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RESEARCH ARTICLE

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Soil amendment with insect frass and exuviae affects rhizosphere bacterial community, shoot growth and carbon/nitrogen ratio of a brassicaceous plant

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Abstract
Aims In terrestrial ecosystems, deposition of insect frass and cadavers in the soil influences soil characteristics, including microbial community composition, with consequences for plant growth and development. Insect frass and exuviae are also a major residual stream from insect production for food and feed, that may be used as soil amendment. However, only few studies have thoroughly examined the effect of soil amendment with insect frass and exuviae on rhizosphere microbial communities and plant growth.
Methods We studied the effects of soil amendment with frass and/or exuviae originating from three insect species, *Tenebrio molitor*, *Acheta domesticus*, and *Hermetia illucens*, at three different concentrations, compared to synthetic fertiliser. At several time points we analysed the rhizosphere bacterial community and assessed multiple plant-growth parameters of a brassicaceous plant.
Results Soil amendment with frass and/or exuviae improved plant growth at least as well as synthetic fertiliser, *A. domesticus* exuviae having the strongest impact. The origin (insect species), type (frass or exuviae) and concentration of soil amendment influenced the effects on plant traits. The rhizosphere bacterial community differed between amended and unamended soil. Bacterial genera that contain plant growth-promoting species were more abundant in the rhizosphere of plants grown in amended soil.
Conclusions Addition of insect frass and/or exuviae to the soil differentially affects the bacterial rhizosphere community and promotes plant growth in these soils, underlining their unique roles in the aboveground-belowground feedback loop, and their potential use as soil amendment in circular agriculture.

Keywords Belowground-aboveground interactions · *Brassica oleracea* · Insect frass and exuviae · Plant growth · Rhizosphere microbiome · Soil amendment

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Introduction

Aboveground and belowground communities in terrestrial ecosystems interact via plants (Swift et al. 1979; Van der Putten et al. 2001; Frost and Hunter 2004; Friman et al. 2021a). Soil microbes are dominant members of the belowground community and herbivorous insects are important members of the aboveground community. Soil microbes affect plant phenotype, or interact with members of the plant-associated community (Berendsen et al. 2012; Heinen et al. 2018; Friman et al. 2021b). A well-studied group of plant-associated microbes are plant growth-promoting rhizobacteria (PGPR). PGPR may provide various benefits to plants, such as nitrogen fixation, phytohormone production, increased root growth, enhanced nutrient uptake, stimulation of other beneficial bacteria and fungi, and enhanced resistance to diseases, and insect herbivory, all of which ultimately result in increased plant growth (Goswami and Deka 2020; Khatoon et al. 2020; Friman et al. 2021b). Remarkably, also products from insect herbivores can stimulate plant growth. For instance, frass (faeces) deposition by herbivorous insects and decomposition of their cadavers in the soil affect soil nutrient dynamics, increasing nitrogen availability to the benefit of plants (Belovsky and Slade 2000; Frost and Hunter 2004; Behie and Bidochka 2013; Kos et al. 2017). Frass and cadavers also stimulate growth of soil microbes (Lovett and Ruesink 1995; Frost and Hunter 2004; Yang 2004), and increase decomposition rate, soil respiration, nitrogen immobilisation and mineralisation (Lovett and Ruesink 1995; Frost and Hunter 2007; Fielding et al. 2013; Song et al. 2015). Interestingly, several genera containing PGPR have been identified in insect frass (Poveda et al. 2019).

In agricultural settings we tend to reduce the number of herbivores, diminishing the beneficial effects of insect frass deposition and cadaver decomposition. There is a growing interest in producing insects for food and feed on organic residual streams as a contribution to the transition towards a circular agriculture (Varelas 2019; van Huis 2021; Barragán-Fonseca et al. 2022a). The production of insects yields a residual stream consisting of left-over feed combined with insect faeces (frass) and moulted cuticles (exuviae). This residual stream may be used to restore the natural feedback loop and promote crop production. To prevent the spread of diseases, the current fertiliser legislation in Europe requires the insect residual streams to be hygienised (70 °C for 1 h) prior to their use as a soil amendment (European Commission 2021). This treatment serves to reduce microbial concentrations by a factor 10^5 (European Commission 2011) , leading to a substantial reduction in numbers of frass-associated microbes. However, there are indications that hygienised frass may increase microbial diversity and activity when applied as a soil amendment (Houben et al. 2020; Watson et al. 2021), suggesting a positive effect of frass on indigenous soil microbes.

Soil amendment with insect exuviae may also affect plant growth. Insect exuviae contain a relatively large proportion of chitin, a polysaccharide that is found in fungal cell walls, crustacean shells and arthropod cuticles and stimulates plant-beneficial bacteria (Manjula and Podile 2001). The effects of soil amendment with crustacean-derived chitin on soil microbial communities and plant nutrient availability have been studied widely (Sharp 2013; Shamshina et al. 2020). It increases the relative abundance of various bacterial taxa that include PGPR, and promotes plant growth (Debode et al. 2016; Andreo-Jimenez et al. 2021). Research on chitin from other sources, however, is scarce. Differences in co-occurring compounds may result in unique effects of certain chitinous amendments on bacterial communities. During decomposition in soil, the chitin-rich exuviae of mealworms, unlike chitin from fungi and shrimp, stimulated a wide diversity of chitinolytic bacteria, with a notable increase in the abundance of Bacilli (Bai 2015). This class of bacteria includes several species that are already known for their plant protective and growth-promoting properties, from genera such as *Paenibacillus*, *Lysinibacillus* and *Bacillus* (Borriss 2015; Grady et al. 2016; Ahsan and Shimizu 2021). Furthermore, soil amendment with residues from either mealworm, black soldier fly or house cricket production increased the relative abundance of bacterial taxa in the rhizosphere that are known to be stimulated by chitin and include PGPR, such as *Pseudomonas*, *Devosia,* or *Nocardioides* (Wantulla et al. 2023). Interestingly, exuviae from different insect species were found to cause distinct changes in rhizosphere communities (Wantulla et al. 2023). The differential effects of soil amendment with exuviae originating from different insect species on bacterial rhizosphere communities are likely caused by differences in the composition of the exuviae. For example, while black soldier fly exuviae have a chitin content of 10.8-11.5 %, house cricket and mealworm exuviae contain 9.4-10.1 % and 7.8-8.7% chitin, respectively(Nurfikari and de Boer 2021). Furthermore, black soldier fly exuviae seem to have a relatively low lipid content, whereas house cricket and mealworm exuviae appear to be rich in lipids. Thus, although insect exuviae stimulate bacterial taxa that include PGPR, exuviae of different species may be colonised and degraded by specific microbes, which may differentially affect plant growth and health.

To understand the effects of insect residues on soil microbes and their effects on belowground-aboveground interactions, it is essential to compare how frass and exuviae of different insect species affect rhizosphere bacterial communities and plant growth. Differential stimulation of microbial taxa by insect frass and exuviae of different species may clarify differences in plant responses to soil amendment with these materials. The aim of the present study was to elucidate the effects of soil amendment with frass and exuviae of black soldier fly larvae, *Hermetia illucens* L., house crickets, *Acheta domesticus* L., or yellow mealworms, *Tenebrio molitor* L. on the diversity and composition of the rhizosphere bacterial community and growth of *Brassica oleracea*. While *B. oleracea* is the main vegetable species produced after tomatoes, onions and cucumbers in terms of global production, black soldier fly, house cricket and mealworm are among the most important commercially produced insect species (van Huis 2021; FAO 2022). Based on previous research with insect exuviae (Wantulla et al. 2023), the amendments were hypothesised to improve plant growth, diminish bacterial diversity in the rhizosphere and result in distinct bacterial communities.

Materials and methods

Soil

Agricultural soil was collected from the upper 10-20 cm mineral layer of the organically managed Droevendaal experimental field of Wageningen University (the Netherlands; 51.9899634° N 5.6652231° E) on 1 October 2018. The field had been used to grow various brassicaceous plants since 2011, most recently black mustard (*Brassica nigra*). Soil composition as assessed for this field by Eurofins Agro (Wageningen, the Netherlands) in 2018 was 81 % sand, 14 % silt and 2 % clay, while the soil organic matter content was 3.2 %. The soil was homogenised by sieving (< 5 mm).

Treatments

The agricultural soil was subjected to one of nine treatments or left untreated as the control. Three different amendment concentrations (1 g kg^{-1}, 2 g kg^{-1}; 5 g kg^{-1}) of exuviae (shed cuticles), frass (mixture of faeces, left over feed and remaining exuviae) and a 1:1 mixture of exuviae and frass were used, in the following designated as insect residues for short, originating from black soldier fly larvae, *Hermetia illucens* L., house crickets, *Acheta domesticus* L., or yellow mealworms, *Tenebrio molitor* L. that were produced by commercial insect mass-rearing companies in the Netherlands (Table S1). These amendments were inspected for the presence of insects and insect fragments, which were removed, dried at 60 ^{0}C for 24 h and subsequently ground to a fine powder (SM 100 cutting mill; Retsch, Haan, Germany). The different soil amendments were manually mixed through the agricultural soil in bags containing 10 kg of soil. To get an indication of the effect solely caused by nutrients contained in the amendments, a synthetic fertiliser treatment with 30 ml $week^{-1}$ (Table S2) was included. The amount of synthetic fertiliser added per kg of agricultural soil was based on measurements of NH_4^+ and NO_x release from the insect exuviae applied at 10 g kg^{-1} over time (Nurfikari 2022) and roughly corresponded to the nitrogen release of 2 g insect exuviae per kg soil. Amendment concentrations of 5 g kg^{-1} field soil were based on a nitrogen fertilisation advice for cabbage of 230 kg ha^{-1} provided by Eurofins Agro (Wageningen, the Netherlands), assuming a topsoil depth of 35 cm and taking into account the nitrogen release per insect residue (Nurfikari 2022) for the duration of the experiment (Table S3).

