .065$; *PR1*: GLM: $\chi^2 = 0.45$, $df = 1$, $p = .50$; Figure 1e).

3.2 | Effects of *P. simiae* soil inoculation on *M. brassicae* larval biomass and plant traits

The effects of rhizobacterial soil inoculation did neither influence the body mass of *M. brassicae* larvae after 10 days of feeding (GLMM: $\chi^2 = 0.25$, $df = 1$, $p = .61$; Figure 2c), nor plant shoot dry weight, carbon or nitrogen leaf content (shoot dry weight: GLM: $\chi^2 = 0.08$, $df = 1$, $p = .77$; Figure 2a; carbon content: t test: $t = -0.86$, $df = 4.44$, $p = .43$; nitrogen content: t test: $t = -0.35$, $df = 8$, $p = .74$; Figure 2b) of *M. brassicae*-infested plants. Rhizobacterial inoculation did not affect phytohormone levels of JA, total jasmonates, SA or ABA in leaves (JA: t test: $t = 0.73$, $df = 8$, $p = .48$; total jasmonates: t test: $t = 1.16$, $df = 8$, $p = .28$; SA: GLM: $\chi^2 = 0.78$, $df = 1$, $p = .38$; ABA: GLM: $\chi^2 < 0.01$, $df = 1$, $p = .93$; Figure 2d) of plants infested by *M. brassicae*. Finally, rhizobacterial soil inoculation affected transcript levels of marker gene *MYC2*, whereas the levels of the three other marker genes were not affected in leaves after 24 hr of caterpillar feeding (*LOX2*: GLM: $\chi^2 = 0.05$, $df = 1$, $p = .83$; *MYC2*: GLM: $\chi^2 = 6.02$, $df = 1$, $p = .014$; *PAL1*: GLM: $\chi^2 < 0.01$, $df = 1$, $p = .96$; *PR1*: GLM: $\chi^2 = 2.65$, $df = 1$, $p = .10$; Figure 2e).

3.3 | Effects of *P. simiae* soil inoculation on *D. radicum* adult biomass and plant traits

Rhizobacterial inoculation resulted in increased adult weight of *D. radicum* (LMM: $\chi^2 = 6.85$, $df = 1$, $p < .001$; Figure 3c). The effect size of the random factor plant was 25%, indicating that the individual plant was important for the fly weight. The effect size of random factor of emergence day was 6%. Development time until insect adult emergence was similar for inoculated and non-inoculated plants (non-inoculated: median = 37 days post-infestation DPI, 3rd quartile = 39 DPI, $n = 38$; inoculated: median = 38 DPI, 3rd quartile = 39 DPI; GLMM: $\chi^2 = 2.07$, $df = 1$, $p = .15$, $n = 39$). Shoot dry weight of *D. radicum*-infested plants

