

The potential of soil amendment with insect exuviae and frass to control the cabbage root fly

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Abstract

Reliable options to control the cabbage root fly, *Delia radicum* L., are lacking in many countries as restrictions on insecticide use have tightened due to environmental concerns. Although microbial control agents are often considered as a sustainable alternative, their application in agriculture is constrained by inconsistent efficacy owing to low field persistence. To stimulate naturally occurring beneficial microbes, soil amendment with the residual streams of insect production has been suggested as an alternative to synthetic fertilization and a new approach to microbial crop protection. In a set of greenhouse experiments, exuviae and frass of black soldier fly larvae, *Hermetia illucens* L., house crickets, *Acheta domesticus* L. and exuviae of mealworms, *Tenebrio molitor* L., were added to soil from an organically managed field. Exuviae and frass treatments were compared to treatments with synthetic fertilizer. Brussels sprouts, *Brassica oleracea* L., plants were grown in amended soil for 5 weeks before being infested with cabbage root fly larvae. Insect and plant performance were assessed by recording cabbage root fly survival, biomass and eclosion time and seed germination and plant biomass, respectively. Whereas soil amendment with black soldier fly frass or exuviae reduced cabbage root fly survival and biomass, respectively, amendment with house cricket or mealworm residual streams did not negatively affect root fly performance. Furthermore, seed germination was reduced in soil amended with house cricket exuviae, while amendment with either residual stream derived from black soldier fly larvae or house crickets resulted in lower plant shoot biomass compared with the synthetic fertilizer treatment. Amending soil with black soldier fly residual streams could become a novel and low-cost tool to be integrated in cabbage root fly management programmes, especially where methods currently available are insufficient. Therefore, the mechanisms underlying the effects of insect-derived soil amendments described here should be the focus of future research.

KEYWORDS

Brassica oleracea, *Delia radicum*, *Hermetia illucens*, insect residual streams, pest management, soil amendment

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1 | INTRODUCTION

The cabbage root fly or cabbage maggot, *Delia radicum* L. (Diptera: Anthomyiidae), is a major pest of cruciferous crops in northern temperate regions and is considered to be the most damaging pest of turnip and swede (Santolamazza-Carbone et al., 2017). Female flies oviposit in soil close to the base of the host plant, from where the larvae start to feed on root tissue upon hatching. After up to 3 weeks of feeding, pupation occurs in the soil and adult flies eclose between 2 and 4 weeks later (Joseph & Ludice, 2020). Root feeding by *D. radicum* larvae disrupts the transport of water and nutrients and can lead to secondary damage due to the facilitation of pathogen invasion. Symptoms associated with *D. radicum* infestation include yellowing, wilting, stunting and slow growth, which often result in plant mortality (Herbst et al., 2017; Shuhang et al., 2016). As a consequence, crop losses can be as high as 100%, especially among young plants (Razinger et al., 2017). While some damage to roots may be tolerable for leaf or inflorescence vegetables such as cabbage or cauliflower, even slight damage can substantially reduce the quality of root vegetables like radish, turnip, or swede (Collier et al., 2020).

Over the past decades, management of the cabbage root fly has largely relied on the use of different synthetic insecticides (Herbst et al., 2017; Shuhang et al., 2016). As the root-feeding larvae are difficult to reach, insecticide application is mostly limited to methods such as seed, granule, or drench treatments, particularly using systemic active ingredients (Collier et al., 2020). However, stricter regulations due to environmental concerns as well as the development of insecticide resistance in *D. radicum* have reduced the availability of effective products worldwide (Joseph & Ludice, 2020; Shuhang et al., 2016). As a result of bans and restrictions on the use of relevant insecticides, reliable options for chemical control are lacking in several European countries (Collier et al., 2020; Herbst et al., 2017). With levels of plant resistance to *D. radicum* generally being too low in brassica crops, growers thus increasingly need to rely on physical, cultural and biological control strategies (Santolamazza-Carbone et al., 2017). Unfortunately, methods such as the use of physical barriers, parasitoids, or predators of *D. radicum* are often considered to be not effective enough and in some cases can have adverse effects on the crop. While microbial control agents are considered to be more promising alternatives, problems in their practical application and inconsistent effects in the field have prevented their use from becoming widespread (Collier et al., 2020).

In comparison with conventional pesticides, beneficial microorganisms such as entomopathogens or plant mutualists are generally regarded as more sustainable (Kupferschmied et al., 2013). So-called microbial biopesticides commonly exhibit multiple distinct modes of action, which can be helpful in managing pest resistance. Furthermore, they tend to be environmentally safe, not least due to relatively low persistence (Cawoy et al., 2011). At the same time, however, limited field persistence also represents a major weakness of microbial control agents. As introduced microbes frequently fail to establish in soil, their efficacy has largely remained unreliable under field conditions (Kaminsky et al., 2019). Therefore, instead

of applying non-native biocontrol agents, it has been suggested to make use of indigenous soil microbiomes. For this purpose, different organic soil inputs can serve as substrates to selectively enrich endemic microbes with biological control potential (Mazzola & Freilich, 2017).

Many of the properties of substrate inputs would render them particularly suitable for the management of soil pests. Infestations with root-feeding insects are extremely difficult to predict and react to in a timely manner, which often makes it necessary to apply crop protection products prophylactically (Johnson et al., 2016). While this might not always be practical with a control agent, it is convenient for the application of organic substrates, as soil usually has to be amended before or during planting. Although consistent efficacy of crop protection measures through a certain functional period is essential, increased persistence and the possible spread of microorganisms can also pose environmental risks (Kaminsky et al., 2019). Substrate-mediated recruitment of beneficial microbes, however, should limit the presence of colonizers to a definite period. Possible effects of organic inputs on soil organisms should thus wear off as soon as most of the substrate has been decomposed. Another advantage of substrate-based soil treatments is the promotion of potentially useful microbiomes rather than single microbial strains (Mazzola & Freilich, 2017). By stimulating functional consortia of microbes, the risk of resistance development in pests can be reduced greatly.