Plants

Seeds of Brussels sprouts plants (*Brassica oleracea* L. var. *gemmifera* cv. Cyrus) were obtained from Unifarm (Wageningen, the Netherlands). Two seeds were sown per pot containing agricultural soil, and germinated in a greenhouse (20 ± 2 °C, 60-70 % RH and 16:8 h L:D). After germination one of the two seedlings was removed to retain one viable seedling per pot. Due to the size of the experiment the plants were divided over six blocks (Fig. 1a), which were sown with 2-week intervals and contained two replicates

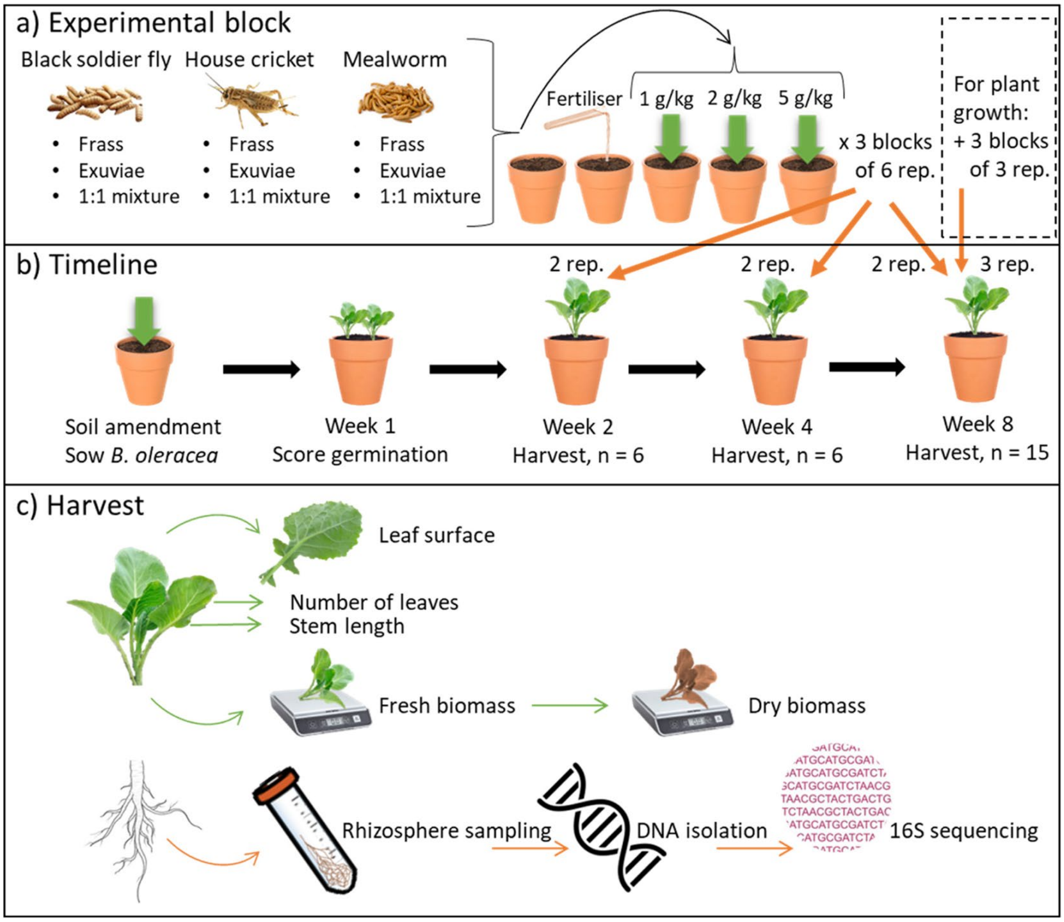


Fig. 1 Schematic overview of the experimental block structure (**a**), the timeline (**b**), and the harvest (**c**). Of each treatment, two replicates (rep.) were harvest per timepoint for three blocks (total of six replicates per treatment per timepoint). Furthermore, three replicates of each treatment were harvested at the last timepoint for three extra blocks (total of 15 replicates per treatment at the week 8 harvest). Blocks were sown in two-week intervals

of each treatment for each timepoint (blocks 1-3) or three replicates of each treatment for the 3rd timepoint (blocks 4-6). Plants were watered three times per week, by filling the saucer underneath each pot with water and allowing the soil to absorb it during 2 h.

Growth measurements and rhizosphere sampling

One week after sowing, the number of germinated seeds per pot was counted (Fig. 1b). Every week the number of leaves of each plant was counted and the length of the shoot was measured. After 2, 4 and 8 weeks of growth, plants of each treatment were harvested (Fig. 1c) by gently kneading the soil from the roots with sterile gloves (sprayed with 70 % EtOH). The shoot was cut from the roots at the root-shoot interface and collected in an aluminium tray, while rhizosphere samples were collected from the roots as described by Lundberg et al. (2012). Briefly, soil was shaken off the roots so that a ca. 1 mm thick layer of soil remained on the surface. The roots were collected in 50 ml tubes containing 25 ml of sterile phosphate buffer (6.33 g $NaH_2PO_4 \cdot H_2O$, 10.96 g $Na_2HPO_4 \cdot 2H_2O$ and 200 µl Silwet L-77 per l). Subsequently, the roots were vortexed for 15 s at maximum speed and removed from the tubes before centrifuging all samples at 1800 *g* for 15 min. Supernatants were discarded and rhizosphere samples were stored at -25 °C until DNA extraction. The total leaf area of the shoots was measured within 10 min after harvest (LI-3100C area meter, LI-COR, USA), directly followed by weighing the fresh shoot biomass (UW6200HV, Shimadzu Corporation, Japan). All shoot material was oven dried at 70 °C for 72 h and weighed to determine the dry biomass (New classic MF ML54, Mettler Toledo, Switzerland).

Carbon and nitrogen content

To assess the nutrient content of the plant material, total carbon and nitrogen contents of roots and shoots of the 8-week-old plants were measured using dry combustion and an elemental analyser (TruSpec CN Analyzer 630-100-100, LECO, Germany). Before analysis the dried plant material was ground to a fine powder, dried again at 70 °C for 16 h and a sample of 150 mg was loaded in the machine.

DNA isolation and amplicon sequencing of the 16S rRNA V4 region

DNA was extracted from rhizosphere samples using the DNeasy PowerSoil Pro Kit (QIAGEN, Venlo, the Netherlands) and quantified using the DeNovix DS-11 Fluorometer and dsDNA Broad Range assay (DeNovix, Wilmington, Delaware, USA). Samples of all exuviae or frass amendments with 5 g kg^{-1} were sent to the Centre d'expertise et de services Génome Québec (Montréal, Québec, Canada) for 16S rRNA amplicon sequencing with the Illumina MiSeq system. The V4 region was amplified using the primers 515F/806R (Caporaso et al. 2011) and demultiplexed FASTQ files without non-biological nucleotides were provided by the sequencing provider. Primers were removed with the *filterAndTrim* function of the dada2 package in R (Version 3.6.3; R Core Team 2020) and further processing of the data was done using the DADA2 pipeline (Callahan et al. 2016). The Silva reference database version 132 (Yilmaz et al. 2014) was used for taxonomy assignment to amplicon sequence variants (ASVs).

Statistical analyses

All statistical analyses were performed using R version 4.2.1.(R Core Team 2022). Normalization of the ASV table was done by cumulative-sum scaling with the *cumNorm* function of the package metagenomeSeq (Paulson et al. 2013). To assess the effect of soil amendment on bacterial alpha diversity, Shannon indices were calculated with the phyloseq package (McMurdie and Holmes 2013). For beta diversity, principal coordinate analyses (PCoA) and permutational multivariate analyses of variance (PERMANOVA) were performed using Bray-Curtis dissimilarity matrices with the packages phyloseq and vegan (Oksanen et al. 2020). In order to visualise differences between treatments in the abundance of the main occurring genera, relative abundances of genera contributing more than 1% to the total number of sequences were used to order samples within heatmaps using ordination based on PCoA on Bray-Curtis dissimilarities. Heatmaps were created with the phyloseq implementation of the NeatMap approach (Rajaram and Oono 2010).

The effects of soil amendment on Shannon indices, relative abundances of bacterial genera above 1% and plant growth parameters were analysed with linear mixed effects models (LMM) using the package nlme (Pinheiro et al. 2020). Similarly, LMM were used to test the main effects and possible interactions between the factors insect species, type of material (frass, exuviae or their 1:1 mixture) and concentration of material on fresh shoot biomass as a proxy for plant growth, and on shoot carbon/nitrogen ratio as a proxy for shoot quality, the control and fertilised treatment were excluded from the analysis. To compare the different soil amendments with the control and synthetic fertiliser treatments, additional models were fitted with soil treatment as the only fixed factor. The effect of soil amendment on seed germination and leaf number were analysed using generalised linear mixed effects models (GLMM) with binomial and Poisson distributions, respectively, using the package lme4 (Bates et al. 2015). In all models, sowing block was used as a random factor. P-values for main effects were obtained using the *Anova* function from the car package (Fox and Weisberg 2019). Pairwise comparisons were performed using estimated marginal means (EMM) with the package emmeans (Lenth et al. 2023). Models were validated by plotting residuals and, where necessary, homogeneity of variances and normality were confirmed with Levene's test and the Shapiro-Wilk test, respectively, using the packages car and DHARMa (Hartig and Lohse 2022). Where needed, the data was square-root or log-transformed to meet the model assumptions.

Results

Germination

Soil treatment with insect residues, or synthetic fertiliser affected the germination of *Brassica oleracea* seeds (GLMM, $df = 28$, $\chi^2 = 99.162$, $P < 0.001$). Compared with the control, two treatments, i.e. 5 g kg^{-1} house cricket exuviae and synthetic fertiliser (Fig. 2) inhibited germination significantly.

