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RESEARCH ARTICLE

BugBook: Critical considerations for evaluating and applying insect frass

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Abstract

Insect frass is one of the most abundant products in the insect industry, regardless of the farmed species. Rich in organic matter and plant nutrients, frass is a promising organic amendment that can be used in agriculture. While many benefits and challenges have been reported, several knowledge gaps remain unanswered, regarding the specific characteristics and mechanisms of its effects on soils and plants, particularly in terms of the processes driving these effects and interactions. This article aims to discuss recent research on frass and identify existing knowledge gaps, and propose strategies to improve research methodologies, increase comparability across studies, and generate more robust results. The topics covered include the evaluation of frass effects on soil physical properties, microbiological dynamics and fertility, as well as its effects on plant growth, development, and metabolism. The article also explores the potential of frass as an antimicrobial agent and highlights the need for stabilising and improving its quality and safety before it is used in agricultural activities. Looking forward, insect frass holds great promise as a sustainable input for agriculture, food production, and feed systems.

Keywords

experimental design – fertiliser – hygienisation – protocol – soil amendment

1 Introduction

Climate change poses a wide-ranging threat to global food security, affecting crop yields, food distribution networks, and market stability through extreme climatic events, changing precipitation dynamics and rising temperatures (Dasgupta and Robinson, 2022). In addition, an expanding world population with shifting dietary preferences, along with geopolitical tensions further intensify the challenges associated with food secu-

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rity, thereby requiring a multidisciplinary approach to address agricultural productivity, socio-economic factors, and environmental sustainability (Mbow *et al.*, 2019). As one measure to secure proper food supply, global agriculture is urged to provide higher yields (Ren *et al.*, 2019). The increase in agricultural output is expected to come from intensifying production on existing farmland, in addition to the need for new areas (FAO, 2017). Improving agricultural production and boosting its yield involves the adequate use of fertilisers, which are becoming more expensive. The prices of fertilisers, food and energy interact closely, creating a feedback loop within which rising costs in one sector increase costs in others, as typically observed in linear economies, leading the whole system to instability (Alexander *et al.*, 2023; Ott, 2012). From an environmental perspective, the use of synthetic fertilisers such as ammonium, nitrate, and urea have significant impacts. This includes high water and energy consumption, substantial use of fossil fuels, and a generally high potential for contributing to global warming throughout its production and use, justifying the need for a greater use of organic inputs (Chien *et al.*, 2009).

A growing interest in finding solutions to replace synthetic fertilisers – at least partially, from both academia and industry – has been observed over the last few decades. Partial replacements of synthetic inputs with organic amendments could enhance agricultural sustainability by improving soil health and resilience, reduce environmental footprints of current production systems, and boost long-term productivity. While synthetic fertilisers have a more rapid nutrient release upon application in the soil, these are more prone to nutrients runoff. For instance, NO_3^- is easily soluble in water and is not adsorbed by the soil organic matter's negative charges. Conversely, organic fertilisers are typically known to act as slow-release fertilisers, seen that they need to "break down" and its nutrients need to mineralise before absorption, providing those in a lower amount over an extended period of time and preventing runoff (Shaji *et al.*, 2021). Their rational and proper use may therefore provide a means of long-term protection to agricultural fields, not only by enhancing nutrient availability but also by preventing the risk of eutrophication and groundwater contamination. Besides, organic fertilisers and soil amendments are known to contain an endogenous active microbiome that may exert long-term effects on the productivity and sustainability of agro-ecosystems (Mas-Carrió *et al.*, 2018), contributing to soil organic matter (SOM) buildup and increasing microbial activity and diversity. Therefore, the integration of organic fertilisers into current systems might contribute to reducing the dependency on synthetic inputs, bolstering ecosystem resilience (Badagliacca *et al.*, 2024; Liu *et al.*, 2024; Tang *et al.*, 2022). In addition to these well-known benefits, the use of organic fertilisers, such as compost, manure, and bio-based waste products can enhance circularity in food production systems by bringing valuable nutrients back. However, as pointed by Leytem *et al.* (2024), several challenges exist from this perspective, including unbalanced and inconstant nutrient composition, the possible presence of pathogenic microorganisms, heavy metals, and other undesired substances such as antibiotics.