A relatively new approach to managing indigenous microbial resources is the amendment of soil with by-products of commercial insect production (Barragán-Fonseca et al., 2022). In recent years, mass rearing of insects for food and feed has become an expanding industry that continues to develop rapidly (Poveda, 2021; van Huis, 2021). Growing interest in the production of insects mainly owes to their capacity to convert low-quality organic material into animal protein. Nevertheless, insect rearing also generates other outputs, namely a mixture of unconsumed substrate, exuviae (moulted exoskeletons) and insect faeces, which are thought to be valuable products themselves (Houben et al., 2020). The combination of these three residual products of insects produced for food or feed is commonly referred to as 'frass' (Barragán-Fonseca et al., 2022). Frass has been the primary topic of research as it is produced in large quantities during insect production. In particular, frass of larvae of the black soldier fly, *Hermetia illucens* L. and of yellow mealworms, *Tenebrio molitor* L., are investigated for potential uses in agriculture (Barragán-Fonseca et al., 2022; Poveda, 2021; Schmitt & de Vries, 2020).

While it has been noted that soil amendment with insect frass could stimulate the activity of beneficial microbes, it is mostly considered for application as a fertilizer (Poveda et al., 2019). In fact, the capacity of frass to supply nutrients to plants and enhance plant growth has been compared to that of synthetic fertilizer and its potential to replace conventional fertilizers has been pointed out (Houben et al., 2020). Whether the use of insect residual streams as fertilizers can also contribute to the management of agricultural pests, however, remains to be investigated. Interestingly, mealworm exuviae, unlike other chitin-containing materials, were found to be

colonized by high numbers of Bacilli in both forest and former arable soils (Bai, 2015). Different species and various strains in this diverse group of bacteria are commonly applied for crop protection to control insects as well as plant diseases (Cawoy et al., 2011).

Overall, a great potential to promote soil and crop health has been attributed to insect residual streams, though this has yet to be proven by research (Torgerson et al., 2021). As there might be crucial differences in the composition of exuviae and frass between insect species, it is essential to study the residual streams of various species to determine possible uses beyond fertilization for each of them. Therefore, the objective of the present study was to investigate the effects of different exuviae and frass soil amendments as a replacement for synthetic fertilizers on the root-feeding insect pest *D. radicum*. To this end, the amendments were directly compared with the application of synthetic fertilizer.

2 | MATERIALS AND METHODS

2.1 | Plants and growth conditions

Brassica oleracea L. var. *gemmifera* cv. Cyrus (Brussels sprouts) plants were kept in a greenhouse compartment at 20°C, 60%–80% relative humidity and 16-h light/8-h dark photoperiod. Plants were grown in 1-L plastic pots, which were placed in saucers. For all experiments, pots containing the different treatments were randomly distributed over a single greenhouse bench. Two seeds were sown per pot and gently pressed down. If both seeds germinated, one seedling was randomly removed from each pot after 1 week. Excess seedlings were transplanted to pots of the same treatment in which no seeds had germinated and were used for experiments or were discarded together with ungerminated seeds if not needed. Plants growing in unamended soil were fertilized three times per week with 20 ml of an optimized fertilizer solution (see Table S1) in Experiment 1 and 15 ml per pot in Experiments 2 and 3. Fertilizer amounts per 1 L of field soil were based on a nitrogen fertilization advice of 230 kg/ha for cabbage provided by Eurofins Agro (Wageningen, the Netherlands), assuming a topsoil depth of 25 cm and taking into account the duration of each experiment. Plants growing in soil amended with insect residual streams did not receive any synthetic fertilizer. All plants were watered three times per week by filling saucers and emptying them after 2 h. Plants were grown for 5 weeks before being infested with *D. radicum* larvae.

2.2 | Insect rearing and plant infestation

The *D. radicum* population used was collected in Zeewolde (Flevoland, the Netherlands) in 2013. All life stages were kept in a climate cabinet at 20 ± 1°C and 16-h light/8-h dark photoperiod. Larvae were kept on *Brassica napus* L. subsp. *rapifera* (swede) roots of 10-week-old plants until pupation. Eclosed adult root flies were kept in gauze cages and were fed with a 1:1:1 mixture of sugar,

milk powder and yeast. In addition, a solution of honey in tap water was offered in a Petri dish and tap water was offered in a Petri dish with moist filter paper on top of wet cotton wool. Oviposition was stimulated by providing slices of swede in Petri dishes to the flies in the cages. Eggs were collected and placed on a new swede prior to hatching.

To obtain larvae for plant infestation, eggs were incubated in Petri dishes with moist filter paper. Larvae hatched from the eggs after 4 days and plants were infested by carefully placing five neonate larvae on plastic plant labels that were inserted into the soil surface close to the stem. Labels were checked after 30 min, and remaining larvae were replaced. This was repeated until five larvae had moved into the soil in every pot.

2.3 | Insect residual streams

Five insect residual streams were used in this study: exuviae and frass of larvae of black soldier flies, *H. illucens* (Bestico, Berkel en Rodenrijs, the Netherlands), exuviae and frass of house crickets, *Acheta domesticus* L. (Protix, Bergen op Zoom, the Netherlands) and exuviae of yellow mealworms, *T. molitor* (Nijenkamp Voederdieren, Hellendoorn, the Netherlands). Exuviae and frass were separated by the supplying companies before being delivered. All materials were first inspected for the presence of insects or insect fragments, which were removed, and were then oven-dried at 60°C for 24 h to allow for soil amendment on a dry matter basis. The dried materials were subsequently ground to a powder with an SM 100 cutting mill (Retsch, Haan, Germany).