Fresh shoot biomass

Fresh shoot biomass of *B. oleracea* plants was affected by the soil amendments (Fig. 3). The insect species from which the residues originated affected plant shoot biomass at all time points, and increasing concentration of insect residues in the soil positively affected shoot biomass (Table 1, Fig. S1a). Furthermore, the type of residue material (frass, exuviae or mixture) used as soil amendment affected fresh shoot biomass after 4 and 8 weeks (Table 1). After 4 weeks of plant growth, amending soil with 5 g kg^{-1} of black soldier fly exuviae, house cricket mixture and mealworm frass resulted in an increased shoot biomass compared to control plants and plants that received synthetic fertiliser (EMM, $P < 0.003$) (Fig. 3). Unfortunately, at this timepoint, no plants growing on soil amended with 5 g kg^{-1} house cricket exuviae could be harvested, due to the low germination rate of seeds on this treatment (Fig. 2). When soil was amended with 2 g kg^{-1}, all amendments derived from house crickets significantly increased shoot biomass (EMM, $P < 0.03$) compared to control plants and plants that received synthetic fertiliser (Fig. 3). After 8 weeks, plants of all treatments had a higher biomass than control plants. Moreover, plants growing in soil amended with house cricket exuviae at 2 g kg^{-1} and 5 g kg^{-1} of black soldier fly exuviae, and 5 g kg^{-1} of all materials from mealworm or house cricket, had a higher biomass than plants that received synthetic fertiliser (Fig. 3). In general, soil amendment with residues originating from house crickets resulted in significantly heavier plants, compared to amendment with residues from mealworms and black soldier fly larvae (Fig. S1a). At week 8, the plants amended with 5 g kg^{-1} of house cricket frass and the 1:1 mixture of house cricket frass and exuviae, were

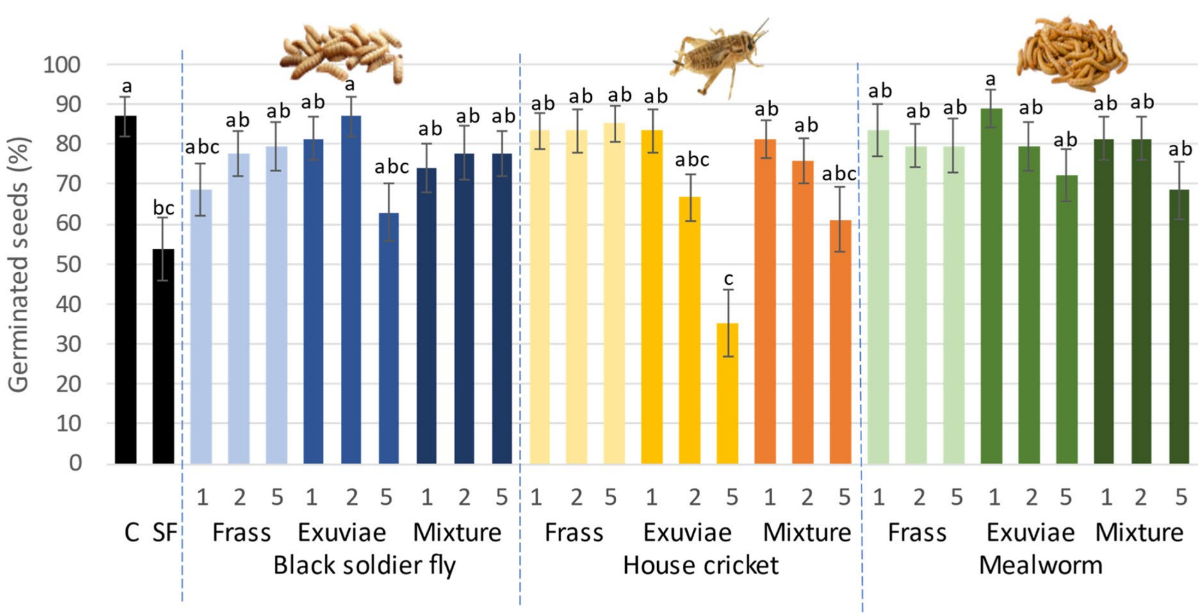


Fig. 2 Percentage of germinated *Brassica oleracea* seeds 1 week after sowing two seeds per replicate in untreated soil (control; C), soil treated with synthetic fertiliser (SF), or soil amended with insect residues (frass, exuviae or a 1:1 mixture of the two) derived from black soldier fly larvae (*Hermetia illucens*), house crickets (*Acheta domesticus*) or mealworms (*Tenebrio molitor*). Numbers underneath the bars indicate the concentration of material in grams per kg of soil. Treatments denoted with the same letter do not differ significantly (EMM, α = 0.05). Error bars represent standard error (SE), $n = 24$

heavier than plants growing in the same amendments originating from mealworm (EMM, $P < 0.001$) and black soldier fly larvae (EMM, $P < 0.001$) (Fig. 3). After 2 and 8 weeks, plants growing in soil amended with residues from black soldier fly larvae were the smallest of all treated plants (Fig. S1a). The strength of the effect of insect residues also depended on the residue material that was used as soil amendment. After 4 weeks of plant growth, plants growing in soils amended with exuviae or with the 1:1 mixture of exuviae and frass were significantly heavier than plants growing in frass-amended soils (Fig. S1a). The differences became more distinct after 8 weeks of plant growth, with the heaviest plants growing in exuviae-amended soil and the lightest plants growing in frass-amended soil, with an intermediate biomass for plants growing in soil amended with the mixture of both materials (Fig. 3, Fig. S1a). Furthermore, after 4 weeks, there was a significant interaction of insect species from which the residues were derived with the type of material that was used as soil amendment, and with the residue concentration in the soil (Table 1). Moreover, after 8 weeks, there were clear interaction effects between all combinations of the three main effects (Table 1).

Plant carbon/nitrogen ratio

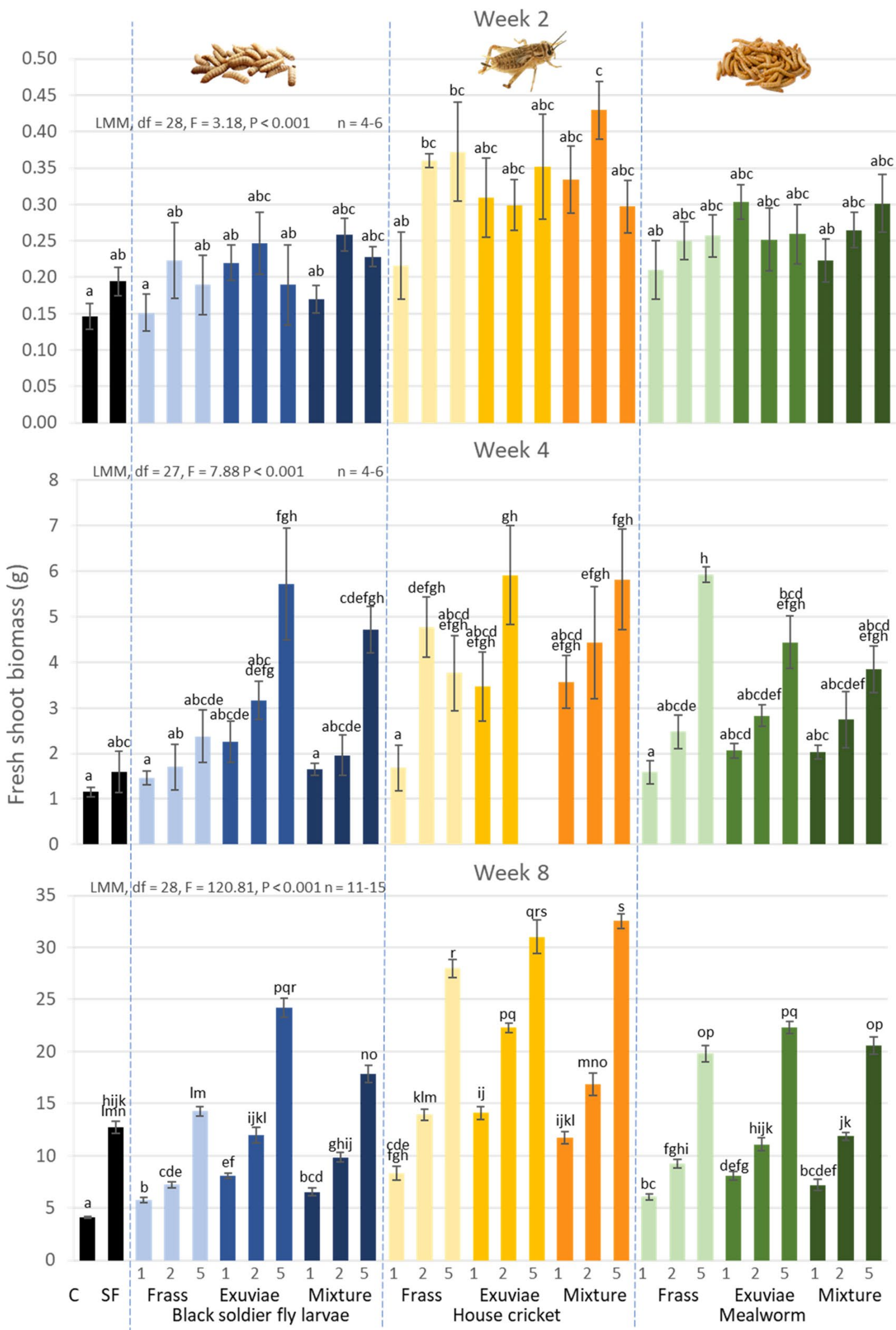
Soil amendment with insect residues affected *B. oleracea* shoot carbon/nitrogen (C/N) ratio (LMM, $df = 28$, $F = 16.23$, $P < 0.001$). Soil amendment with residues originating from house crickets resulted in plants with a significantly lower C/N ratio, compared to plants amended with residues from mealworms or black soldier fly larvae (Fig. S2a). Soil amendment with exuviae lowered the shoot C/N ratio, compared to amendment with frass or the mixture. Furthermore, the shoot C/N ratio was negatively affected by higher concentrations of amendment. There was a strong interaction between the effects of insect species, type of material and concentration of residue (Fig. S2b). Four treatments resulted in a lower C/N ratio compared to plants growing in unamended soil: synthetic fertiliser, and 5 g kg^{-1} of house cricket exuviae, house cricket 1:1 mixture and mealworm exuviae (Fig. S2c, Fig. 4). Amendment with 5 g kg^{-1} of house cricket exuviae was the only amendment that resulted in a lower shoot C/N ratio compared to plants that received synthetic fertiliser. The carbon/nitrogen ratio of plant roots was affected by soil amendment with insect residues (LMM, $df = 28$, $F = 2.318$, $P = 0.0037$; Fig. S3). Soil amendment with 5 g kg^{-1} of house cricket exuviae resulted in a significantly lower root C/N ratio compared to plants in untreated soil.

Other plant growth parameters

Soil amendment with insect residues and with synthetic fertiliser significantly affected leaf surface area, stem length, dry shoot biomass and number of leaves of *B. oleracea* (Table S4), following the same patterns as found for fresh shoot biomass. After 2 weeks of plant growth, there was a 300 % increase in leaf surface area of the largest plants growing in amended soil compared to untreated soil. After 8 weeks of growth, the increase in leaf surface area was up to 616 %. The difference in stem length was significant after 2 weeks of plant growth at an increase of 36 % for the tallest plants in amended soils compared to untreated soil, and a 159 % increase after 8 weeks of growth. Dry shoot biomass was only measured after 4 and 8 weeks of plant growth; after 4 weeks the increase was 475 % and after 8 weeks it was 595 % for the heaviest plants in amended soil compared to untreated soil. The number of leaves per plant was increased after 8 weeks of plant growth (95 %) by soil amendment with insect residues.