Interest in insect farming has been rapidly growing since the last few decades, as it addresses several global challenges by connecting waste management with food security and sustainability (Alfiko *et al.*, 2022; Tomberlin and van Huis, 2020). Farmed insects, such as various cricket species, yellow mealworm (YM), and black soldier fly (BSF), have high levels of essential amino acids and micronutrients, which makes these valuable food and feed components (Jiang *et al.*, 2023; Oonincx and Finke, 2021). Insects are mainly produced for their biomass, which is rich in protein and fat, but after their harvest, significant amounts of the remaining substrate, exuviae, and insect faeces remain. This blend is called insect frass. Even when insects are provided with a high-quality substrate suitable for production, the amount of frass remaining after harvest can exceed the weight of dry larvae produced (Deruytter *et al.*, 2023; Guidini Lopes *et al.*, 2023; Yakti *et al.*, 2022).

According to EU regulations, frass is defined as a mixture of insect excrement, residual feeding substrate, parts of farmed insects (not more than 5% (v/w) and not more than 3% (w/w)), and dead eggs (Commission Regulation (EU) 2021/1925). This classification further requires that frass undergoes hygienisation measures, such as heat treatment (70 °C for 1 h), anaerobic digestion or further composting, as it is legally considered within the "manures" group (Commission Regulation (EU) 142/2011). Insect frass is a valuable plant fertiliser that contains organic and plant available forms of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), as well as other elements (Amorim *et al.*, 2024; Beesigamukama *et al.*, 2022b). Additionally, chitin-rich exuviae fragments that remain in the frass might have a protective effect against pests, while boosting the plant's immune response (Barragán-Fonseca *et al.*, 2022). Besides potentially reducing the harmful environmental impacts related to the production and use of chemical fertilisers and pesticides, insect frass represents an additional revenue stream for producers, which could increase profitability for both the insect producers and farmers who would utilise this by-product (Beesigamukama *et al.*, 2022a; Niyonsaba *et al.*, 2021, 2023). However, frass can still be considered a novel agricultural input with several unknown traits and effects, from its composition to its use.

Insect frass has been showcased multiple times in the literature as a promising fertiliser and/or soil amendment that can benefit the soil-plant system in distinct ways. However, it is noteworthy that this novel material has a widely varying composition, not only in terms of organic matter and plant nutrients, but also in relation to the presence of bioactive compounds, which might cause this input to be different in comparison to other known organic materials such as compost. Among the knowledge gaps existing around insect frass, the most relevant ones are wide variations in its nutrient content, either from the same or distinct species as affected by the feed substrate used; fluctuating results when using frass as a fertiliser, in both soil and plants; possible effects of insect frass in plant protection; logistical challenges; and market needs (Figure 1). Encompassing all of these gaps is a central matter: how frass-related research is conducted and how replicable the results currently being published are. The aforementioned gaps will certainly be fulfilled in the future, but in order to advance knowledge in an adequate manner, designing experimental protocols in certain ways could enhance the comprehension of the effects of frass in agricultural practices even more. The main objective of this article is to discuss frass-related research up to this moment, highlighting both unsuccessfully and successfully acquired data, while providing useful suggestions for future research around this topic.

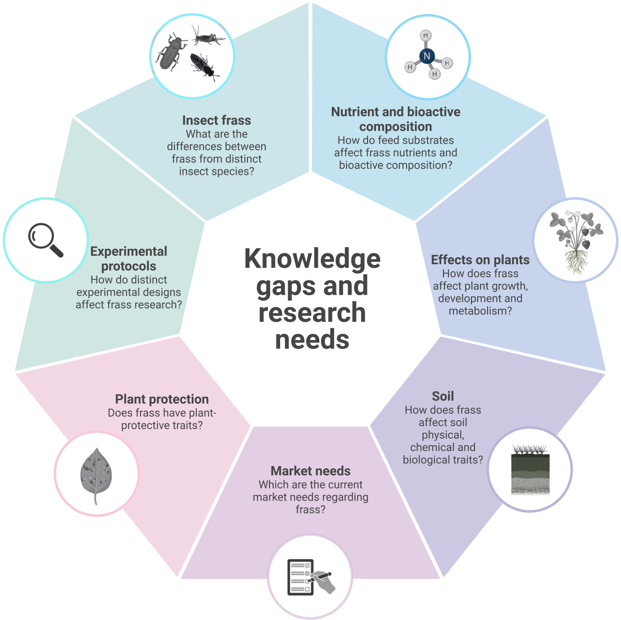

FIGURE 1 Conceptual map of knowledge gaps and research needs related to insect frass. Key challenges in frass research include variability in composition (due to insect species, diets, life stages, and processing techniques), the need for methodological standardisation (e.g. extraction solutions, ratios, and testing protocols), similar to what is done with compost and soils, as well as identification of key parameters, regulatory challenges, and market needs.