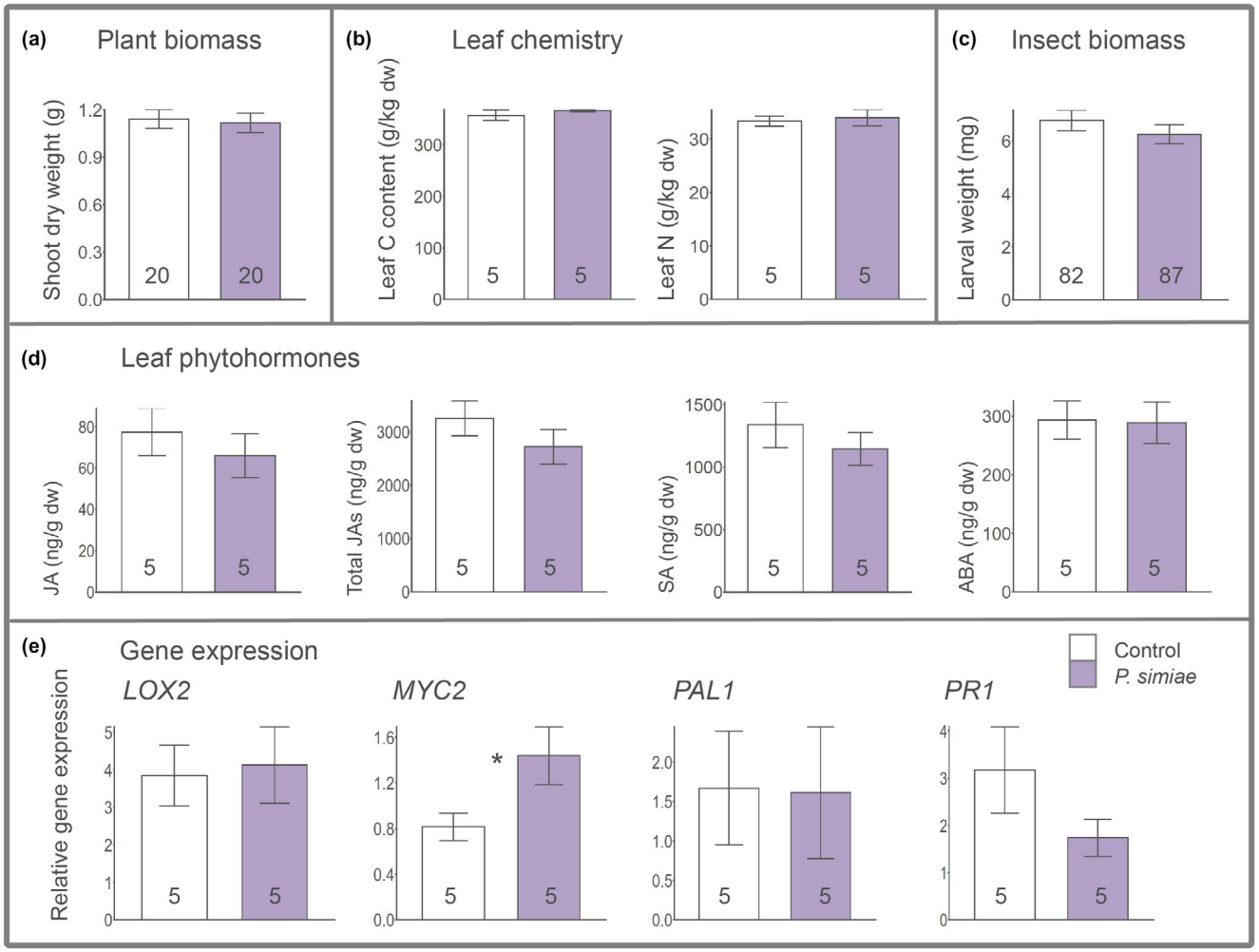

FIGURE 2 Effects of *Pseudomonas simiae* inoculation on *Mamestra brassicae* growth performance and plant parameters. (a) Shoot dry weight of *M. brassicae*-infested cabbage plants growing in inoculated or non-inoculated soil. (b) Carbon and nitrogen content in leaves with *M. brassicae* feeding on inoculated or non-inoculated cabbage plants in g/kg dry weight. (c) *Mamestra brassicae* larval biomass 10 days post-infestation feeding on inoculated or non-inoculated cabbage plants. (d) Concentrations of the plant hormones jasmonic acid (JA) and total jasmonates (JAs), salicylic acid (SA) and abscisic acid (ABA) in leaves of *M. brassicae*-infested plants growing in inoculated or non-inoculated soil in ng/g dry weight. (e) Plant defence marker gene expression in leaves of *M. brassicae*-infested cabbage plants growing in inoculated or non-inoculated soil relative to expression of housekeeping genes. Bars show mean ± *SE*; numbers in bars are number of replicates per treatment. Asterisk (*) indicates significant difference between control plants and plants grown on *P. simiae* inoculated soil [Colour figure can be viewed at wileyonlinelibrary.com]

was similar for inoculated and non-inoculated plants (*t* test: $t = -1.61$, $df = 39$, $p = .12$; Figure 3a). Soil inoculation did not influence leaf carbon or nitrogen content (carbon content: $t = -1.23$, $df = 8$, $p = .25$; nitrogen content: *t* test: $t = -1.62$, $df = 8$, $p = .14$; Figure 3b) of plants infested by *D. radicum*. Insect infestation and rhizobacterial inoculation did not affect the foliar concentrations of the phytohormones JA, total jasmonates or ABA, but did have an effect on SA levels (JA: *t* test: $t = -0.31$, $df = 8$, $p = .76$; total jasmonates: GLM: $\chi^2 = 0.49$, $df = 1$, $p = .48$; SA: GLM: $\chi^2 = 5.34$, $df = 1$, $p = .021$; ABA: GLM: $\chi^2 = 2.46$, $df = 1$, $p = .12$; Figure 3b) or defence marker gene expression in leaves of *D. radicum*-infested plants after 24 hr of herbivory (*LOX2*: GLM: $\chi^2 < 0.01$, $df = 1$, $p = .97$; *MYC2*: GLM: $\chi^2 = 0.47$, $df = 1$, $p = .49$; *PAL1*: GLM: $\chi^2 = 1.00$, $df = 1$, $p = .32$; *PR1*: GLM: $\chi^2 = 0.16$, $df = 1$, $p = .69$; Figure 3e).

3.4 | Effect of *P. simiae* soil inoculation on traits of uninfested plants

The addition of *P. simiae* to the soil increased shoot dry weight of uninfested cabbage plants after 6 weeks of exposure by on average 33% (*t* test: $t = -2.99$, $df = 17$, $p = .008$; Figure 4a), whereas carbon or nitrogen content of leaves were not affected (carbon content: *t* test: $t = -0.79$, $df = 7$, $p = .46$; nitrogen content: *t* test: $t = 0.83$, $df = 7$, $p = .44$; Figure 4b). Foliar concentrations of SA were higher and concentrations of ABA lower for plants inoculated with bacteria compared to control plants, whereas JA concentration was unaffected by bacterial inoculation (JA: *t* test: $t = -0.26$, $df = 7$, $p = .80$; total jasmonates: GLM: $\chi^2 = 0.82$, $df = 1$, $p = .37$; SA: *t* test: $t = -2.64$, $df = 7$, $p = .033$; ABA: GLM: $\chi^2 = 13.3$, $df = 1$, $p < .001$;