Bacterial alpha and beta diversity

The Shannon indices of bacterial communities in the *B. oleracea* rhizosphere were significantly affected by soil amendment after 4 weeks (LMM: $df = 6$, $\chi^2 = 132.9$, $P < 0.001$) and 8 weeks (LMM: $df = 7$, $\chi^2 = 60.913$, $P < 0.001$), but not after 2 weeks of plant growth (LMM: $df = 7$, $\chi^2 = 4.5466$, $P = 0.715$). Shannon indices were significantly lower than in the control following amendment with black soldier fly exuviae (EMM: $P < 0.001$; Fig. 5c) or house cricket frass after 4 weeks (EMM: $P = 0.007$; Fig. 5c) and 8 weeks (EMM: $P < 0.05$; Fig. 5e). Soil amendment with mealworm exuviae or with frass from black soldier fly larvae or mealworms resulted in significantly lower Shannon indices compared to the control only after 4 weeks (EMM: $P < 0.05$; Fig. 5c). After 8 weeks, Shannon indices were significantly lower than in the control or than when soil was amended with mealworm frass, following amendment with black


Week 2
LMM, df = 28, F = 3.18, P < 0.001
n = 4-6
Week 4
LMM, df = 27, F = 7.88 P < 0.001
n = 4-6
Week 8
LMM, df = 28, F = 120.81, P < 0.001 n = 11-15
Fresh shoot biomass (g)
C
SF
Frass
Exuviae
Mixture
Black soldier fly larvae
House cricket
Mealworm

◂**Fig. 3** Fresh shoot biomass of 2-, 4-, and 8-week-old *Brassica oleracea* plants growing in untreated soil (control; C), soil treated with synthetic fertiliser (SF), or soil amended with residues derived from black soldier fly larvae (*Hermetia illucens*), house crickets (*Acheta domesticus*) or mealworms (*Tenebrio molitor*). Numbers underneath the bars indicate the residue concentration in grams per kg of soil. Treatments denoted with the same letter do not significantly differ (EMM, $\alpha = 0.05$). Error bars represent standard error (SE). Range of number of replicates per treatment are indicated at the top of each panel by n

soldier fly or house cricket exuviae (EMM: $P < 0.05$; Fig. 5e).

PCoAs using Bray-Curtis metrics showed that bacterial communities in the rhizosphere of control plants and plants that received synthetic fertiliser clustered together, while the rhizosphere communities of plants grown in soil amended with exuviae or frass formed a separate cluster after 4 weeks (Fig. 5d). Although, in general, clusters were more distinct after 2 weeks, rhizosphere communities of plants grown in soil amended with mealworm exuviae overlapped slightly with communities of the control and synthetic fertiliser treatments (Fig. 5b). After 8 weeks, clusters were less distinct than at either previous time point (Fig. 5f). PERMANOVA based on Bray-Curtis dissimilarity matrices showed that soil amendment had a significant effect on the composition of rhizosphere bacterial communities after 2 weeks (22.18% of the variation, $P < 0.001$; Table 2), 4 weeks (19.41% of the variation, $P < 0.001$; Table 2) and 8 weeks of plant growth (19.38% of the variation, $P < 0.001$; Table 2).

Bacterial community composition

Significant differences in relative abundance of bacteria in the *B. oleracea* rhizosphere between the control and at least one of the soil amendments were observed for 24 genera (Table S5). In the heatmaps presenting relative abundance of these genera, samples from control plants and plants that received synthetic fertiliser grouped together at all time points (Fig. 6). After 2 weeks, samples largely grouped according to the material type used for soil amendment, with two outlying samples from plants grown in soil amended with exuviae and one from a plant grown in soil amended with frass (Fig. 6a). Within the group of samples from plants grown in soil amended with exuviae, samples grouped according to insect species as well. After 4 weeks, samples from plants grown in soil amended with mealworm exuviae or frass grouped together, with the exception of two outlying samples (Fig. 6b). After 8 weeks, samples no longer grouped according to material type or insect species (Fig. 6c).

Soil amendment with exuviae or frass of any of the three insect species significantly increased the relative abundance of the genera *Pseudomonas*, *Acinetobacter* and *Devosia* at one or more time points (EMM: $P < 0.05$; Table S5). Amendment with exuviae of any species significantly increased the relative abundance of the genus *Sphingomonas*, whereas amendment with frass of any species significantly increased the relative abundance of the genera *Paenibacillus* and *Chthoniobacter* at one or more time points (EMM: $P < 0.05$; Table S5). Only soil amendment with either black soldier fly frass or exuviae significantly increased the relative abundance of the genus *Paenibacillus* already after 2 weeks (EMM: $P < 0.05$; Table S5). The two black soldier fly amendments were the only ones that significantly increased the relative abundances of a group of different genera in the *Burkholderiaceae* (*Burkholderia-Caballeronia-Paraburkholderia*) and of the genus *Chthoniobacter* not only after 2 weeks but also after 4 and 8 weeks, respectively (EMM: $P < 0.05$; Table S5). Soil amendment with black soldier fly exuviae was the only amendment that significantly increased the relative abundance of the genus *Lysinibacillus* after 8 weeks (EMM: $P = 0.011$; Table S5). Compared to the synthetic fertiliser treatment, only amendment with black soldier fly frass significantly increased the relative abundance of a group of different genera belonging to the *Rhizobiaceae* (*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*) after 2 weeks (EMM: $P < 0.001$; Table S5). Amendment with either house cricket frass or exuviae significantly increased the relative abundance of the genus *Flavobacterium* after 2 weeks (EMM: $P < 0.05$; Table S1). Compared to the synthetic fertiliser treatment, the two house cricket amendments were the only ones that significantly increased the relative abundance of the genus *Pedobacter* after 2 weeks (EMM: $P < 0.05$; Table S5). Soil amendment with house cricket exuviae was the only amendment that significantly increased the relative abundance of

Table 1 Main and interaction effects of soil amendment with insect residues on fresh shoot biomass of *Brassica oleracea* after 2, 4 and 8 weeks of plant growth and shoot C/N ratio after 8 weeks of plant growth

LMM	Fresh shoot biomass									Shoot C/N ratio		
	Week 2			Week 4			Week 8			Week 8		
	df	*F*-value	*P*-value	*df*	*F*-value	*P*-value	*df*	*F*-value	*P*-value	*df*	*F*-value	*P*-value
S	2	21.28	< 0.001	2	13.44	< 0.001	2	315.48	< 0.001	2	10.69	< 0.001
M	2	1.57	0.213	2	7.59	< 0.001	2	183.29	< 0.001	2	5.14	0.021
C	2	3.92	0.022	2	33.51	< 0.001	2	108.17	< 0.001	2	106.9	< 0.001
S : M	4	0.35	0.845	4	2.89	0.025	4	14.54	<0.001	4	6.32	< 0.001
S : C	4	0.77	0.545	4	3.36	0.012	4	72.66	<0.001	4	13.41	< 0.001
M : C	4	1.8	0.133	4	0.25	0.912	4	16.61	<0.001	4	9.16	< 0.001
S : M : C	8	0.99	0.4498	-	-	-	8	5.41	<0.001	8	1.49	0.185

Soil amendments were grouped by insect species from which the residues originated (S: black soldier fly larvae (*Hermetia illucens*), house cricket (*Acheta domesticus*) and mealworm (*Tenebrio molitor*)), by type of material that was used (M: frass, exuviae, or 1:1 mixture), and by concentration of material in g per kg of soil (C: 1 g kg^{-1}, 2 g kg^{-1}, or 5 g kg^{-1})

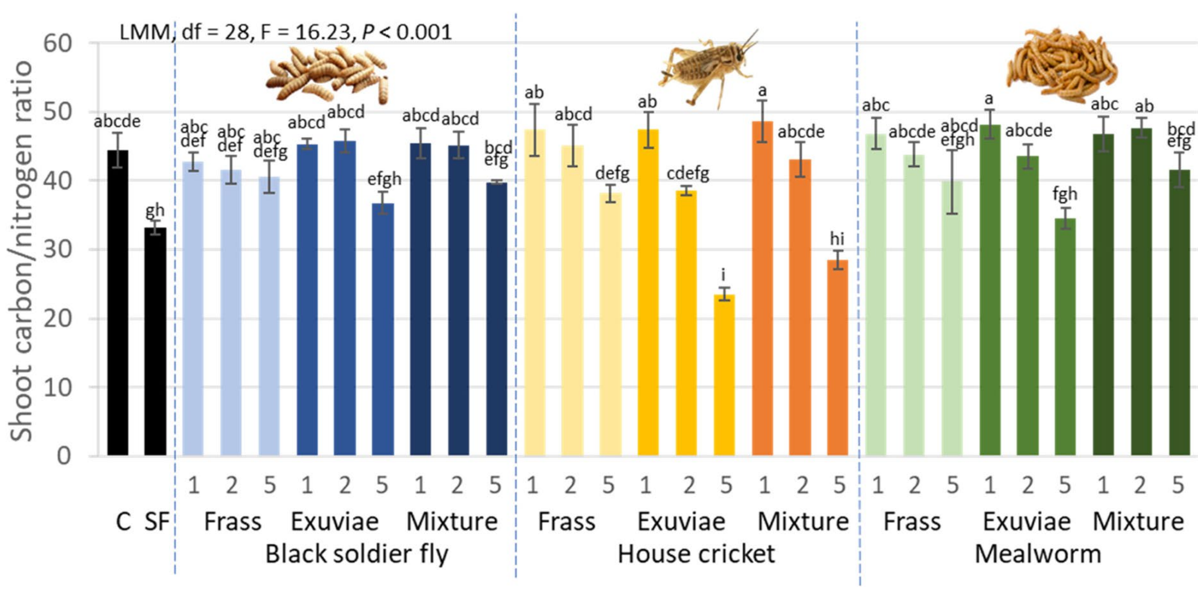


Fig. 4 Carbon/nitrogen ratio in the shoots of *Brassica oleracea* grown for 8 weeks in untreated soil (control; C), soil treated with synthetic fertiliser (SF), or soil amended with residues derived from black soldier fly larvae (*Hermetia illucens*), house crickets (*Acheta domesticus*) and mealworms (*Tenebrio molitor*). Numbers underneath the bars indicate the residue concentration in grams per kg of soil. Treatments denoted with the same letter do not significantly differ (EMM, α = 0.05). Error bars represent standard error, $n = 6$

the genus *Nocardioides* after 8 weeks (EMM: $P < 0.05$; Table S5) and of the genus *Chryseobacterium* already after 2 weeks (EMM: $P = 0.003$; Table S5). It caused a significantly greater increase in relative abundance of the genus *Flavobacterium* than amendment with house cricket frass after 2 weeks (EMM: $P = 0.004$; Table S5) and was the only amendment that significantly increased the relative abundance of the genus *Clostridium* (*Clostridium_sensu_stricto_13*) after 8 weeks (EMM: $P < 0.001$; Table S5). Both increases were also significant compared to all other soil amendments (EMM: $P < 0.05$; Table S5). Only amendment with house cricket frass significantly increased the relative abundance of the genus *Pirellula* and of an unclassified genus belonging to the *Blastocatellaceae* (JGI_0001001-H03) after 4 and 8 weeks, respectively (EMM: $P < 0.05$; Table S5).