2.4 | Soil

Agricultural soil was collected from the topsoil layer of an organically managed field in Wageningen, the Netherlands, in March 2019 for Experiments 1 and 2 and in December 2019 for Experiment 3. The field had been used to grow various brassicaceous plants since 2011 and black mustard (*Brassica nigra* L.) had recently been grown at the location selected for soil collection. Soil composition as assessed for the same 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% with a nitrogen delivery capacity of 80 kg/ha. The soil was homogenized by sieving (particle size <5 mm) and stored at ambient temperature in a non-heated warehouse for 2–8 months before being used.

2.5 | Experiment 1: Effects of black soldier fly and mealworm exuviae on *D. radicum* survival and larval biomass

Soil was mixed with black soldier fly or mealworm exuviae at a ratio of 5 g/kg of dry soil or left unamended for the application of

synthetic fertilizer as described above. Exuviae rates were chosen based on previous soil nitrogen content measurements so that the amount of plant-available nitrogen added to soil by amendment approximately corresponded to the amount applied as synthetic fertilizer (Nurfikari, 2022). As plants only grew large enough to sustain feeding *D. radicum* larvae in amended or fertilized soil, an untreated control was not included in the experiment. In a completely randomized design, 11 plants were grown per treatment and infested with *D. radicum* after 5 weeks. Plants were uprooted 2 weeks after infestation, and roots were rinsed to remove adhering soil. Roots were checked for remaining larvae, and all soil was washed through a Fenwick can (Metaalgaas Twente, Hengelo, the Netherlands) and a 0.5 mm aperture sieve to collect larvae and pupae. Living larvae were counted and weighed on a CP2P-F microbalance (Sartorius, Göttingen, Germany). Plants were oven-dried at 105°C for 24 h before measuring shoot and root dry biomass.

2.6 | Experiments 2 and 3: Effects of black soldier fly and house cricket residual streams on *D. radicum* survival, fly eclosion time and biomass

For Experiment 2, soil was mixed with black soldier fly exuviae or frass at a ratio of 5 g/kg or 10 g/kg of dry soil, respectively, or left unamended for the application of synthetic fertilizer as described above. In Experiment 3, soil was mixed with house cricket exuviae or frass, both at a ratio of 5 g/kg of soil, or left unamended for the application of synthetic fertilizer. Exuviae and frass rates were chosen based on previous soil nitrogen content measurements so that the amount of plant-available nitrogen added to soil by amendment approximately corresponded to the amount applied as synthetic fertilizer (Nurfikari, 2022). As plants only grew large enough to sustain feeding *D. radicum* larvae in amended or fertilized soil, an untreated control was not included in the experiments. For each experiment, 21 plants were grown per treatment in a completely randomized design and were infested with *D. radicum* larvae after 5 weeks. Plants were enclosed in mesh sleeves 3 weeks after infestation, and eclosing flies were counted and collected daily until no more flies had eclosed for at least 7 days. Flies were stored at -20°C before being dried at 50°C for 48 h. Fly dry biomass was then measured on a CP2P-F microbalance (Sartorius, Göttingen, Germany). Plant shoots were harvested 7 weeks after infestation and were oven-dried at 105°C for 24 h before measuring shoot dry biomass.

2.7 | Statistical analysis

Statistical tests were performed using R (Version 3.6.3; R Core Team, 2020) and the packages car (Fox & Weisberg, 2019), dunn.test (Dinno, 2017), emmeans (Lenth, 2021), nlme (Pinheiro et al., 2020) and stats (R Core Team, 2020). Seed germination and *D. radicum* survival were analysed using generalized linear models (GLM) with binomial or quasibinomial distributions, depending on possible overdispersion of the data. Pairwise comparisons were performed

using estimated marginal means (EMM). Larval and adult *D. radicum* biomass were analysed using linear mixed effects models (LMM) with plant as a random factor and EMMs for pairwise comparisons. Average fly eclosion time per plant as well as shoot and root biomass were analysed using linear models (LM) and pairwise comparisons of EMMs or Kruskal–Wallis and Dunn's tests if the assumption of normality was not met. Models were validated by plotting residuals and, where necessary, homogeneity of variances and normality were confirmed using Levene's test and the Shapiro–Wilk test, respectively.

3 | RESULTS

3.1 | Experiment 1: Effects of black soldier fly and mealworm exuviae on *D. radicum* survival and larval biomass

3.1.1 | *D. radicum* performance

The main effect of soil amendment with insect exuviae on the survival of *D. radicum* was significant (GLM: $\chi^2 = 14.827$, $df = 2$, $p < 0.001$). Whereas amendment with black soldier fly exuviae resulted in a significantly lower survival than amendment with mealworm exuviae (EMM: $p = 0.002$; Figure 1a), survival was not significantly different from the synthetic fertilizer treatment for either of the amendments. Soil amendment had a significant main effect on larval fresh biomass (LMM: $\chi^2 = 28.107$, $df = 2$, $p < 0.001$), and amendment with black soldier fly exuviae significantly reduced larval biomass compared with both the synthetic fertilizer treatment (EMM: $p = 0.021$; Figure 1b) and amendment with mealworm exuviae (EMM: $p < 0.001$; Figure 1b).