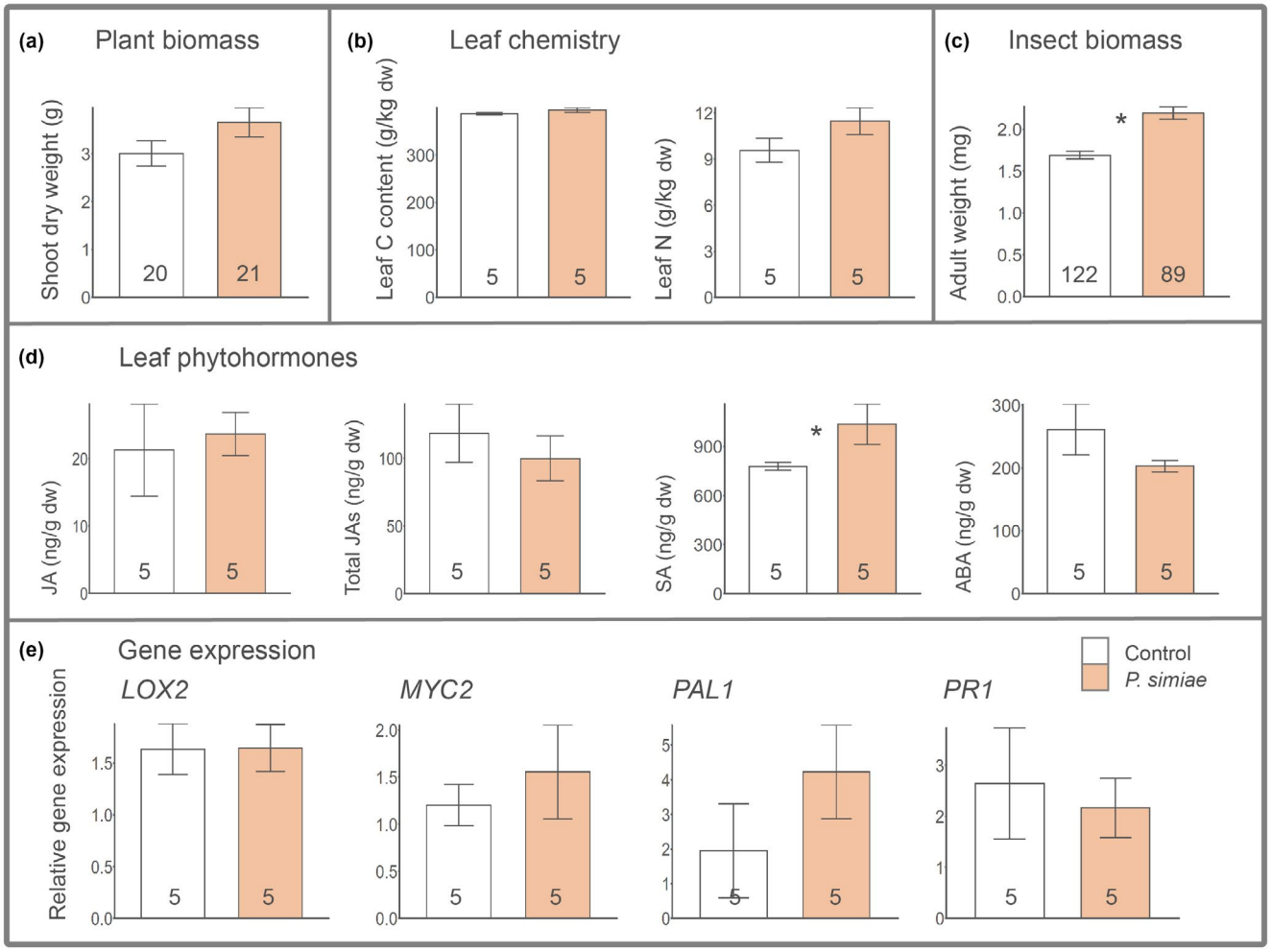

FIGURE 3 Effects of *Pseudomonas simiae* inoculation on *Delia radicum* growth performance and plant parameters. (a) Shoot dry weight of *D. radicum*-infested cabbage plants growing in inoculated or non-inoculated soil. (b) Carbon and nitrogen content in leaves with *D. radicum* feeding on inoculated or non-inoculated cabbage plants in g/kg dry weight. (c) *Delia radicum* adult weight by emergence feeding on inoculated or non-inoculated cabbage plants. (d) Concentrations of the plant hormones jasmonic acid (JA) and total jasmonates (JAs), salicylic acid (SA) and abscisic acid (ABA) in leaves of *D. radicum*-infested plants growing in inoculated or non-inoculated soil in ng/g dry weight. (e) Plant defence marker gene expression in leaves of *D. radicum*-infested cabbage plants growing in inoculated or non-inoculated soil relative to expression of housekeeping genes. Bars show mean ± *SE*; numbers in bars are number of replicates per treatment. Asterisk (*) indicates significant difference between control plants and plants grown on *P. simiae* inoculated soil [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 4c). Bacterial inoculation resulted in lower expression level of the marker gene *PAL1* in leaves, whereas the expression levels of *PR1*, *LOX2* and *MYC2* were unaffected (*LOX2*: GLM: $\chi^2 = 3.76$, $df = 1$, $p = .053$; *MYC2*: GLM: $\chi^2 = 0.26$, $df = 1$, $p = .61$; *PAL1*: GLM: $\chi^2 = 7.07$, $df = 1$, $p = .008$; *PR1*: GLM: $\chi^2 = 0.01$, $df = 1$, $p = .91$; Figure 4d).