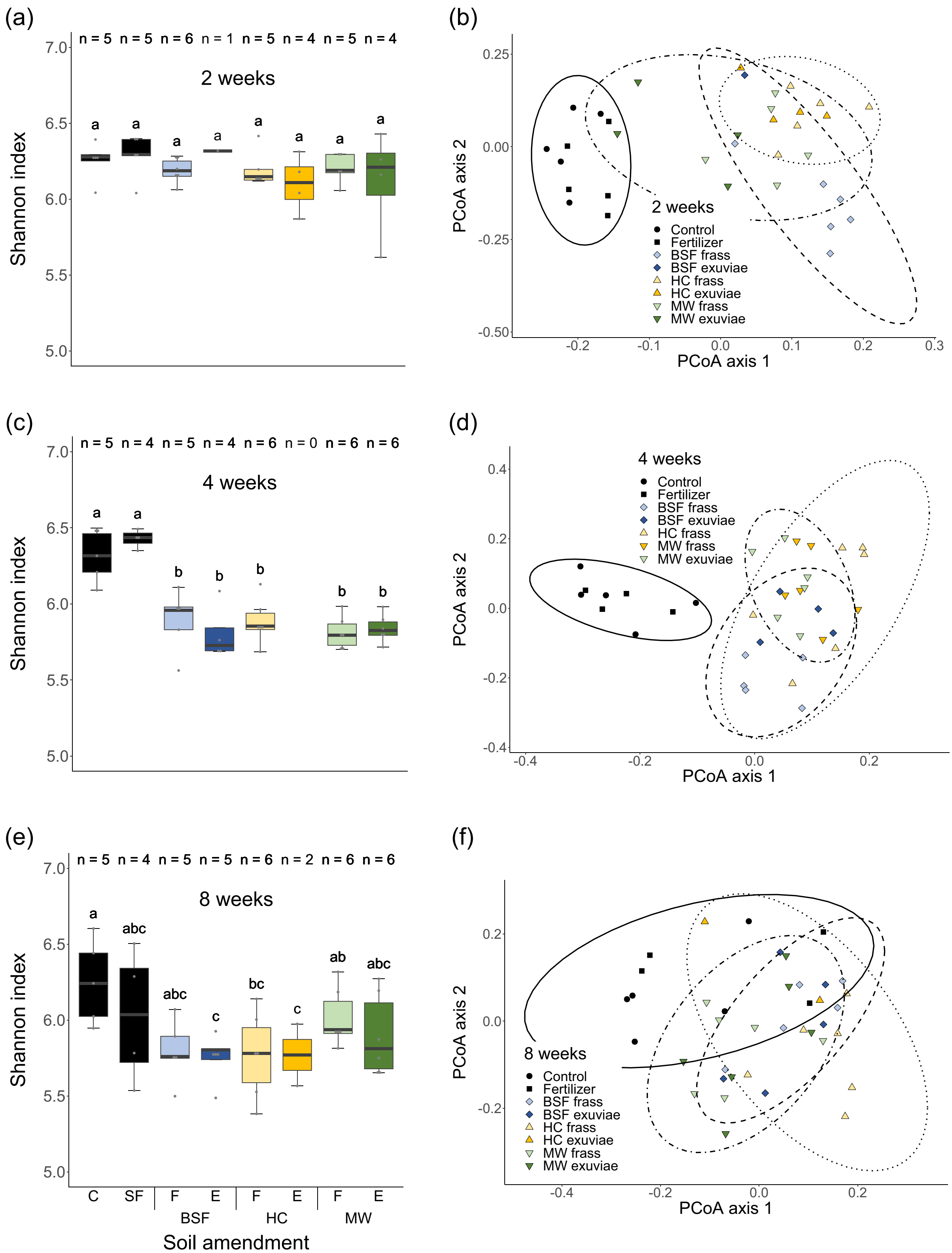
Discussion

Our study shows that seed germination, plant growth, plant C/N ratio and the bacterial community in the rhizosphere of *B. oleracea* are affected by soil amendment with insect exuviae, frass and their 1:1 combination. Similar to results found in natural terrestrial ecosystems (Belovsky and Slade 2000; Frost and Hunter 2007; Behie and Bidochka 2013; Kos et al. 2017), the presence of insect frass in the soil stimulates plant growth. Soil amendment with insect exuviae decreases plant C/N ratio, thus increasing plant nutritional quality (Mattson 1980; Couture et al. 2010). Although all soil amendments with insect residues increased the relative abundance of several genera that include PGPR, soil amendment with house cricket exuviae uniquely promoted several of them and resulted in the largest plants. Therefore, the composition of the residues seems to be crucial for the strength of the plant growth-promoting effect of soil amendment. Our results underline the importance of insect residual material in aboveground-belowground interactions and show opportunities for utilising this knowledge in agricultural systems, by valorising residual streams from the production of insects for food and feed (Barragán-Fonseca et al. 2022a).

Plant growth and C/N ratio

Soil amendment with insect residues stimulated the growth of *B. oleracea*. Insect species from which a residue originates, type of residual material and the residue concentration in the soil are determining factors for plant growth promotion. The effects of soil amendment with 2 g kg^{-1} of soil are comparable to those of synthetic fertiliser with a similar nitrogen content. Although amendment with house cricket exuviae clearly outperformed the synthetic fertiliser, amendment with black soldier fly frass resulted in a less strong increase in plant biomass. Insect frass and exuviae are rich in plant nutrients (Nurfikari 2022; Beesigamukama et al. 2022) and insect frass can improve the growth of leafy vegetables and barley (Poveda et al. 2019; Houben et al. 2020; Izza Bukari et al. 2021; Butnan et al. 2022), while soil amendment with black soldier fly exuviae increased shoot growth and flower production of *Brassica nigra* (Barragán-Fonseca et al. 2022b, 2023). Furthermore, soil amendment with chitin, a major component of exuviae, can stimulate the growth of lettuce and rockcress among other plant species (Debode et al. 2016; Winkler et al. 2017; Li et al. 2022). The differential effects of residues from different insect species, or of different insect-derived materials – frass, exuviae or the mixture – do not seem to strongly depend on chitin content in this study. Soil amendment with black soldier fly material, resulted in the lowest increase in plant growth, while amendment with house cricket material resulted in the largest plants, despite house cricket exuviae having a lower chitin content. Besides nutrient and chitin content, the effects of the composition of the residual material on the stimulation of indigenous plant growth-promoting rhizobacteria (PGPR), may thus be crucial for the strength of the plant-growth promoting effect of soil amendment.

Interestingly, soil amendment with house cricket material not only resulted in the largest plants, amendment with house cricket exuviae also resulted in the lowest carbon to nitrogen (C/N) ratio in shoot and root tissues. Soil treatment with synthetic fertiliser, mixture of house cricket exuviae and frass, or mealworm exuviae reduced the ratio compared with plants growing in untreated soil. This reduction of C/N ratios in plant tissue may be due to an increase in nitrogen availability in the soil upon fertiliser application, or decomposition of insect residues by soil microbes. Nitrogen fertilisation is well known to increase nitrogen concentrations in plant tissue with consequences for insect herbivore performance (Awmack and Leather 2002; Ebeling et al. 2013). Frass deposition in terrestrial ecosystems affects carbon and nitrogen cycling (Belovsky and Slade 2000; Frost and Hunter 2004; Behie and Bidochka 2013; Kos et al. 2017). The highly labile carbon source in frass fuels rapid microbial growth, leading to significant immobilisation of nitrogen in microbial tissue, thereby reducing the potential of nitrogen mineralisation and subsequent utilisation by the plant (Lovett et al. 2002), which would explain the lack of C/N reduction in plants growing in frass-amended soil. In contrast, exuviae do not contain highly labile carbon sources but have


(a)
n = 5 n = 5 n = 6 n = 1 n = 5 n = 4 n = 5 n = 4
2 weeks
a a a a a a a a
Shannon index
7.0 6.5 6.0 5.5 5.0
(b)
PCoA axis 2
0.25 0.00 -0.25 -0.50
2 weeks
Control
Fertilizer
BSF frass
BSF exuviae
HC frass
HC exuviae
MW frass
MW exuviae
-0.2 -0.1 0.0 0.1 0.2 0.3
PCoA axis 1
(c)
n = 5 n = 4 n = 5 n = 4 n = 6 n = 0 n = 6 n = 6
4 weeks
a a b b b b b
Shannon index
7.0 6.5 6.0 5.5 5.0
(d)
4 weeks
Control
Fertilizer
BSF frass
BSF exuviae
HC frass
MW frass
MW exuviae
PCoA axis 2
0.4 0.2 0.0 -0.2 -0.4
-0.4 -0.2 0.0 0.2
PCoA axis 1
(e)
n = 5 n = 4 n = 5 n = 5 n = 6 n = 2 n = 6 n = 6
8 weeks
a abc abc c bc c ab abc
Shannon index
7.0 6.5 6.0 5.5 5.0
C SF F E F E F E
BSF HC MW
Soil amendment
(f)
PCoA axis 2
0.2 0.0 -0.2
8 weeks
Control
Fertilizer
BSF frass
BSF exuviae
HC frass
HC exuviae
MW frass
MW exuviae
-0.4 -0.2 0.0 0.2
PCoA axis 1