2 Challenges in classifying, characterising and testing insect frass

Rearing insects is *per se* a highly variable subject, with outcomes influenced by factors such as insect species, rearing methods, production scale, feed substrates used, and the type of processing applied to the resulting biomass and frass. Even when focusing on a single insect species, high variations in larval biomass and frass quality have been observed (Gärttling and Schulz, 2022; Lalander *et al.*, 2019). While balancing the proximate composition of larval biomass is somewhat easier, given the provision of diets with stable composition, maintaining consistent frass composition is more challenging, as even minor adjustments in process parameters, such as the bioconversion efficiency, ventilation, temperature, and humidity could affect its characteristics.

Based on the benefits reported so far in the literature regarding the use of insect frass in agriculture, its high concentration of organic matter, plant nutrients (which may vary in availability depending on the insect species and post-treatment method), and beneficial microorganisms are particularly prominent (Lopes *et al.*, 2022). Due to the varying life cycles of insect species, which directly influence the time required for frass production (e.g. 10–15 days for BSFL and 40–60 days for YM per batch for a first-use sort of say), it is even more challenging to understand and predict the differences observed in frass composition. Therefore, it is noteworthy that, at first glance, 'insect frass' types should not be treated the same when it comes to understanding their potential. Even though legal definitions are necessary (as stated above), the characteristics of frass and its effects on soils and plants must be considered, at least regarding its origin, i.e. the species from which it was produced. Nevertheless, a series of parameters can be considered and evaluated when reporting the effects and results of frass use, in the attempt of increasing the understanding of frass effects in crop production within a circular perspective, as performed by Ashworth *et al.* (2025). These are listed below as "recommended parameters" (which should always be reported in research on frass or commercially), "additional parameters" (which could be of great benefit for understanding the potential of the product) and "useful supplementary parameters" that are more specific. Based on those parameters, the effects and potentialities of frass in the soil and in plant cultivation are subsequently discussed.

Recommended parameters for frass characterisation:
(1) Organic matter content,
(2) C, N, P, and K concentrations.

Additional recommended parameters:
(1) pH and electrical conductivity (EC),
(2) Ammonium and nitrate concentration,
(3) Organic matter degradability (CO_2 and NH_3 emissions, self-heating capacity).

Useful supplementary parameters:
(1) Concentration of other macro- and micronutrients (Ca, Mg, S, Cu, Na, Zn, and B),
(2) Concentration of heavy metals (e.g. Cd, Hg, As, Pb, Cr),
(3) Microbial abundance and functionality,
(4) Biostimulants (e.g. amino acids, humic substances and phytohormones).

The assessment and disclosure of frass characteristics will primarily depend on two possibilities: for research purposes and the marketing context it is introduced to. When it comes to scientific efforts, it is advisable that the recommended and additional recommended parameters mentioned above are always disclosed to ensure a thorough understanding of frass composition given a certain rearing protocol and conditions, which can be translated later on to other realities and production scales. Conversely, when frass is marketed as a soil amendment or organic fertiliser, it must be minimally in agreement with the respective country's regulatory requirements.

Chemical and biological analysis of frass

Insect frass is a rich source of organic matter, nutrients, and bioactive substances in close interactions that can contribute to the fertility of soils and plant health in distinct ways (Lopes *et al.*, 2022). However, the understanding of frass effects depends directly on the proper chemical and biological characterisation of this input. Such characterisation can be a challenging step towards frass analysis, as there are several methods used to characterise soil and other organic amendments, such as vermicompost, compost and animal manures, which are currently being used as they are or with small adaptations. This chapter aims to bridge existing knowledge on analytical methods with the unique considerations that frass demands, assisting newcomers in accurate sample analysis. While standard procedures for soil amendments are applicable to frass, its distinct properties – such as lower maturity due to rapid production, the diversity of insect species and their digestion, and the variety of feed substrates – should be carefully accounted for, with attention to how these factors differentiate frass from other organic amendments. A thorough portrayal of methods for characterising insect frass is described in another chapter of the BugBook (Smets *et al.*, 2025). Nevertheless, we highlight some methods for the above-mentioned parameters, which are paramount for frass characterisation.