For 12-week-old plants, rhizobacterial inoculation did neither affect shoot dry weight ($t = -0.75$, $df = 18$, $p = .46$; Figure 5a) nor foliar carbon or nitrogen content (carbon: *t* test: $t = -1.80$, $df = 7$, $p = .11$; nitrogen: GLM: $\chi^2 = 1.31$, $df = 1$, $p = .25$; Figure 5b), nor foliar levels of JA, SA and ABA (JA: *t* test: $t = 0.55$, $df = 7$, $p = .60$; total jasmonates: GLM: $\chi^2 = 0.52$, $df = 1$, $p = .47$; SA: GLM: $\chi^2 = 1.61$, $df = 1$, $p = .20$; ABA: GLM: $\chi^2 = 0.43$, $df = 1$, $p = .51$; Figure 5c).

3.5 | Re-isolation of *Pseudomonas* from plant rhizosphere

Pseudomonas bacterial concentration was higher in inoculated soil than in non-inoculated soil after both 6 weeks and 12 weeks of plant growth on agar media selective for *Pseudomonas*. The control soil contained on average 2.7×10^6 *Pseudomonas* bacterial CFU/g of rhizosphere after 6 weeks. The inoculated soil contained approximately 76 times more, that is on average 2.05×10^8 CFU/g of rhizosphere. After 12 weeks of plant growth, control soil had on average 5.74×10^6 CFU/g, and inoculated rhizosphere soil had 2.5×10^7 CFU/g.