◂**Fig. 5** Shannon indices and PCoA plots of bacterial communities in the *Brassica oleracea* rhizosphere after 2 weeks (**a** and **b**), 4 weeks (**c** and **d**) and 8 weeks (**e** and **f**) of plant growth. Plants were grown in untreated soil (C; solid ellipse), received synthetic fertiliser (SF; solid ellipse) or were grown in soil amended with exuviae (E) or frass (F) from black soldier fly larvae (BSF, *Hermetia illucens*; dashed ellipse), house crickets (HC, *Acheta domesticus*; dotted ellipse) or mealworms (MW, *Tenebrio molitor*; dotdashed ellipse) at a concentration of 5 g kg^{-1}. Shannon indices of treatments denoted with the same letter are not significantly different (EMM, $P > 0.05$). Box plot whiskers represent largest values within 75% quantiles + 1.5 × interquartile range (IQR) and smallest values within 25% quantiles – 1.5 × IQR. Numbers of replicate plants are indicated at the top of the panels by n. Statistical significance of PCoAs was assessed by PERMANOVA ($P < 0.001$)

a high nitrogen content. The nitrogen content of mealworm and house cricket exuviae approaches that of insect cadavers (± 10 %), although cadavers will contain more fat and more diverse proteins (Brodbeck et al. 1999; Yang 2004; Nurfikari et al. 2023). Therefore, the effects of exuviae amendment might be more comparable to those of decomposing insect cadavers, which cause long-lasting increases in plant-available ammonium and nitrate levels in the soil (Hunter 2001; Schimel and Bennett 2004; Yang 2004; Song et al. 2015). Our results indicate that soil amendment with insect residues not only affects plant growth, but also the C/N ratio of plant tissues and thus likely the performance of herbivorous insects (Mattson 1980; Couture et al. 2010).

Remarkably, soil treatment with synthetic fertiliser or amendment with house cricket exuviae at 5 g kg^{-1}, reduced the germination of *B. oleracea* seeds, while the other amendments did not differ from the untreated control soil. Similarly, Wantulla (2023) found a reduced germination of *B. oleracea* seeds after soil amendment with house cricket exuviae, compared to treatment with synthetic fertiliser. In contrast, seed treatment with chitin increases the germination of grasses and pigeon pea (Shamshina et al. 2020). One explanation might be that the dried house cricket material hampered water uptake into the soil due to its hydrophobic properties, which were observed during our and a previous study (Nurfikari and de Boer 2021). Similarly, the application of synthetic fertiliser solution to the soil surface, near the seeds, might have resulted in the soil being too wet, causing a reduced oxygen availability, conditions that may have led to suboptimal germination (Dasberg and Mendel 1971; Finch-Savage et al. 2005).

Rhizosphere bacterial community

In general, soil amendment with insect residues at 5 g kg^{-1} reduced bacterial diversity in the rhizosphere of *B. oleracea*. This supports the hypothesis that only specific groups of bacteria were able to utilise the residues as a substrate (Wantulla et al. 2023). Similarly, soil treatment with purified chitin decreased bacterial diversity in bulk soil and the rhizosphere of soy bean plants (Andreo-Jimenez et al. 2021; Fan et al. 2022), although it did not affect bacterial diversity in the rhizosphere of lettuce and eggplant (Debode et al. 2016; Inderbitzin et al. 2018). The fact that bacterial diversity in the *B. oleracea* rhizosphere was reduced by exuviae and frass indicates that these effects cannot solely be explained by a high chitin content of the amendments. Remarkably, soil amendment with black soldier fly frass either reduces (Chiam et al. 2021) or increases (Fuhrmann et al. 2022) bacterial diversity of bulk soil, whereas amendment with mealworm frass resulted in an increase in bacterial diversity only upon combined application with fertiliser (Houben et al. 2020). This indicates a potential relevance of nutrient composition of the insect frass, which is known to depend on nutrient quality of the insect diet (Fielding et al. 2013; Gebremikael et al. 2022; Arabzadeh et al. 2022).

Soil amendment with insect residues clearly altered the bacterial community structure in the *B. oleracea* rhizosphere. Remarkably, soil treatment with synthetic fertiliser did not change bacterial community structure, while rhizosphere bacterial communities in soil amended with insect frass or exuviae formed a distinct group. In general, microorganisms associated with untreated insect residues may be introduced to the soil by amendment (Poveda et al. 2019; Fuhrmann et al. 2022). However, due to the heat treatment used in the present study, it can be assumed that any microbes remaining on the materials had a negligible effect on bacterial communities in the amended soil (Nurfikari et al. 2023). Similar to our results, bacterial communities in soil amended with different concentrations of black soldier fly frass differed from the bacterial communities in untreated soil and soil treated with organic fertiliser (Chiam et al. 2021). Such a difference in bacterial communities persisted

Table 2 PERMANOVA of bacterial communities in the rhizosphere of *Brassica oleracea* plants that were grown in soil amended with insect exuviae or frass at 5 g kg^{-1} or received synthetic fertiliser for 2-8 weeks

Time point	Factor	*Df*	Sum of squares	R^2	Pseudo-*F*	*P*
2 weeks	Treatment	7	3.3652	0.2218	1.1012	< 0.001
	Block	2	0.8833	0.0582	1.0117	0.113
4 weeks	Treatment	6	2.9790	0.1941	1.1685	< 0.001
	Block	2	0.8871	0.0578	1.0439	0.034
8 weeks	Treatment	7	3.2583	0.1938	1.0703	< 0.001
	Block	2	0.9382	0.0558	1.0786	0.125

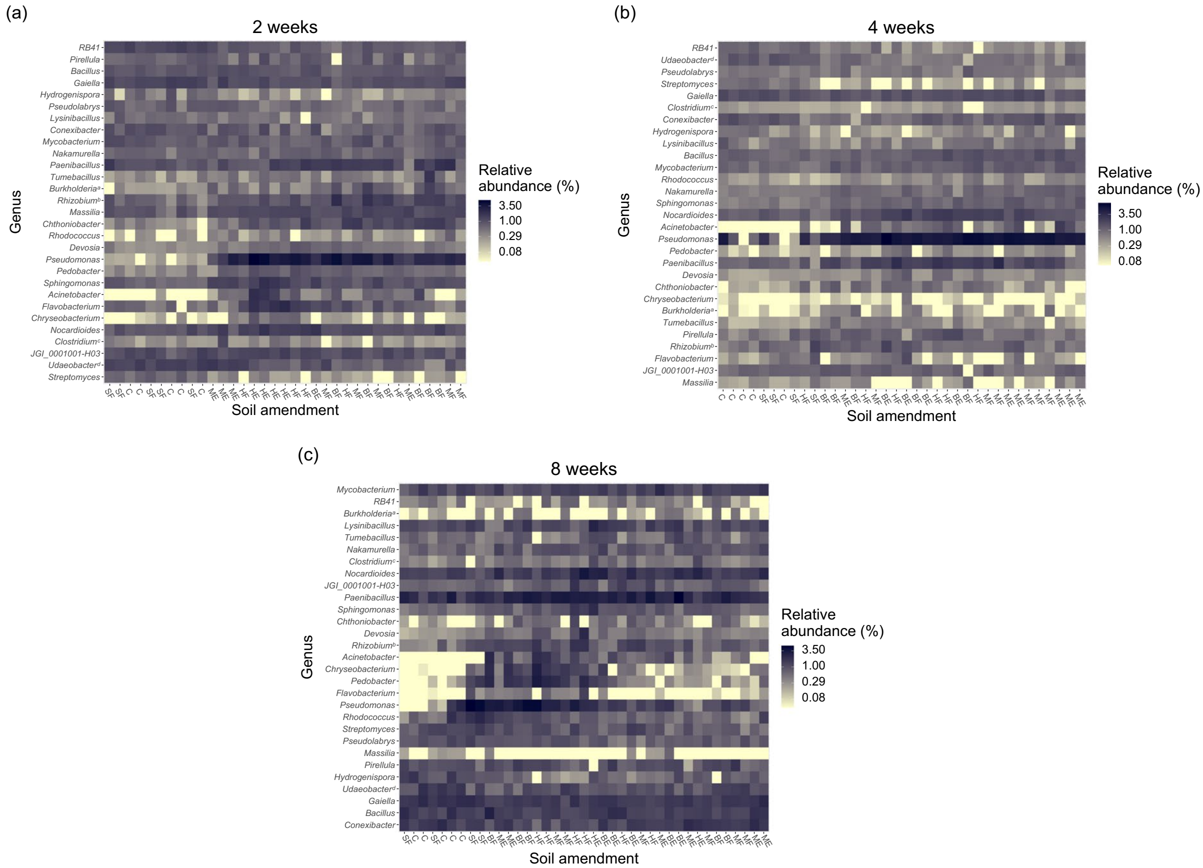


Fig. 6 Relative abundances of bacterial genera in the *Brassica oleracea* rhizosphere after 2 weeks (**a**), 4 weeks (**b**) and 8 weeks (**c**) of plant growth. Plants were grown in untreated soil (C), received synthetic fertiliser (SF) or were grown in soil amended with exuviae or frass from black soldier fly larvae (*Hermetia illucens*, BE and BF, respectively), house crickets (*Acheta domesticus*, HE and HF, respectively) and mealworms (*Tenebrio molitor*, ME and MF, respectively) at a concentration of 5 g kg^{-1}. Genera and individual samples are shown in rows and columns, respectively, and are ordered based on PCoA ordination. [a]Including *Caballeronia* and *Paraburkholderia*. [b]Including *Allorhizobium*, *Neorhizobium* and *Pararhizobium*. [c]*Clostridium_sensu_stricto_13*. [d]*Candidatus_Udaeobacter*

between the rhizosphere of plants amended with black soldier fly frass and those treated with conventional compost, independently of whether the amendments were sterilised before use (Fuhrmann et al. 2022). Differential effects of fungal and arthropod chitin resources on soil bacterial communities were suggested to be the result of differences in the ultrastructure of chitin fibres and co-occurring structural compounds (Bai 2015). The same could hold for differences between residues originating from different insect species.