It is strongly recommended to determine the organic matter content, for which the loss-on-ignition method is the simplest and most commonly used method. A dry frass sample (dried at 60–105 °C to constant weight) is gradually and slowly heated in a muffle furnace to 550 °C and maintained at this temperature for 4 h. The volatile solids (VS) content, representing the amount of organic matter in the sample, is then calculated, as the remaining material consists solely of ash. Besides organic matter determination, elemental analysis (CNS) is a rapid and reliable method for characterising basic composition of frass. In elemental analysers, the samples are burned and the generated gases, related to the concentrations of C, N and sulphur (S), for example, are then analysed. The carbon-to-nitrogen ratio (C/N ratio) gives a first indication of how quickly the organic matter in frass can be decomposed, releasing nutrients to the soil, even though a recent study has demonstrated that for BSF larvae frass, a low C/N ratio does not necessarily indicate that this organic material is completely digested and considered a biologically stable material (Lopes *et al.*, 2024).

Ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) are important forms of available nitrogen in insect frass and are essential for plants and microorganisms. Colorimetric methods (e.g. based on the Berthelot reaction) are widely used, but ion chromatography can also be applied. The procedure for determining $N\text{-}NO_3^-$ (and even nitrite $N\text{-}NO_2^-$) is based on the "cadmium reduction method", in which a sample is buffered to a pH of 8.2 and passed through a column containing copper-cadmium to reduce the $N\text{-}NO_3^-$ to $N\text{-}NO_2^-$, which is then measured photometrically following diazotisation and coupling. An automated and precise method is the continuous flow analysis principle, which is based on international standard regulations such as the ISO. The extraction of N fractions (e.g. for continuous flow analysis) typically involves a KCl solution (2 M), K_2SO_4, $CaCl_2$, or deionised water. However, the choice of extraction ratio should be carefully investigated prior to the analysis itself, and depending on frass type and physical composition, distinct methods should be evaluated and validated.

Regarding the analysis of P, owing to the availability of both organic and inorganic forms of this macronutrient, it is paramount to estimate its concentration to properly understand its availability to plants. A simple and well-established method is the determination of phosphate (PO_4^{3-}), which is based on the reaction of ammonium heptamolybdate and potassium antimony(III) oxide tartrate in an acidic medium with dialysed and diluted solutions of PO_4^{3-}, to form an antimony-phospho-molybdate complex. This complex is then reduced to an intensely blue complex, which can be analysed spectrophotometrically. When the aim is to infer the bioavailable fraction of P, alkaline or neutral extraction is recommended. Irrespective of the type of fertiliser, several extractants for determining plant-available phosphorus in recycled P fertilisers have been proposed and compared by Hernandez-Mora *et al.* (2025), including H_2O, NAC (neutral ammonium citrate), $NaHCO_3$, Ca-lactate and others. According to those authors, the results of different methods might differ according to chemical properties, with the extraction with H_2O being reported as not reliable for determining P availability. However, the use of NAC and LiCl is suitable for a wide range of pH values, while H_2O often underestimates the concentration of available P, given that no ion exchange is favoured. The concentration of K in a frass sample can be best assessed by AAS (flame photometry) or inductively coupled plasma analysis after extracting the samples in a neutral salt solution (usually 1M ammonium acetate).

Laboratory-based analysis of fertilisers (including insect frass) includes pH and EC measurements, which are carried out using aqueous extracts (with a recommended ratio of 1:5 (w/v)) with deionised water. The contents are vortexed for homogenisation, allowed to sit for 30–60 min, and then analysed (ISO, 1994).