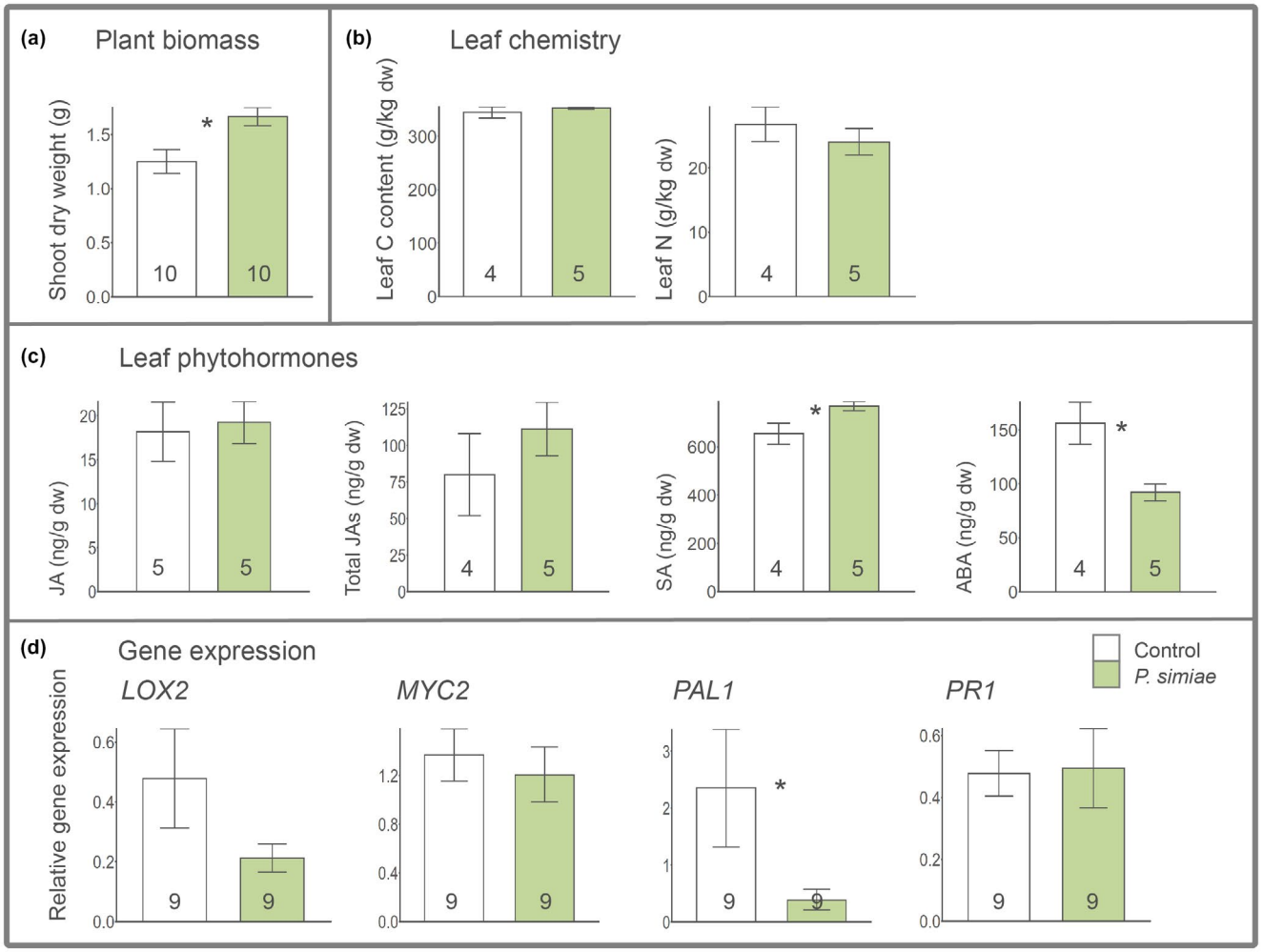

FIGURE 4 Effects of *Pseudomonas simiae* inoculation on 6-week-old cabbage plants. (a) Shoot dry weight of cabbage plants growing in inoculated or non-inoculated soil. (b) Carbon and nitrogen content in leaves of cabbage plants growing in inoculated or non-inoculated soil in g/kg dry weight. (c) Concentrations of the plant hormones jasmonic acid (JA) and total jasmonates (JAs), salicylic acid (SA) and abscisic acid (ABA) in leaves of cabbage plants growing in inoculated or non-inoculated soil in ng/g dry weight. (d) Plant defence marker gene expression in leaves of cabbage plants growing in inoculated or non-inoculated soil relative to expression of housekeeping genes. Bars show mean ± *SE*; numbers in bars are number of replicates per treatment. Asterisk (*) indicates significant difference between control plants and plants grown on *P. simiae* inoculated soil [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Our study shows that inoculation of *Pseudomonas simiae* WCS417r into the soil differentially influences the performance of three chewing insect herbivores feeding on cabbage plants. Insect biomass data for *P. xylostella* showed decreased growth when cabbage plants were colonized by *P. simiae* WCS417r, suggesting an induced systemic plant defence response. This suggestion is supported by higher transcript levels of the defence marker gene *LOX2* in *P. xylostella*-infested leaves of rhizobacteria-inoculated plants. *Plutella xylostella* feeding has been previously shown to upregulate *LOX2* as part of plant defence in cabbage (Li et al., 2016). Upregulation of plant defence marker genes has previously been reported in conjunction with *P. simiae*-treatment in *A. thaliana* plants (Pangesti et al., 2016). Concentrations of JA, SA or ABA in *P. xylostella*-infested leaves were not affected by rhizobacterial inoculation. A plausible explanation of why JA and ABA concentrations were not affected whereas the expression of *LOX2*, functioning in the biosynthetic pathway of JA and ABA biosynthesis, was upregulated is the long time interval between taking tissue samples for gene expression analysis (24 hr) and phytohormone quantification (6–12 weeks).

Rhizobacterial soil inoculation did not affect biomass of *M. brassicae* larvae. Previous work shows that upregulation of *LOX2* is part of plant defence against *M. brassicae* feeding on cabbage (Li et al., 2016). In our study, *M. brassicae*-induced transcription of *LOX2* nor jasmonate production was affected by rhizobacterial inoculation; however, JA-dependent transcription of *MYC2*, was upregulated by rhizobacterial inoculation without an effect on growth of *M. brassicae* larvae. This finding shows that the three chewing herbivores have different effects on the regulation of expression of genes in the JA pathway. Studies on other plants show various effects of *P. simiae* inoculation on *M. brassicae* performance. When feeding on