Different bacterial genera that are known to include plant growth-promoting species were stimulated by all insect residues, e.g., *Pseudomonas*, *Acinetobacter* or *Devosia* (Mercado-Blanco and Bakker 2007; Rokhbakhsh Zamin et al. 2011; Chhetri et al. 2022). Other genera associated with plant growth promotion were stimulated by all exuviae, e.g. *Sphingomonas* (Asaf et al. 2020), or by the frass of each insect species, e.g. *Paenibacillus* (Grady et al. 2016). The stimulation of *Pseudomonas, Devosia* and *Sphingomonas* species by soil amendment with insect exuviae or with chitin has been reported previously (Andreo-Jimenez et al. 2021; Wantulla et al. 2023). Interestingly, the genus *Paenibacillus*, was found to respond to certain chitin degradation products but not to chitin polymers (Shimoi et al. 2020). The enrichment of this genus by frass in the present study suggests that chitin is present in this material in a more degraded form.

Soil amendment with house cricket residues resulted in the largest plants and the unique enrichment of several bacterial genera that are known to include plant growth-promoting species. For example, the relative abundance of the genus *Flavobacterium* (Kolton et al. 2016) was uniquely increased by amendment with house cricket frass and exuviae, while the genus *Clostridium* (Figueiredo et al. 2020) was only stimulated upon amendment with house cricket exuviae. Furthermore, the genera *Chryseobacterium* (Chhetri et al. 2022) and *Nocardioides* (Nafis et al. 2019) were enriched earlier or longer by soil amendment with house cricket exuviae than by any other amendment. Similarly, amendment with black soldier fly residues stimulated a group of different *Burkholderiaceae* for a longer period of time than other amendments, as was reported for black soldier fly exuviae (Wantulla et al. 2023). Remarkably, the genus *Lysinibacillus* was only stimulated by black soldier fly exuviae, whereas it was previously found to be uniquely enriched by amendment with house cricket exuviae (Wantulla et al. 2023). For different species belonging to the genera *Burkholderia* and *Lysinibacillus*, plant growth-promoting properties were described (Eberl and Vandamme 2016; Ahsan and Shimizu 2021). While it is possible to identify likely candidates for plant growth promotion, which of these stimulated bacteria may be most important for enhancing plant growth cannot be determined based on the present study. Nonetheless, the differential stimulation of specific PGPR-including genera by amendment with different insect residues underlines their unique roles in nutrient cycling and the aboveground-belowground feedback loop. It moreover creates opportunities to utilise this knowledge for agricultural purposes by choosing specific insect residues as soil amendments to stimulate select indigenous PGPR.

Conclusion and future perspectives

In conclusion, this study shows that soil amendment with insect residues has great potential to promote plant growth and affect the composition of rhizosphere bacterial communities. Most insect residues had a comparable effect on plant C/N ratio and growth parameters (fresh and dry shoot biomass, leaf surface, leaf number and stem length) as synthetic fertiliser with a similar nitrogen content, although soil amendment with house cricket exuviae clearly outperformed the synthetic fertiliser treatment. In contrast to treatment with synthetic fertiliser, all soil amendments with insect residues increased the relative abundance of several bacterial taxa that include PGPR, highlighting their potential to promote the activity and growth of indigenous PGPR. However, insect residues originating from different insect species, and the different insect-derived materials (frass and exuviae) differentially stimulated specific bacterial genera. Future studies should focus on how these effects influence the interaction of plants with members of higher trophic levels, such as herbivores and their natural enemies. Such studies on multi-trophic effects will be valuable to evaluate the unique role of insect residues in the aboveground-belowground feedback loop, and their potential use as soil amendment in circular agriculture.

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Author contributions Els M. van de Zande, Max Wantulla, Joop J. A. van Loon and Marcel Dicke designed the study. Els M. van de Zande and Max Wantulla collected and analysed data. Els M. van de Zande, Max Wantulla, Joop J. A. van Loon and Marcel Dicke interpreted the results. Els M. van de Zande and Max Wantulla wrote the manuscript and all authors commented and approved the final version.

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Data availability The raw sequencing data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB67463 (https://www.ebi.ac.uk/ena/browser/view/PRJEB67463).

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahsan N, Shimizu M (2021) *Lysinibacillus* species: Their potential as effective bioremediation, biostimulant, and biocontrol agents. Rev Agric Sci 9:103–116. https://doi.org/10.7831/ras.9.0_103

Andreo-Jimenez B, Schilder MT, Nijhuis EH et al (2021) Chitin- and keratin-rich soil amendments suppress *Rhizoctonia solani* disease via changes to the soil microbial community. Appl Environ Microbiol 87:e00318. https://doi.org/10.1128/AEM.00318-21

Arabzadeh G, Delisle-Houde M, Tweddell RJ et al (2022) Diet composition influences growth performance, bioconversion of black soldier fly larvae: agronomic value and in vitro biofungicidal activity of derived frass. Agronomy 12:1765. https://doi.org/10.3390/agronomy12081765

Asaf S, Numan M, Khan AL, Al-Harrasi A (2020) *Sphingomonas*: from diversity and genomics to functional role in environmental remediation and plant growth. Crit Rev Biotechnol 40:138–152. https://doi.org/10.1080/07388551.2019.1709793

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol 47:817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300

Bai Y (2015) Ecological functioning of bacterial chitinases in soil. PhD thesis, Leiden University, Leiden, The Netherlands

Barragán-Fonseca KY, Greenberg LO, Gort G et al (2023) Amending soil with insect exuviae improves herbivore tolerance, pollinator attraction and seed yield of *Brassica nigra* plants. Agric Ecosyst Environ 342:106219. https://doi.org/10.1016/j.agee.2022.108219

Barragán-Fonseca KY, Nurfikari A, van de Zande EM et al (2022a) Insect frass and exuviae to promote plant growth and health. Trends Plant Sci 27:646–654. https://doi.org/10.1016/j.tplants.2022.01.007

Barragán-Fonseca KY, Rusman Q, Mertens D et al (2022b) Insect exuviae as soil amendment affect flower reflectance and increase flower production and plant volatile emission. Plant Cell Environ 46:931–954. https://doi.org/10.1111/pce.14516

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Beesigamukama D, Subramanian S, Tanga CM (2022) Nutrient quality and maturity status of frass fertilizer from nine edible insects. Sci Rep 12:7182. https://doi.org/10.1038/s41598-022-11336-z

Behie SW, Bidochka MJ (2013) Insects as a nitrogen source for plants. Insects 4:413–424. https://doi.org/10.3390/insects4030413

Belovsky GE, Slade JB (2000) Insect herbivory accelerates nutrient cycling and increases plant production. Proc Natl Acad Sci USA 97:14412–14417. https://doi.org/10.1073/pnas.250483797

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Borriss R (2015) *Bacillus*, a plant-beneficial bacterium. In: Lugtenberg B (ed) *Principles of plant-microbe interactions*. Springer International Publishing, Cham, Switzerland, pp 379–391

Brodbeck BV, Andersen PC, Mizell RF (1999) Effects of total dietary nitrogen and nitrogen form on the development of xylophagous leafhoppers. Arch Insect Biochem Physiol 42:37–50. https://doi.org/10.1002/(SICI)1520-6327(199909)42:1<37::AID-ARCH5>3.0.CO;2-9

Butnan S, Duangpukdee J, Sriraj P (2022) Temporal effects of the combined use of cricket frass and eucalyptus biochar on the yield and tissue nitrate content in chinese kale. Pertanika J Trop Agric Sci 45:697–710. https://doi.org/10.47836/pjtas.45.3.10

Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Caporaso JG, Lauber CL, Walters WA et al (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108:4516–4522. https://doi.org/10.1073/pnas.1000080107

Chhetri G, Kim I, Kim J et al (2022) *Chryseobacterium tagetis* sp. nov., a plant growth promoting bacterium with an antimicrobial activity isolated from the roots of medicinal plant (*Tagetes patula*). J Antibiot Res 75:312–320. https://doi.org/10.1038/s41429-022-00525-7

Chiam Z, Lee JTE, Tan JKN et al (2021) Evaluating the potential of okara-derived black soldier fly larval frass as a soil amendment. J Environ Manage 286:112163. https://doi.org/10.1016/j.jenvman.2021.112163

Couture JJ, Servi JS, Lindroth RL (2010) Increased nitrogen availability influences predator–prey interactions by altering host-plant quality. Chemoecology 20:277–284. https://doi.org/10.1007/s00049-010-0058-y

Dasberg S, Mendel K (1971) The effect of soil water and aeration on seed germination. J Exp Bot 22:992–998. https://doi.org/10.1093/jxb/22.4.992

Debode J, De Tender C, Soltaninejad S et al (2016) Chitin mixed in potting soil alters lettuce growth, the survival of zoonotic bacteria on the leaves and associated rhizosphere microbiology. Front Microbiol 7:565. https://doi.org/10.3389/fmicb.2016.00565

Ebeling A, Allan E, Heimann J et al (2013) The impact of plant diversity and fertilization on fitness of a generalist grasshopper. Basic Appl Ecol 14:246–254. https://doi.org/10.1016/j.baae.2013.01.006

Eberl L, Vandamme P (2016) Members of the genus *Burkholderia*: Good and bad guys. F1000Res 5:1007. 10.12688/F1000RESEARCH.8221.1

European Commission (2021) Amending certain annexes to regulation (EU) No 142/2011 as regards the requirements for placing on the market of certain insect products and the adaptation of a containment method. Office for Official Publications of the European Communities, Luxembourg

Fan Y, Liu J, Liu Z et al (2022) Chitin amendments eliminate the negative impacts of continuous cropping obstacles on soil properties and microbial assemblage. Front Plant Sci 13:1067618. https://doi.org/10.3389/fpls.2022.1067618

FAO (2022) Agricultural production statistics. 2000-2020. FAOSTAT Analytical Brief 41

Fielding DJ, Trainor E, Zhang M (2013) Diet influences rates of carbon and nitrogen mineralization from decomposing grasshopper frass and cadavers. Biol Fertil Soils 49:537–544. https://doi.org/10.1007/s00374-012-0702-5

Figueiredo GGO, Lopes VR, Romano T, Camara MC (2020) *Clostridium*. In: Amaresan N, Senthil Kumar M, Annapurna K et al (eds) *Beneficial microbes in agro-ecology: bacteria and fungi*. Academic Press, London, pp 477–491