The degradability of frass organic matter and its readiness for use can be assessed using soil CO_2 and NH_3 emission tests to estimate the microbial activity in a frass sample. This can be achieved using Solvita® tests, as suggested by Lopes *et al.* (2024). Although established for compost, the Dewar flask self-heating test (Brinton *et al.*, 1995; Niese, 1996) can be used to determine the stability of organic matter based on the rise over ambient temperature of frass in an insulated thermos flask. In brief, the Dewar flask, a type of insulated container designed to minimise heat exchange with the surroundings, reheat test measures self-heating potential of materials. Sieved (4–5 mm) and moistened samples (50% moisture) are placed in 2-litre insulated Dewar flasks, and max daily temperatures are recorded alongside ambient temperatures over 5–7 days using min-max thermometers. However, the conditions under which this method is applied must be carefully controlled. In particular, the water content plays a critical role in heat development. Moreover, heat development should ultimately be assessed by integrating the area under the heat curve rather than relying solely on the maximum temperature achieved.

Other macro- and micronutrients (e.g. Ca, Mg, Cu, Mn, Zn, and others) can be analysed by atomic absorption spectrometry (AAS) after dissolving the ashes of a sample in an acid solution, such as HNO_3 (0.1 M). In addition, the fibre content of frass can be analysed by the sequential method with detergents, as proposed by Robertson and Van Soest (1981). Regarding heavy metal concentrations in frass, several elements (e.g. Al, As, Cd, Cr, Fe, Pb, etc.) can be evaluated by inductively coupled optical emission spectroscopy (ICP-OES), as suggested by Zarcinas *et al.* (1987) and validated by Amorim *et al.* (2024).

Several methods can be used to characterise the biological and bioactive properties of frass samples. Both nutrient dynamics and microbial activities in frass-treated and untreated soils (e.g. in pot experiments) can be evaluated by measuring carbon and nitrogen mineralisation as a function of CO_2 release and $N\text{-}NH_4^+$ concentration, respectively, as conducted by Praeg and Klammsteiner (2024) and Gebremikael *et al.* (2022). In addition, the characterisation of microbial abundance in frass can be done using similar methods as for soil, as performed by Nurfikari *et al.* (2024). However, it is of paramount importance that when using soil DNA extraction kits and sequencing samples, thorough quality checks should be carried out in the obtained data sets. The composition of bacterial and fungal communities can be determined by combining quantitative PCR and amplicon sequencing approaches (e.g. Illumina MiSeq) as standard methods.

Secondary metabolites, such as phenolic compounds, terpenoids, and alkaloids may reflect the plant material consumed by insects. Insects that feed on plants often pass on plant-derived bioactive compounds through their frass, including phenolic compounds, terpenoids, and alkaloids. Gas chromatography-mass spectrometry (GC-MS) is applied to detect volatile organic compounds and non-volatile organic compounds such as fatty acids, terpenoids, and phenolics in frass. To date, scarce resources are available of phytoactive substances and biostimulants in frass fertilisers and to the author's knowledge, a single study published by Green (2023) described a small share of this bioactive composition, including biogenic amines and some phytohormones (e.g. indoleacetic acid, abscisic acid, jasmonic acid, methyl jasmonate and gibberellins). This demonstrates the large knowledge gap that exists in frass-related research from this perspective. The bioactive composition of frass can be determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as discussed by Vrobel and Tarkowski (2023).

Considering the discussions made above, once related to standardised protocols for soil and other organic materials, such as compost and manure, it is highly advisable to set appropriate standards for insect frass, considering its widely varying composition. Establishing standard protocols for insect frass is essential to accurately reflect its unique features – influenced by rapid production, diverse insect species, and varying feed substrates. Developing standardised protocols facilitates interdisciplinary collaboration by providing clear guidelines accessible to a wider audience, resulting in more robust and comparable research outcomes. It is clear that several soil- and compost-analysis methods are applicable for frass analysis. However, protocols based on soil or compost are often used depending on the context of fertiliser application, and there is currently no clear guideline on which protocols or standardised methods should be universally applied. Therefore, we suggest that the scientific community either agrees on a common approach using composting methods or considers developing specific protocols tailored to insect frass. This lack of information and standardisation hampers proper comparison between studies conducted in different laboratories. Thus, we advocate the need for standardisation of protocols in frass research.