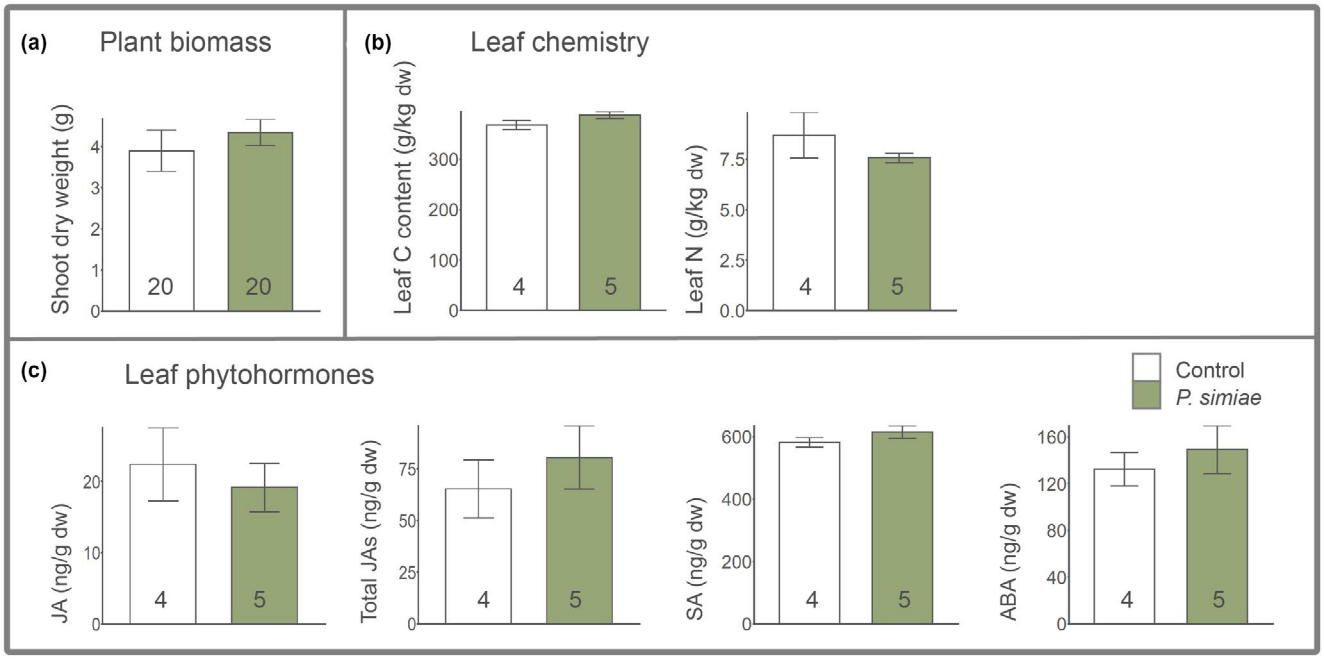

FIGURE 5 Effects of *Pseudomonas simiae* inoculation on 12-week-old cabbage plants. (a) Shoot dry weight of cabbage plants growing in inoculated or non-inoculated soil. (b) Carbon and nitrogen content in leaves of cabbage plants growing in inoculated or non-inoculated soil in g/kg dry weight. (c) Concentrations of the plant hormones jasmonic (JA) and total jasmonates (JAs), salicylic acid (SA) and abscisic acid (ABA) in leaves ng/g dry weight. Bars show mean ± *SE*; numbers in bars are number of replicates per treatment [Colour figure can be viewed at wileyonlinelibrary.com]

Arabidopsis thaliana plants, *P. simiae* WCS417r colonization can both negatively or positively affect *M. brassicae* larvae, depending on soil nutrient concentration and drought stress (Fernández de Bobadilla et al., 2017; Pangesti et al., 2015). Addition of fertilizer in our study may have offset potential negative effects of *P. simiae* on *M. brassicae* growth.

Delia radicum performed better on plants in rhizobacteria-treated soil compared to *D. radicum* on control plants. The effects of rhizobacterial inoculation of plants on *D. radicum* have not been studied so far. However, root microbial community structure is altered when *D. radicum* is feeding on oilseed rape (*Brassica napus*) (Ourry et al., 2018). Additionally, another belowground chewer, the beetle *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae), is negatively affected by rhizobacterial inoculation of *Azospirillum brasilense* in maize (Santos et al., 2014).

We propose three non-mutually exclusive mechanisms that may explain the increased *D. radicum* biomass. First, cabbage root biomass may have increased due to rhizobacterial inoculation, thereby increasing food availability to *D. radicum* larvae. How cabbage root biomass is affected by *P. simiae* is not yet known, although in the related plant *A. thaliana*, *P. simiae* WCS417r increased root and shoot biomass (Pangesti et al., 2017). Second, root production of secondary metabolites such as glucosinolates may be affected by PGPR colonization. PGPR inoculation may reduce glucosinolate levels in *A. thaliana* roots when colonized by *Kosakonia radicincitans* rhizobacteria (Witzel et al., 2017). However, glucosinolate levels did not influence *D. radicum* performance in five wild cabbage populations (Van Geem et al., 2015). Yet, glucosinolates stimulate oviposition by *D. radicum* (Roessingh et al., 1992), but it is not known whether the behaviour of *D. radicum* larvae is affected by glucosinolate levels. A third explanation may be found in direct interactions between the PGPR and the insect. Compounds secreted by *P. simiae* may either act as feeding stimulants, or interact with insect gut microbes to increase nutrient acquisition. The gut microbiome of *D. radicum* larvae may contain PGPR. For example the PGPR strain *Pseudomonas* sp. PRGB06 has been found in the gut of *P. xylostella* (Indiragandhi et al., 2008), but has yet to be found in belowground feeders. Further research is needed to determine whether *P. simiae* WCS417r stimulates feeding by *D. radicum*.

Uninfested rhizobacteria-inoculated plants exhibited downregulated expression of the marker gene *PAL1*, whereas expression of JA-associated marker genes was similar to that in uninfested control plants. Previous studies showed an upregulation of JA-related marker genes in *P. simiae* colonized *A. thaliana* plants (Pangesti et al., 2016).

We found that rhizobacterial inoculation increased plant biomass, a result in line with previous research. Our results present the first report of cabbage growth promotion in response to colonization by *P. simiae* WCS417r. Cabbage plants have previously been shown to respond to rhizobacterial colonization by increased growth; several rhizobacterial species increase plant growth (Turan et al., 2014). Plant growth promotion by *P. simiae* WCS417r has been shown in other plant species such as grapevine, radish and banana (Berendsen et al., 2015). Yet, for some plant species this strain did not promote growth, such as tobacco (van Loon et al., 2008). Hence, the strain possesses specificity to plant species. This study's observed growth-promoting effect

was lacking in herbivore-infested plants. This may be explained by increase of plant defence in infested plants. According to the hypothesis of a growth-defence trade-off, plant resources will be distributed to either expansion or protection, where one will be adjusted for the other. An increase of defence in the experimental plants would thus be expected to result in lowered plant growth.

In conclusion, growth and defence of cabbage plants can benefit from the beneficial rhizobacterium *P. simiae* WCS417r. Interestingly, we found a positive effect on the performance of the root herbivore *D. radicum* whereas for other chewing root herbivores negative effects have been recorded. Thus, promotion of plant growth and improving resistance to chewing insect herbivores through soil inoculation with rhizobacteria has potential to be applied in the protection of cabbage crops against the diamondback moth. Our experiments did not demonstrate similar protective effects of rhizobacterial inoculation with *P. simiae* against cabbage moth larvae or larvae of cabbage root fly.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

JF conducted the research and wrote the manuscript with input of AP, JG, MD and JJAL. MR conducted the plant hormone analysis. All authors read and approved the manuscript.