3.1.2 | Plant performance

Soil amendment with insect exuviae had a significant main effect on both shoot dry biomass (LM: $F = 15.073$, $df = 2$, $p < 0.001$) and root dry biomass (LM: $F = 4.0746$, $df = 2$, $p = 0.027$) of *B. oleracea* plants. Amendment with black soldier fly exuviae resulted in significantly lower shoot biomass than the synthetic fertilizer treatment or amendment with mealworm exuviae (EMM: $p < 0.001$; Figure 2a). Root biomass of plants grown in soil amended with black soldier fly exuviae was significantly higher than that of plants grown in soil amended with mealworm exuviae (EMM: $p = 0.045$; Figure 2b).

3.2 | Experiment 2: Effects of black soldier fly residual streams on *D. radicum* survival, fly eclosion time and biomass

3.2.1 | *D. radicum* performance

The main effect of soil amendment with black soldier fly residual streams on *D. radicum* survival was significant (GLM: $\chi^2 = 7.7423$,

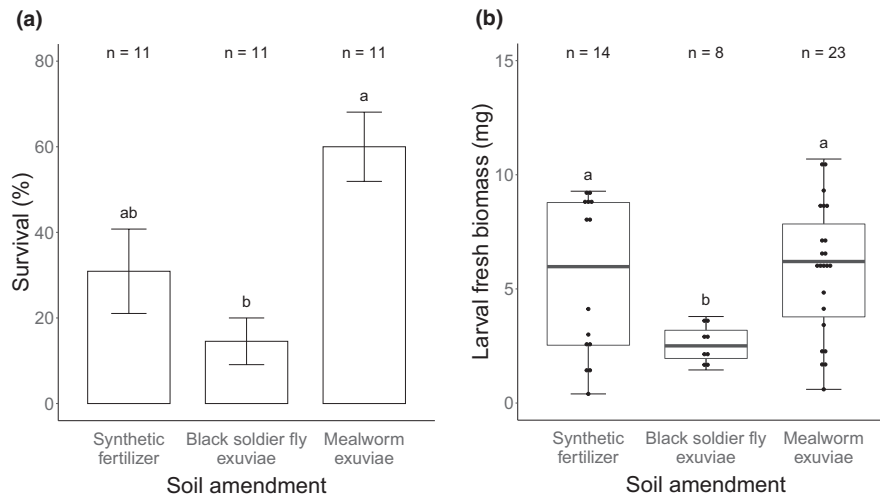
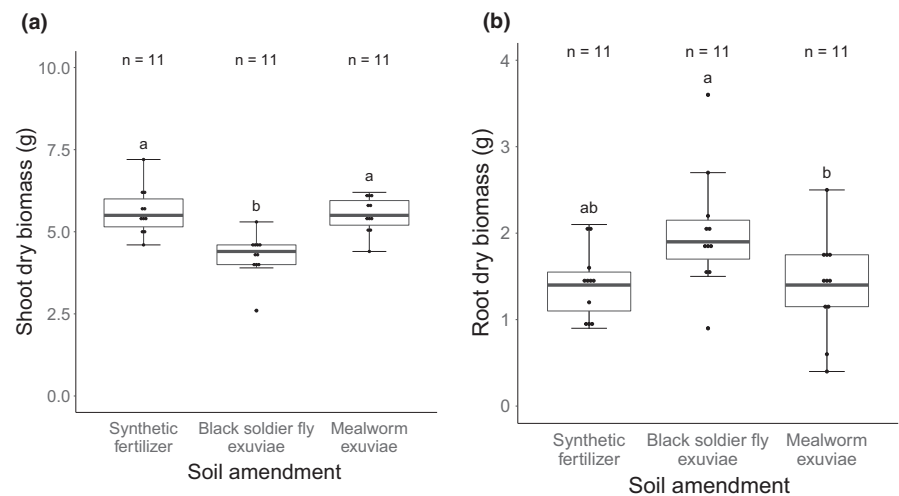


FIGURE 1 *Delia radicum* survival (a) and larval fresh biomass (b) after 2 weeks of feeding on *Brassica oleracea* plants growing in soil amended with insect exuviae (5 g/kg). Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (EMM, $p > 0.05$). Error bars (a) represent standard errors. Box plot whiskers (b) represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate plants (a) or larvae (b) are indicated at the top of the panels by n

FIGURE 2 Shoot (a) and root (b) dry biomass of *Brassica oleracea* plants grown in soil amended with insect exuviae (5 g/kg) 7 weeks after planting and 2 weeks after infestation with *Delia radicum* larvae. Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (EMM, $p > 0.05$). Box plot whiskers represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate plants are indicated at the top of the panels by n



$df = 2$, $p = 0.021$). Amendment with black soldier fly frass almost halved fly emergence from the soil as compared to the synthetic fertilizer treatment (EMM: $p = 0.02$; Figure 3a). Soil amendment had no significant effect on fly dry biomass (LMM: $\chi^2 = 2.1391$, $df = 2$, $p = 0.343$; Figure 3b). Soil amendment with black soldier fly residual streams did not affect *D. radicum* eclosion time (Kruskal–Wallis test: $H = 3.8389$, $df = 2$, $P = 0.15$; Figure 4).

3.2.2 | Plant performance

While soil amendment with black soldier fly residual streams did not affect *B. oleracea* seed germination (GLM: $\chi^2 = 0.96686$, $df = 2$, $p = 0.617$; Figure 5a), amendment significantly affected shoot dry biomass of *B. oleracea* plants (Kruskal–Wallis test: $H = 7.6925$, $df = 2$, $p = 0.02$). Both amendments with black soldier fly exuviae (Dunn's

test: $p = 0.044$; Figure 5b) and with black soldier fly frass (Dunn's test: $p = 0.04$; Figure 5b) resulted in lower shoot biomass than the synthetic fertilizer treatment.