Testing frass as an organic input on plant performance

Once the insect frass is properly characterised, its effects on plants can be evaluated. As previously mentioned, insect frass is typically rich in nutrients, especially N-NH_4^+, P, K, and micronutrients, in addition to organic matter. Therefore, it can benefit soil and plants in multiple ways. However, assessing these effects under greenhouse or field conditions is a complex subject due to variations in soil and cultivation media properties, climatic conditions, microbial interactions, and application methods, all of which influence plant development. In addition, as demonstrated by Lopes *et al.* (2024), some traits that are typically considered as indicators of a compost's maturity and stability in compost or other organic amendments might not be entirely valid for BSF larvae frass, as well as other potential characteristics, justifying the need for specific ways of approaching insect frass. In this section, the main properties and plant performance indexes that should be considered when evaluating plant growth and development experimentally are discussed, while in Section 3.0 ("Assessing the influence of insect frass on plant performance") we elaborate in-depth on how to conduct experiments in greenhouse and field conditions.

A seed germination test is a common way to evaluate frass maturity and to detect any phytotoxic effects (Luo *et al.*, 2018). Potential phytotoxicity existing in frass can be due to lack of organic matter stabilisation, presence of phytotoxic substances (metals, high load of nutrients) or other reasons. Seed germination bioassays can be conducted according to the methods described in Lopes *et al.* (2024) and Bohm *et al.* (2023). Briefly, frass extracts are prepared by dissolving 5 g of fresh frass in 50 ml of deionised water and shaking continuously for 1 h, followed by centrifugation (4000–10 000 rotations per minute) for 15 min and subsequent filtering (0.45 μm filter). Then, 10 ml of the extracts are placed in Petri dishes or germination boxes, with a deionised water variant as control, and seeds are germinated over time, enabling the calculation of the germination index proposed by Zucconi *et al.* (1981). Seeds from distinct species should be used (e.g. watercress, tomato, lettuce, *Brassica* spp. seeds), considering that species show varying sensitivity to distinct compounds that might be present in frass.

General growth parameters (e.g. biomass accumulation, stem diameter, leaf area) can be assessed at the beginning, during and after an experiment, and compared with an established control. For instance, aboveground and root biomass can be sampled and oven-dried (60–70 °C until constant weight) to determine the fresh and dry weights of a plant. Plant height, stem diameter, and leaf area (estimated by digital imaging or by using specific leaf area metres), are good indicators of vegetative growth. To determine nutrient uptake and development, it is important to measure the total carbon and nitrogen content of the plant (which can be performed in elemental analysers, as described in the previous section) (Oxborough and Baker, 1997). Pérez-Harguindeguy *et al.* (2013) provided useful standard protocols for the determination of several parameters of interest in plant growth, including shoot and root biomass, leaf dry mass, leaf area, relative growth rates, and root-to-shoot ratio. Additional parameters to be assessed, depending on the plant species, are the number and quality of flowers and fruits, and physiological parameters.

As stated above, plant performance can be assessed through various developmental stages over the course of an experiment, and what will stipulate the specific stage that should be evaluated is the experiment's objective, overall. For instance, when simply evaluating frass as a source of nutrients for short-cycle crops, plant performance can be assessed by measuring growth parameters and nutrient use efficiency, which requires the plant to be harvested and chemically analysed. Conversely, in case frass is being evaluated for its effects in the emission of volatile compounds in a stressed plant, or even for its effects in abiotically-stressed plants (e.g. evaluating drought or heat stress), non-destructive methods can be used (e.g. photosynthetic efficiency and gas collection in closed chambers). Therefore, the plant's developmental stage in which frass should be tested must be considered case-by-case, as there is no general recommendation.

Insect frass affects plant growth through pathways that go beyond simple nutrient supplementation. Many of the effects verified following frass application are mediated by microorganisms, which affect plant metabolism and induce effects from photosynthetic performance improvements to increase plant defence. These aspects can be addressed by measuring distinct physiological parameters of plants, such as chlorophyll content, which can be assessed by using a Soil Plant Analysis Development (SPAD) metre to infer potential improvements in a plant's photosynthetic performance. Chlorophyll fluorescence is an easy and accessible method for assessing the efficiency of photosystem II (PSII), potentially identifying stress-related situations. This can be achieved using portable fluorometers. The maximum fluorescence yield is measured by exposing the leaves to a saturating flash of 3500 $\mu mol/m^2$ per s during exposure to natural light, and the effective quantum yield of PSII is recorded. After these measurements, the maximum quantum efficiency of PSII (called the Fv/Fm ratio) is determined for the dark-adapted leaves. Then, the non-photochemical quenching coefficient (NPQ) is calculated, and photochemical quenching (qP) and the intrinsic efficiency of open PSII centres are estimated (Oxborough and Baker, 1997).