Finch-Savage WE, Côme D, Lynn JR, Corbineau F (2005) Sensitivity of *Brassica oleracea* seed germination to hypoxia: A QTL analysis. Plant Science 169:753–759. https://doi.org/10.1016/j.plantsci.2005.05.026

Fox J, Weisberg S (2019) An R Companion to Applied Regression, 3rd edn. Sage, Thousand Oaks

Friman J, Karssemeijer PN, Haller J et al (2021a) Shoot and root insect herbivory change the plant rhizosphere microbiome and affects cabbage–insect interactions through plant–soil feedback. New Phytologist 232:2475–2490. https://doi.org/10.1111/nph.17746

Friman J, Pineda A, van Loon JJA, Dicke M (2021b) Bidirectional plant-mediated interactions between rhizobacteria and shoot-feeding herbivorous insects: a community ecology perspective. Ecol Entomol 46:1–10

Frost CJ, Hunter MD (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. Ecology 85:3335–3347. https://doi.org/10.1890/04-0003

Frost CJ, Hunter MD (2007) Recycling of nitrogen in herbivore feces: Plant recovery, herbivore assimilation, soil retention, and leaching losses. Oecologia 151:42–53. https://doi.org/10.1007/s00442-006-0579-9

Fuhrmann A, Wilde B, Conz RF et al (2022) Residues from black soldier fly (*Hermetia illucens*) larvae rearing influence the plant-associated soil microbiome in the short term. Front Microbiol 13:994091. https://doi.org/10.3389/fmicb.2022.994091

Gebremikael MT, van Wickeren N, Hosseini PS, De Neve S (2022) The impacts of black soldier fly frass on nitrogen availability, microbial activities, C sequestration, and plant growth. Front Sustain Food Syst 6:795950. https://doi.org/10.3389/fsufs.2022.795950

Goswami M, Deka S (2020) Plant growth-promoting rhizobacteria—alleviators of abiotic stresses in soil: A review. Pedosphere 30:40–61. https://doi.org/10.1016/S1002-0160(19)60839-8

Grady EN, MacDonald J, Liu L et al (2016) Current knowledge and perspectives of *Paenibacillus*: A review. Microb Cell Fact 15:203. https://doi.org/10.1186/s12934-016-0603-7

Hartig F, Lohse L (2022) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package v.0.4.6

Heinen R, Biere A, Harvey JA, Bezemer TM (2018) Effects of soil organisms on aboveground plant-insect interactions in the field: patterns, mechanisms and the role of methodology. Front Ecol Evol 6:106. https://doi.org/10.3389/fevo.2018.00106

Houben D, Daoulas G, Faucon MP, Dulaurent AM (2020) Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Sci Rep 10:4659. https://doi.org/10.1038/s41598-020-61765-x

Hunter MD (2001) Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. Agric For Entomol 3:77–84

Inderbitzin P, Ward J, Barbella A et al (2018) Soil microbiomes associated with verticillium wilt-suppressive broccoli and chitin amendments are enriched with potential biocontrol agents. Phytopathology 108:31–43. https://doi.org/10.1094/PHYTO-07-17-0242-R

Izza Bukari N, Abd Ghani I, Mustaffa M et al (2021) The effect of cricket (Orthoptera: Gryllidae) frass on the growth of leafy vegetables. ASM Sci J 14:175–181

Khatoon Z, Huang S, Rafique M et al (2020) Unlocking the potential of plant growth-promoting rhizobacteria on soil health and the sustainability of agricultural systems. J Environ Manage 273:111118. https://doi.org/10.1016/j.jenvman.2020.111118

Kolton M, Erlacher A, Berg G, Cytryn E (2016) The *Flavobacterium* genus in the plant holobiont: ecological, physiological, and applicative insights. In: Castro-Sowinski S (ed) *Microbial models: from environmental to industrial sustainability*. Springer, Singapore, pp 189–207

Kos M, Jing J, Keesmaat I et al (2017) After-life effects: living and dead invertebrates differentially affect plants and their associated above- and belowground multitrophic communities. Oikos 126:888–899. https://doi.org/10.1111/oik.03734

Lenth RV, Bolker B, Buerkner P et al (2023) Estimated marginal means, aka least-squares means. R package v.1.8.5

Li H, Yoshida S, Mitani N et al (2022) Disease resistance and growth promotion activities of chitin/cellulose nanofiber from spent mushroom substrate to plant. Carbohydr Polym 284:119233. https://doi.org/10.1016/j.carbpol.2022.119233

Lovett GM, Christenson LM, Groffman PM et al (2002) Insect defoliation and nitrogen cycling in forests. Bioscience 52:335–341. https://doi.org/10.1641/0006-3568(2002)052[0335:IDANCI]2.0.CO;2

Lovett GM, Ruesink AE (1995) Carbon and nitrogen mineralization from decomposing gypsy moth frass. Oecologia 104:133–138. https://doi.org/10.1007/BF00328577

Lundberg DS, Lebeis SL, Paredes SH et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90. https://doi.org/10.1038/nature11237

Manjula K, Podile AR (2001) Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF 1. Can J Microbiol 47:618–625. https://doi.org/10.1139/cjm-47-7-618

Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Evol Syst 11:119–161. https://doi.org/10.1146/annurev.es.11.110180.001003

McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8:e61217. https://doi.org/10.1371/journal.pone.0061217

Mercado-Blanco J, Bakker PAHM (2007) Interactions between plants and beneficial Pseudomonas spp.: Exploiting bacterial traits for crop protection. Antonie van Leeuwenhoek, Intl J General Mol Microbiol 92:367–389. https://doi.org/10.1007/s10482-007-9167-1

Nafis A, Raklami A, Bechtaoui N et al (2019) Actinobacteria from extreme niches in Morocco and their plant growth-promoting potentials. Diversity (Basel) 11:139. https://doi.org/10.3390/d11080139

Nurfikari A (2022) Prospects of residual streams from insect cultivation for sustainable crop management. PhD thesis, Wageningen University, Wageningen, The Netherlands

Nurfikari A, de Boer W (2021) Chitin determination in residual streams derived from insect production by LC-ECD and LC-MS/MS methods. Front Sustain Food Syst 5:795694. https://doi.org/10.3389/fsufs.2021.795694

Nurfikari A, Leite MFA, Kuramae EE, de Boer W (2023) Microbial Community Dynamics During Decomposition of Insect Exuviae and Frass in Soil. SSRN Electron J. https://doi.org/10.2139/ssrn.4422505

Oksanen J, Blanchet FG, Friendly M et al (2020) Vegan: community ecology package. R package v2.5-7

Paulson JN, Stine OC, Bravo HC, Pop M (2013) Differential abundance analysis for microbial marker-gene surveys. Nat Methods 10:1200–1202. https://doi.org/10.1038/nmeth.2658

Pinheiro J, Bates B, DebRoy S et al (2020) nlme: linear and nonlinear mixed effects models. R package v.3.1-147

Poveda J, Jiménez-Gómez A, Saati-Santamaría Z et al (2019) Mealworm frass as a potential biofertilizer and abiotic stress tolerance-inductor in plants. Applied Soil Ecology 142:110–122. https://doi.org/10.1016/j.apsoil.2019.04.016

R Core Team (2020) The R project for statistical computing. Vienna, Austria: R foundation for Statistical Computing. http://www.r-project.org. v3.6.3

R Core Team (2022) R: A language and environment for statistical computing. Vienna, Austria: R foundation for Statistical Computing. URL http://www.r-project.org. v4.2.1

Rajaram S, Oono Y (2010) NeatMap - non-clustering heat map alternatives in R. BMC Bioinformatics 11:45. https://doi.org/10.1186/1471-2105-11-45

Rokhbakhsh Zamin F, Sachdev D, Kazemi-Pour N et al (2011) Characterization of plant-growth-promoting traits of Acinetobacter species isolated from rhizosphere of Pennisetum glaucum. J Microbiol Biotechnol 21:556–566. https://doi.org/10.4014/jmb.1012.12006

Schimel JP, Bennett J (2004) Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85:591–602. https://doi.org/10.1890/03-8002

Shamshina JL, Kelly A, Oldham T, Rogers RD (2020) Agricultural uses of chitin polymers. Environ Chem Lett 18:53–60. https://doi.org/10.1007/s10311-019-00934-5

Sharp RG (2013) A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. Agronomy 3:757–793. https://doi.org/10.3390/agronomy3040757

Shimoi Y, Honma D, Kurematsu A et al (2020) Effects of chitin degradation products N-acetylglucosamine and N,N′-diacetylchitobiose on chitinase activity and bacterial community structure in an incubated upland soil. Soil Sci Plant Nutr 66:429–437. https://doi.org/10.1080/00380768.2020.1767488

Song J, Wu D, Shao P et al (2015) Ecosystem carbon exchange in response to locust outbreaks in a temperate steppe. Oecologia 178:579–590. https://doi.org/10.1007/s00442-015-3248-z

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley

Van der Putten WH, Vet LEM, Harvey JA, Wäckers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens and their antagonists. Trends Ecol Evol 16:547–554. https://doi.org/10.1016/S0169-5347(01)02265-0

van Huis A (2021) Prospects of insects as food and feed. Organ Agric 11:301–308. https://doi.org/10.1007/s13165-020-00290-7

Varelas V (2019) Food wastes as a potential new source for edible insect mass production for food and feed: A review. Fermentation 5:81. https://doi.org/10.3390/fermentation5030081

Wantulla M, van Loon JJA, Dicke M (2023) Soil amendment with insect exuviae causes species-specific changes in the rhizosphere bacterial community of cabbage plants. Appl Soil Ecol 188:104854. https://doi.org/10.1016/j.apsoil.2023.104854

Watson C, Schlösser C, Vögerl J, Wichern F (2021) Excellent excrement? Frass impacts on a soil's microbial community, processes and metal bioavailability. Appl Soil Ecol 168:104110. https://doi.org/10.1016/j.apsoil.2021.104110

Winkler AJ, Dominguez-Nuñez JA, Aranaz I et al (2017) Short-chain chitin oligomers: Promoters of plant growth. Mar Drugs 15:40. https://doi.org/10.3390/md15020040

Yang LH (2004) Periodical cicadas as resource pulses in North American forests. Science (1979) 306:1565–1567. DOI: https://doi.org/10.1126/science.1103114

Yilmaz P, Parfrey LW, Yarza P et al (2014) The SILVA and "All-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648. https://doi.org/10.1093/nar/gkt1209