3.3 | Experiment 3: Effects of house cricket residual streams on *D. radicum* survival, fly eclosion time and biomass

3.3.1 | *D. radicum* performance

Soil amendment with house cricket residual streams affected neither *D. radicum* survival (GLM: $\chi^2 = 4.7617$, $df = 2$, $p = 0.093$; Figure 6a) nor fly dry biomass (LMM: $\chi^2 = 3.7022$, $df = 2$, $p = 0.157$; Figure 6b). Soil amendment had a significant main effect on average *D. radicum* eclosion time (LM: $F = 5.7051$, $df = 2$, $p = 0.01$) and amendment

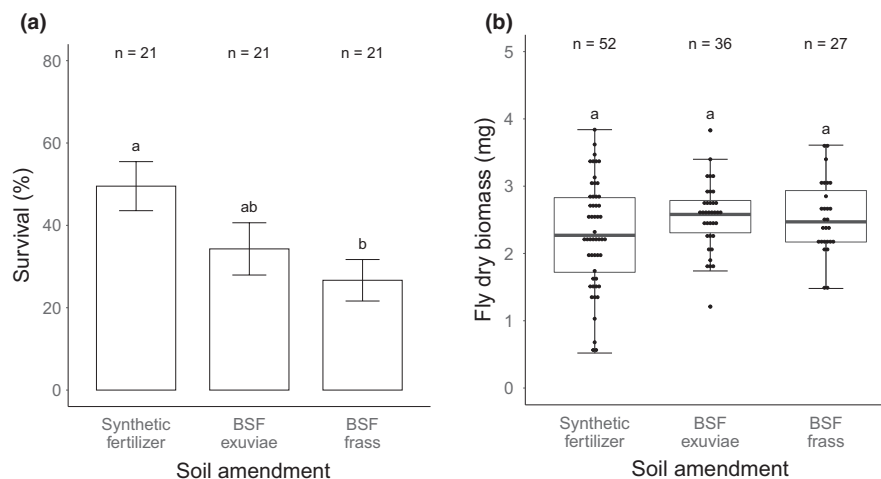


FIGURE 3 Survival (a) and adult fly dry biomass (b) of *Delia radicum* after larval feeding on *Brassica oleracea* plants grown in soil amended with black soldier fly (BSF) exuviae (5 g/kg) and frass (10 g/kg). Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (EMM, $p > 0.05$). Error bars (a) represent standard errors. Box plot whiskers (b) represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate plants (a) or flies (b) are indicated at the top of the panels by n

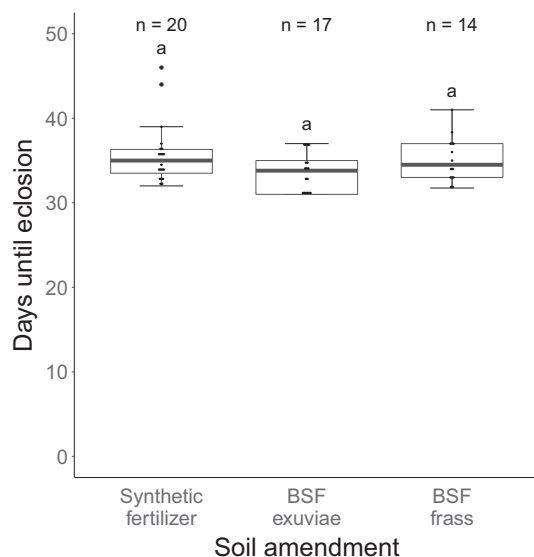


FIGURE 4 Time until eclosion of *Delia radicum* flies after larval feeding on *Brassica oleracea* plants grown in soil amended with black soldier fly (BSF) exuviae (5 g/kg) and frass (10 g/kg). Plants grown in unamended soil received synthetic fertilizer. Time until eclosion did not differ significantly among treatments (Kruskal-Wallis test, $p = 0.15$). Box plot whiskers represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate plants are indicated at the top of the panels by n

with house cricket frass reduced eclosion time by ca. 3 days (EMM: $p = 0.011$; Figure 7).

3.3.2 | Plant performance

Soil amendment with house cricket residual streams significantly affected *B. oleracea* seed germination (GLM: $\chi^2 = 29.142$, $df = 2$,

$p < 0.001$) and shoot dry biomass (LM: $F = 17.472$, $df = 2$, $p < 0.001$). Amendment with house cricket exuviae reduced seed germination by more than half as compared to the synthetic fertilizer treatment (EMM: $p < 0.001$; Figure 8a). Shoot biomass was significantly lower than in the synthetic fertilizer treatment after soil amendment with cricket exuviae (EMM: $p = 0.024$; Figure 8b) or frass (EMM: $p < 0.001$; Figure 8b) and was significantly lower with frass than with exuviae (EMM: $p = 0.006$; Figure 8b).

4 | DISCUSSION

Of the different insect-derived soil amendments tested here, only black soldier fly residual streams had a clear negative effect on the performance of *D. radicum*. While soil amendment with black soldier fly frass nearly halved cabbage root fly emergence as compared to the synthetic fertilizer treatment, amendment with black soldier fly exuviae resulted in a reduction of larval biomass. However, adult fly biomass was affected neither by amendment with frass nor by amendment with exuviae. A possible explanation is that larvae with a reduced biomass are less likely to complete their development and are not represented in the population of emerging flies. Furthermore, soil amendment with neither black soldier fly residual stream affected adult *D. radicum* eclosion time. It should be noted that black soldier fly frass was applied to soil at a mass ratio twice as high as the one for exuviae, leaving the possibility open that a higher content of exuviae in the soil might result in a significant reduction of *D. radicum* survival. However, as exuviae are produced in considerably smaller amounts in insect farming than frass, it is questionable whether soil amendment with larger quantities per kg would be feasible. Altogether, these results show that soil amendment with different black soldier fly residual streams can reduce *D. radicum* survival or impair larval development.