An outstanding tool for assessing a plant's physiological status are gas exchange analysers, which can measure for instance CO_2 exchange, stomatal conductance and transpiration, among other parameters that help infer photosynthetic performance in a highly accurate manner. It has been suggested that the application of insect frass can rapidly affect a plant's capacity to assimilate CO_2, thus improving photosynthetic performance, owing to the action of the distinct groups of biostimulants existing in this organic amendment (Radzikowska-Kujawska *et al.*, 2023). However, it is paramount to disclose values that are expected according to the type of plants used, given that C_3, C_4, and plants using crassulacean acid metabolism (CAM) have very distinct photosynthetic performances, which are also influenced by temperature, as thoroughly discussed by (Yamori *et al.*, 2014). Therefore, it is highly recommended that when gas exchange measurements are carried out experimentally, the study should describe the complete setup of the equipment, including the temperature, air and CO_2 flows, and daylight conditions.

Another way to determine a plant's metabolism, growth, and stress responses is by the analysis of biochemical markers that can indicate carbon allocation and storage, including non-structural carbohydrates such as sugars and starch, which are critical for osmoprotection, long-term carbon storage, and rhizodeposition, among other traits (Chlumská *et al.*, 2014, 2022). Complementarily, antioxidant enzymes can be analysed as an indication of stresses, such as superoxide dismutase, peroxidase, and catalase, which respond to environmental stress (e.g. extreme temperatures). These can be measured in leaves as described in (Cavalcanti *et al.*, 2004). In addition, lipid peroxidation can be measured in fresh leaves as a function of malondialdehyde content, which measures the oxidative stress level and could demonstrate grass-fertilisation effects on plant stress resilience tests (e.g. drought).

Several other parameters can be considered when assessing the effects of insect frass and other organic amendments on plants. In any case, one of the most relevant matters is choosing appropriate controls to distinguish the effects of frass from other soil and environmental factors. Control treatments should reflect all variables tested in an experiment, regardless of being conducted under greenhouse or field conditions, and should demonstrate expected results according to other studies in published and validated literature.

Physical and structural properties of soils and growth substrates as affected by insect frass supplementation

The application of organic materials to soils or growth substrates will inherently change their physical properties, and insect frass will be no exception. Several studies have reported chemical and minor physical shifts in soils fertilised with insect frass, such as changes in fertility levels, organic matter content, bulk density, pH and EC (Antoniadis *et al.*, 2023; Ashworth *et al.*, 2025). However, the authors are not aware of any study that directly tackled the topic on soil structure being affected by insect frass, but there is no question that its addition will affect the physical properties and structure of plant growing media. Instead, the questions to be answered are (i) how strongly insect frass affects soil physical properties and (ii) how important the changes in physical properties are for plant performance. If this is known, insect frass, in addition to its potential value for plant nutrition and protection, can also positively influence the physical environment in plant production systems.

Soil physical properties as affected by organic amendments

The physical properties of soils and substrates are determined by their texture (the size distribution of mineral particles) and structure (the secondary 3D arrangement of particles) (Bronick and Lal, 2005). The texture and structure shape the size and geometry of the soil pore space in which water and solutes move. While texture is constant in time and does not change due to tillage or other measures, soil structure is dynamic in time and changes upon any disturbance, including amendments with organic matter, such as insect frass. Consequently, adding organic carbon, such as frass, influences how water (and its solutes) is retained in and released from soils. In general, soil organic carbon in any form contributes to soil structure and, hence, to soil hydraulic properties (Rawls *et al.*, 2003). Such effects have been described for compost (Pamuru *et al.*, 2024; Rivier *et al.*, 2022), and farmyard manure (Fu *et al.*, 2022), but also for biotic influences, such as the activity of termites (Garg *et al.*, 2023) or for the introduction of plant-derived mucilage (Ahmed *et al.*, 2014; Naveed *et al.*, 2019), and mycorrhizal fungi (Pauwels *et al.*, 2023) that only add organic carbon in the per mile range (on a weight basis) to soils. It is therefore possible that the so-called bio-stimulatory potentials can unfold due to changes in the physical/hydraulic environment even when nutritional effects can be neglected (Bitterlich *et al.*, 2020; Gärttling and Schulz, 2022; Houben *et al.*, 2020).