DATA AVAILABILITY STATEMENT

Data are available on request; raw data and metadata will be uploaded on an open data repository upon final acceptance of the manuscript.

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REFERENCES

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berendsen, R. L., van Verk, M. C., Stringlis, I. A., Zamioudis, C., Tommassen, J., Pieterse, C. M. J., & Bakker, P. (2015). Unearthing the genomes of plant-beneficial *Pseudomonas* model strains WCS358, WCS374 and WCS417. *Bmc Genomics*, *16*, 23.

Biere, A., & Goverse, A. (2016). Plant-mediated systemic interactions between pathogens, parasitic nematodes, and herbivores above- and belowground. *Annual Review of Phytopathology*, *54*, 499–527.

Boutard-Hunt, C., Smart, C. D., Thaler, J., & Nault, B. A. (2009). Impact of plant growth-promoting rhizobacteria and natural enemies on *Myzus persicae* (Hemiptera: Aphididae) infestations in pepper. *Journal of Economic Entomology*, *102*(6), 2183–2191.

Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., & Mauch-Mani, B. (2006). Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions*, *19*(10), 1062–1071. https://doi.org/10.1094/MPMI-19-1062

de Souza, J. T., Arnould, C., Deulvot, C., Lemanceau, P., Gianinazzi-Pearson, V., & Raaijmakers, J. M. (2003). Effect of 2,4-diacetylphloroglucinol on pythium: Cellular responses and variation in sensitivity among propagules and species. *Phytopathology*, *93*(8), 966–975.

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science*, *361*(6405), 916–919. https://doi.org/10.1126/science.aat3466

Disi, J. O., Zebelo, S., Kloepper, J. W., & Fadamiro, H. (2018). Seed inoculation with beneficial rhizobacteria affects European corn borer (Lepidoptera: Pyralidae) oviposition on maize plants. *Entomological Science*, *21*(1), 48–58. https://doi.org/10.1111/ens.12280

Erb, M., Glauser, G., & Robert, C. A. M. (2012). Induced immunity against belowground insect herbivores- activation of defenses in the absence of a jasmonate burst. *Journal of Chemical Ecology*, *38*(6), 629–640. https://doi.org/10.1007/s10886-012-0107-9

Erb, M., Meldau, S., & Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science*, *17*(5), 250–259.

Fernández de Bobadilla, M. F., Friman, J., Pangesti, N., Dicke, M., van Loon, J. J. A., & Pineda, A. (2017). Does drought stress modify the effects of plant-growth promoting rhizobacteria on an aboveground chewing herbivore? *Insect Science*, *24*(6), 1034–1044.

Fox, J., & Weisberg, S. (2019). *Companion to applied regression* (3rd ed.). Sage.

Indiragandhi, P., Anandham, R., Madhaiyan, M., & Sa, T. M. (2008). Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). *Current Microbiology*, *56*(4), 327–333.

Johnson, S. N., Erb, M., & Hartley, S. E. (2016). Roots under attack: Contrasting plant responses to below- and aboveground insect herbivory. *New Phytologist*, *210*(2), 413–418.

Kloepper, J. W., Ryu, C. M., & Zhang, S. A. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, *94*(11), 1259–1266.

Kroes, A., Stam, J. M., David, A., Boland, W., van Loon, J. J. A., Dicke, M., & Poelman, E. H. (2016). Plant-mediated interactions between two herbivores differentially affect a subsequently arriving third herbivore in populations of wild cabbage. *Plant Biology*, *18*(6), 981–991.

Li, Y. H., Dicke, M., Kroes, A., Liu, W., & Gols, R. (2016). Interactive effects of cabbage aphid and caterpillar herbivory on transcription of plant genes associated with phytohormonal signalling in wild cabbage. *Journal of Chemical Ecology*, *42*(8), 793–805.

Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O. H., & Aharoni, A. (2017). Live imaging of root-bacteria interactions in a microfluidics setup. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(17), 4549–4554.

Megali, L., Glauser, G., & Rasmann, S. (2014). Fertilization with beneficial microorganisms decreases tomato defenses against insect pests. *Agronomy for Sustainable Development*, *34*(3), 649–656.

Ourry, M., Lebreton, L., Chaminade, V., Guillerm-Erckelboudt, A. Y., Herve, M., Linglin, J., Marnet, N., Ourry, A., Paty, C., Poinsot, D., Cortesero, A.-M., & Mouge, C. (2018). Influence of belowground herbivory on the dynamics of root and rhizosphere microbial communities. *Frontiers in Ecology and Evolution*, *6*, 21.

Pangesti, N., Pineda, A., Dicke, M., & van Loon, J. J. A. (2015). Variation in plant-mediated interactions between rhizobacteria and caterpillars: Potential role of soil composition. *Plant Biology*, *17*(2), 474–483.