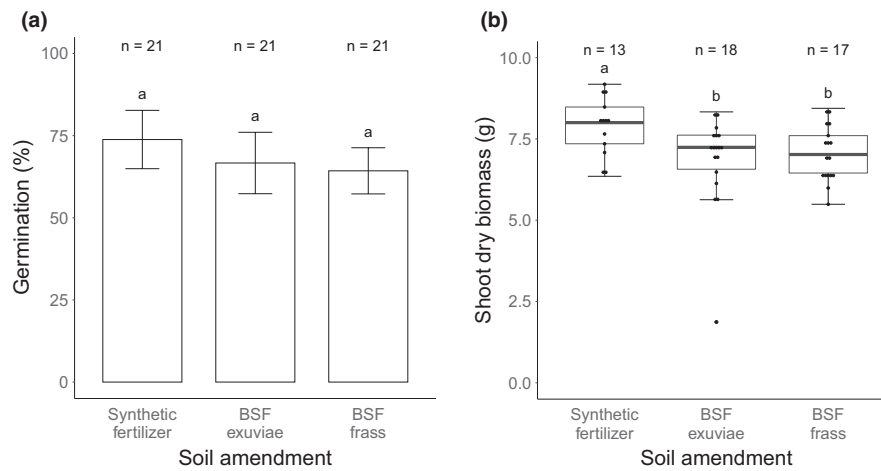


FIGURE 5 Seed germination (a) and shoot dry biomass (b) of *Brassica oleracea* 12 weeks after planting and 7 weeks after infestation with *Delia radicum* larvae in soil amended with black soldier fly (BSF) exuviae (5 g/kg) and frass (10 g/kg). Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (Dunn's test, $p > 0.05$). Error bars (a) represent standard errors. Box plot whiskers (b) represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate pots (a) or plants (b) are indicated at the top of the panels by n

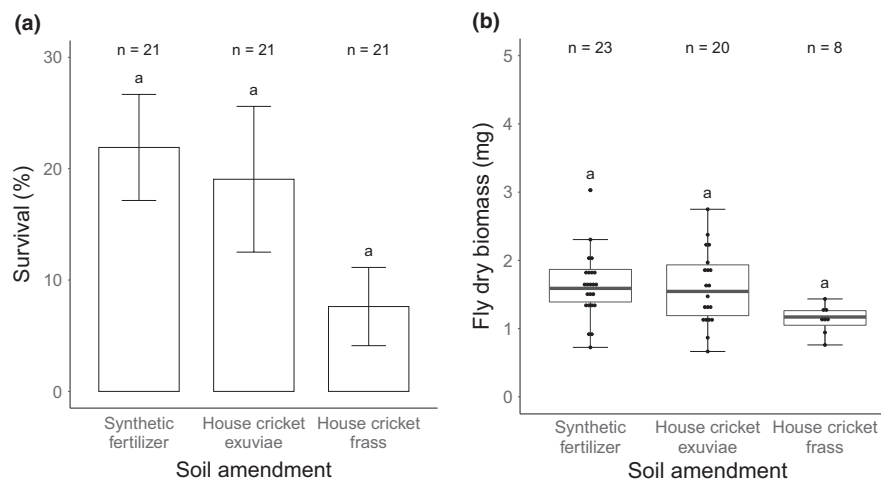


FIGURE 6 Survival (a) and adult fly dry biomass (b) of *Delia radicum* after larval feeding on *Brassica oleracea* plants grown in soil amended with house cricket residual streams (5 g/kg). Plants grown in unamended soil received synthetic fertilizer. Survival and biomass did not differ significantly among treatments (GLM/LMM, $p > 0.05$). Error bars (a) represent standard errors. Box plot whiskers (b) represent largest values within 75% quantiles $+1.5 \times$ interquartile range (IQR) and smallest values within 25% quantiles $-1.5 \times$ IQR. Numbers of replicate plants (a) or flies (b) are indicated at the top of the panels by n

Interestingly, soil amendment with the same residual streams derived from house crickets or mealworms did not significantly reduce *D. radicum* performance. As the effect of insect residual streams on *D. radicum* might depend on the soil microbes colonizing these streams, varying effects might be explained by differences in these microbial communities. Despite a lack of studies investigating how soil microbial communities respond to insect exuviae and frass, it can be assumed that these materials stimulate various microbes including bacteria and fungi. However, it is likely that soil bacteria play a particularly important role, as they are also generally considered to be the main degraders of chitin and respond to chitin addition more quickly than fungi (Cretoiu et al., 2013; Kielak et al., 2013). Insect exuviae and frass contain various compounds such as chitin, proteins

and lipids, the utilization of which as a substrate requires bacteria to have specific properties (Bai, 2015). The exact composition of these compounds may differ between insects so that residual streams derived from different species could also be colonized by specific soil bacteria.

The colonization of black soldier fly, house cricket and mealworm residual streams by distinct bacterial communities seems likely, considering that these species belong to separate insect orders and are thus distantly related. By contrast, the black soldier fly and *D. radicum* are both insects of the order Diptera, which suggests that soil bacteria stimulated by black soldier fly residual streams might potentially colonize *D. radicum*, too. Although many bacterial entomopathogens have a relatively broad host range, some are only

active against specific insect taxa. A well-known example for order specificity of insect-associated bacteria are different subspecies of *Bacillus thuringiensis*, whose host ranges include either lepidopteran, dipteran or coleopteran insects (Sanahuja et al., 2011). Overall, it seems possible that bacteria using black soldier fly exuviae or frass as substrates are detrimental to other fly species and that negative effects of these soil amendments on *D. radicum* may indeed be caused by bacterial colonizers.