Pangesti, N., Reichelt, M., van de Mortel, J. E., Kapsomenou, E., Gershenzon, J., van Loon, J. J. A., Dicke, M., & Pineda, A. (2016). Jasmonic acid and ethylene signaling pathways regulate glucosinolate levels in plants during rhizobacteria-induced systemic resistance against a leaf-chewing herbivore. *Journal of Chemical Ecology*, *42*(12), 1212–1225.

Pangesti, N., Vandenbrande, S., Pineda, A., Dicke, M., Raaijmakers, J. M., & Van Loon, J. J. A. (2017). Antagonism between two root-associated beneficial *Pseudomonas* strains does not affect plant growth promotion and induced resistance against a leaf-chewing herbivore. *FEMS Microbiology Ecology*, *93*(4), 8.

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, *28*, 489–521.

Pieterse, C. M. J., van Wees, S. C. M., van Pelt, J. A., Knoester, M., Laan, R., Gerrits, N., Weisbeek, P. J., & van Loon, L. C. (1998). A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *The Plant Cell*, *10*(9), 1571–1580.

Pieterse, C. M. J., vanWees, S. C. M., Hoffland, E., vanPelt, J. A., & vanLoon, L. C. (1996). Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *The Plant Cell*, *8*(8), 1225–1237.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, *52*, 347–375.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015). Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils*, *51*(4), 403–415.

Pineda, A., Zheng, S. J., van Loon, J. J. A., & Dicke, M. (2012). Rhizobacteria modify plant-aphid interactions: A case of induced systemic susceptibility. *Plant Biology*, *14*, 83–90.

Pineda, A., Zheng, S. J., van Loon, J. J. A., Pieterse, C. M. J., & Dicke, M. (2010). Helping plants to deal with insects: The role of beneficial soil-borne microbes. *Trends in Plant Science*, *15*(9), 507–514. https://doi.org/10.1016/j.tplants.2010.05.007

R Core Team (2018). *A language and environment for statistical computing*. R Foundation for Statistical Computing.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moenne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, *321*(1–2), 341–361. https://doi.org/10.1007/s11104-008-9568-6

Roessingh, P., Stadler, E., Fenwick, G. R., Lewis, J. A., Nielsen, J. K., Hurter, J., & Ramp, T. (1992). Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant-extracts. *Entomologia Experimentalis Et Applicata*, *65*(3), 267–282. https://doi.org/10.1111/j.1570-7458.1992.tb00680.x

Santos, F., Penaflor, M., Pare, P. W., Sanches, P. A., Kamiya, A. C., Tonelli, M., Nardi, C., & Bento, J. M. S. (2014). A novel interaction between plant-beneficial rhizobacteria and roots: Colonization induces corn resistance against the root herbivore *Diabrotica speciosa*. *PLoS One*, *9*(11), e113280. https://doi.org/10.1371/journal.pone.0113280

Soler, R., Harvey, J. A., Rouchet, R., Schaper, S. V., & Bezemer, T. M. (2010). Impacts of belowground herbivory on oviposition decisions in two congeneric butterfly species. *Entomologia Experimentalis Et Applicata*, *136*(2), 191–198. https://doi.org/10.1111/j.1570-7458.2010.01015.x

Turan, M., Ekinci, M., Yildirim, E., Gunes, A., Karagoz, K., Kotan, R., & Dursun, A. (2014). Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. *Turkish Journal of Agriculture and Forestry*, *38*(3), 327–333. https://doi.org/10.3906/tar-1308-62

Vadassery, J., Reichelt, M., Hause, B., Gershenzon, J., Boland, W., & Mithofer, A. (2012). CML42-mediated calcium signaling coordinates responses to *Spodoptera* herbivory and abiotic stresses in *Arabidopsis*. *Plant Physiology*, *159*(3), 1159–1175.

Van Geem, M., Harvey, J. A., Cortesero, A. M., Raaijmakers, C. E., & Gols, R. (2015). Interactions between a belowground herbivore and primary and secondary root metabolites in Wild cabbage. *Journal of Chemical Ecology*, *41*(8), 696–707. https://doi.org/10.1007/s10886-015-0605-7

van Loon, L. C., Bakker, P., van der Heijdt, W. H. W., Wendehenne, D., & Pugin, A. (2008). Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. *Molecular Plant-Microbe Interactions*, *21*(12), 1609–1621. https://doi.org/10.1094/MPMI-21-12-1609

Witzel, K., Strehmel, N., Baldermann, S., Neugart, S., Becker, Y., Becker, M., Berger, B., Scheel, D., Grosch, R., Schreiner, M., & Ruppel, S. (2017). *Arabidopsis thaliana* root and root exudate metabolism is altered by the growth-promoting bacterium *Kosakonia radicincitans* DSM 16656(T). *Plant and Soil*, *419*(1–2), 557–573. https://doi.org/10.1007/s11104-017-3371-1

Zalucki, M. P., Shabbir, A., Silva, R., Adamson, D., Liu, S. S., & Furlong, M. J. (2012). Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just how long is a piece of string? *Journal of Economic Entomology*, *105*(4), 1115–1129.

Zeileis, A., & Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News*, *2*(3), 7–10.

Zhang, H. M., Xie, X. T., Kim, M. S., Kornyeyev, D. A., Holaday, S., & Pare, P. W. (2008). Soil bacteria augment *Arabidopsis* photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. *Plant Journal*, *56*(2), 264–273.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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