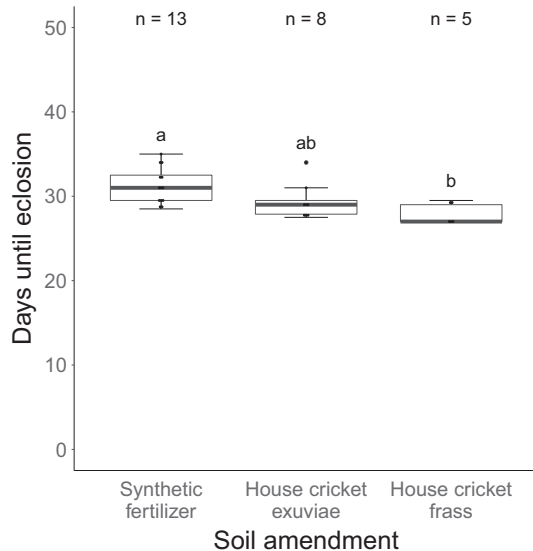


FIGURE 7 Time until eclosion of *Delia radicum* flies after larval feeding on *Brassica oleracea* plants grown in soil amended with house cricket residual streams (5 g/kg). Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (EMM, $p > 0.05$). Error bars represent standard errors. Numbers of replicate plants are indicated at the top of the panels by n

Bacteria that are promoted by insect-derived soil amendments might also be able to colonize insects or plant roots and could be beneficial in different ways. Insect-colonizing bacteria may be entomopathogenic and may control insects such as *D. radicum* by producing insecticidal chemicals (Cawoy et al., 2011; Kupferschmied et al., 2013). Root-colonizing microbes such as beneficial rhizobacteria can trigger so-called induced systemic resistance (ISR), which involves the priming of plants for enhanced defence. This primed state enables plant cells to react more rapidly and strongly to subsequent attacks by pathogens or insects (Pineda et al., 2013). Bacteria triggering ISR as well as entomopathogenic bacteria are found particularly in genera such as *Bacillus*, *Pseudomonas* and *Serratia* (Kupferschmied et al., 2013; Pineda et al., 2010). While there are several examples of single strains that can control insects via both of the above-mentioned mechanisms, it is also possible that different beneficial bacteria act synergistically to provide plant protection (Cawoy et al., 2011; Kupferschmied et al., 2013). However, ISR is mainly known to be effective against generalist herbivores, whereas negative effects on specialist herbivores such as *D. radicum* are usually not observed (Pineda et al., 2013). In fact, soil inoculation with a rhizobacterium causing ISR was found to improve *D. radicum* performance on plants grown in treated soil (Friman et al., 2020). This suggests that bacteria colonizing black soldier fly soil amendments are more likely to affect *D. radicum* due to insecticidal activity and pathogenicity.

Since mealworm exuviae had previously been reported to be colonized by Bacilli in different soils (Bai, 2015), they were expected to have negative effects on *D. radicum*. However, soil amendment with mealworm exuviae resulted in *D. radicum* survival being four times higher than when soil was amended with black soldier fly exuviae. Hence, *D. radicum* may in fact benefit from mealworm exuviae or the bacteria colonizing them in soil. Similarly, soil amendment with house cricket reduced average

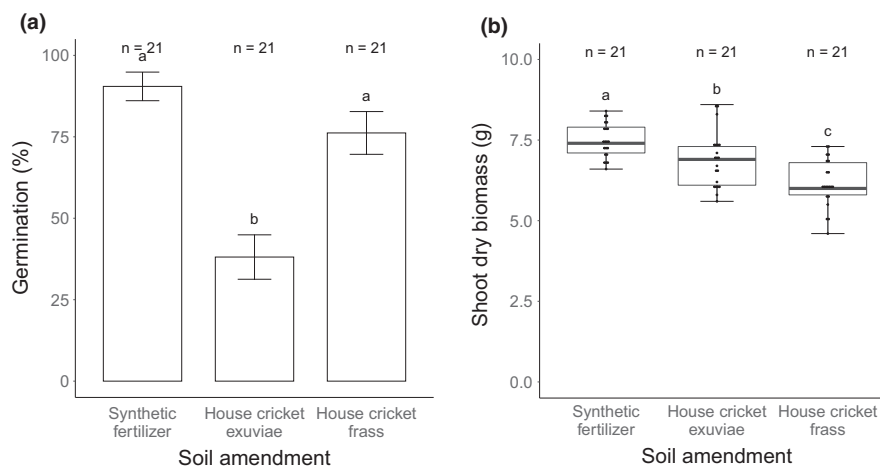


FIGURE 8 Seed germination (a) and shoot dry biomass (b) of *Brassica oleracea* 12 weeks after planting and 7 weeks after infestation with *Delia radicum* larvae in soil amended with house cricket residual streams (5 g/kg). Plants grown in unamended soil received synthetic fertilizer. Treatments denoted with the same letter are not significantly different (EMM, $p > 0.05$). Error bars (a) represent standard errors. Box plot whiskers (b) represent largest values within 75% quantiles +1.5 × interquartile range (IQR) and smallest values within 25% quantiles - 1.5 × IQR. Numbers of replicate pots (a) or plants (b) are indicated at the top of the panels by n

time until fly eclosion by 3 days and thus appears to accelerate *D. radicum* development. Indeed, soil microbes that are typically beneficial to plants are often found to have positive effects on insect herbivores, a phenomenon also known as induced systemic susceptibility (Pineda et al., 2013; Pineda et al., 2017). In the light of this, it seems possible that some insect-derived soil amendments stimulate bacteria that may benefit an insect, whereas others are colonized by bacteria that will reduce its performance. It should be noted that control survival of *D. radicum* was unusually low in all experiments reported here, which suggests that the field soil used may exhibit a certain inherent suppressiveness to *D. radicum*. While black soldier fly residual streams might mediate the recruitment of soil bacteria responsible for this, mealworm exuviae could inhibit these bacteria or serve as a substrate for other bacteria that antagonize them.

Similar to *D. radicum* performance, the influence of different insect residual streams on the performance of *B. oleracea* plants grown in amended soil varied. Whereas soil amendment with mealworm exuviae did not affect plant dry biomass, amendment with black soldier fly or house cricket residual streams resulted in lower shoot dry biomass than the synthetic fertilizer treatment. House cricket exuviae, moreover, reduced the germination of *B. oleracea* seeds in amended soil by more than half as compared to the synthetic fertilizer treatment. Interestingly, soil amendment with either black soldier fly residual stream led to lower shoot biomass despite reducing *D. radicum* performance. It is possible that insect residual streams may not only potentially promote bacteria with insecticidal activity, but that they can stimulate plant growth-inhibiting microbes as well. Although different organic amendments have successfully been used to suppress soilborne plant diseases, organic substrates are generally thought to mediate the assembly of functionally diverse soil microbiomes (Mazzola & Freilich, 2017). While some elements of the soil microbiome colonizing an insect residual stream could contribute to the control of insects such as *D. radicum*, others may in fact be deleterious to crops. Possible plant growth-inhibiting properties of these materials need to be considered in the future and require further investigation.

Although soil amendment with the different insect residual streams tested here was based on previously reported nitrogen release rates (Nurfikari, 2022), it should be noted that soil nitrogen levels were not measured during the experiments. Thus, despite relatively high application rates, the possibility that nitrogen availability was a plant growth-limiting factor in these treatments cannot be excluded. As plants grown in unamended soil, on the other hand, did receive optimal amounts of synthetic fertilizer, the experimental set-up used here is suitable only to a limited extent for explaining effects on plant growth. It should therefore be emphasized that the primary objective of the present study was to investigate possible effects on *D. radicum* rather than effects on plant growth. Based on previous research and considering the relatively low number of larvae added to each plant, it can be assumed that plant size did not limit larval feeding in any of the treatments.

It is striking that root dry biomass of *B. oleracea* plants grown in soil amended with black soldier fly exuviae was higher than when soil was amended with mealworm exuviae and was not different from plants that received synthetic fertilizer, despite shoot dry biomass being lower. This suggests that the supposed benefits of reduced *D. radicum* performance and a reduction in the root damage it caused could outweigh possible nutrient shortage or potentially negative effects of insect-derived soil amendments on plant growth. Finally, it should be pointed out that *B. oleracea* plants were relatively young at the end of the experiments reported here. A clear compensation for plant growth inhibition by stronger *D. radicum* suppression may only become evident after a longer growth period more representative of a *B. oleracea* growing season.

With insect production volumes predicted to grow drastically in the coming years, insect residual streams are soon expected to become available in large amounts (Houben et al., 2020; Schmitt & de Vries, 2020). As soil amendment with the two different black soldier fly residual streams tested here negatively affected *D. radicum* survival and development, these materials could represent a novel tool for managing this insect pest. Although only providing partial control, their implementation in *D. radicum* management programmes seems to offer good prospects in view of presumably low costs and a lack of effective control options in many countries (Collier et al., 2020).

Especially organic growers are likely to adopt new control methods and combine them with existing ones within an integrated pest management context as they cannot resort to synthetic insecticides and have particularly few products for *D. radicum* control at their disposal (Herbst et al., 2017). Likewise, conventional growers are expected to depend increasingly on the integration of alternative tools as new legal restrictions continue to limit insecticide availability (Collier et al., 2020; Johnson et al., 2016). When combined with other control methods that separately may not offer sufficient protection against *D. radicum*, black soldier fly soil amendments could thus contribute to an integrated pest management strategy. Most notably, there is prospect for their application in the production of leafy or flowering crops, as a certain degree of *D. radicum* feeding does not damage the marketable parts of these plants and can be tolerated (Collier et al., 2020; Razinger et al., 2017). On the contrary, a successful implementation in root crop production that demands zero tolerance for feeding damage may be less likely.

The experiments reported here suggest that black soldier fly residual streams can effectively reduce *D. radicum* performance for at least 5 weeks after application. Where introduced microbial agents may not persist long enough to provide effective crop protection, insect-derived soil amendments could indeed ensure the long-term establishment of naturally occurring biocontrol agents. Nonetheless, the true mode of action of these amendments and their potential role as a substrate for beneficial soil microbes remain to be investigated. First approaches to elucidate this should be the metagenomic analysis of microbial communities in amended soil or the identification of microbes associated with *D. radicum* larvae that were exposed to it. In this way, positive or negative

effects on *D. radicum* could be correlated with the presence or absence of certain groups of microbes that may be known to have attributes relevant for crop protection. Finally, even though field soil was used in the experiments reported here, testing the effects of insect-derived soil amendments on *D. radicum* under field conditions will be essential for future applications.

AUTHOR CONTRIBUTIONS

Max Wantulla, Joop van Loon and Marcel Dicke conceived the research. Max Wantulla and Kristian van Zadelhoff conducted experiments. Max Wantulla analysed data and conducted statistical analyses. Max Wantulla wrote the manuscript with input from Joop van Loon and Marcel Dicke. Marcel Dicke secured funding. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DANS-EASY at <https://doi.org/10.17026/dans-xg9-vzpx> (Wantulla et al., 2022).

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