The causes of the response of soil hydraulic properties to organic carbon involve the formation of organo-mineral complexes, particle entanglement and enmeshment by microbes and roots, biopore formation after decomposition of organic matter, the binding of released organic molecules to charged surfaces (clay minerals and polyvalent cations), and the surfactant, often hydrophobic properties of organic matter – see reviewed in Bronick and Lal (2005); Rawls *et al.* (2003); and Rillig and Mummey (2006). From a physical point of view, the expected influences of insect frass application on soil hydraulic properties likely resemble those of compost addition, even though depending on insect species, feed substrate and other variables, the similarity with compost might be reduced. This is because frass is, like compost, derived from organic matter, and its application might come in similar quantities. The C/N ratios of approximately 14 to 15 in insect frass compare well with ratios found in bio-waste compost, and the total N, P, and K contents compare well with those in manure (Gärttling and Schulz, 2022; Houben *et al.*, 2020; Lomonaco *et al.*, 2024). Therefore, to meet the desired nutrient inputs, insect frass has been applied at rates of several tons per hectare (ha) in plant growth studies (Beesigamukama *et al.*, 2020; Houben *et al.*, 2021). Considering the application rates of 5 tons per ha with an organic carbon content of almost 40% – as reported by Houben *et al.* (2020) for mealworm frass – and a soil bulk density of 1.5 g cm^{-3} in the upper 30 cm, the organic matter content in the topsoil would increase by approximately 0.07 to 0.08%. In sandy soils, the global averages of approximately 0.3% organic matter in temperate regions and less than 0.09% organic matter in arid regions have been reported (Yost and Hartemink, 2019). Hence, such organic matter inputs are expected to be non-negligible for soil hydraulics, particularly when applied to coarsely textured soil.

The consequences of altered soil physical properties for plant trials: theoretical considerations

Barring the provision of nutrients, the relevance of frass application to soils for plant resource use is most likely related to the sensitivity of soil hydraulic properties to organic matter (Feifel *et al.*, 2024). For instance, the water retention of sandy soils is most sensitive to increases in soil organic matter, whilst the water retention of finer textured soils is less sensitive (Rawls *et al.*, 2003), which implies a context dependency (Rehan *et al.*, 2024). It depends on the soil or substrate used, and on the dose, whether a meaningful change in soil hydraulic properties due to insect frass application merits consideration. In addition, the influence of added frass might change with time, depending on its mineralisation rate.

Although it is always appropriate to test the usefulness of insect frass in a particular setting against a control treatment with no application, this does not allow extrapolation of the findings to other settings. However, a systematic understanding of the side effects of fertilising with insect frass is desired because nutrient and water availability to plants integrate photosynthesis and growth. If the influence of frass addition on soil hydraulic properties is unknown and cultivation measures are not adjusted, the benefits of nutrient provision could be counteracted by suboptimal irrigation. We continue to illustrate this with theoretical considerations. In Figure 2 we illustrate how insect frass could affect soil hydraulic properties, specifically the soil water retention of a coarsely textured soil or substrate. These generic water retention curves show that frass addition can increase soil water retention due to some of the mechanisms mentioned earlier. While the volumetric soil water content is subject to change upon precipitation, irrigation, and water extraction by any means, the soil water potential is the intrinsic soil property that changes due to frass inputs and determines plant water availability.

Assuming that nutrient leaching by drainage should be prevented (irrigation of water content between FC and PWP) in a planting trial, equal irrigation of potted plants would, according to Figure 2, lead to lower soil water potentials in pots with insect frass than in pots without it. Knowing that plants react to declining water potentials with stomatal closure (Carminati and Javaux, 2020), the even irrigation of all pots in this scenario harbours the risk that the potential benefits of frass-derived nitrogen for photosynthesis are counteracted by lower stomatal conductance. Therefore, the nitrogen use efficiency of plants from insect frass may be unintentionally limited by the mode of irrigation, and the fertilisation potential of insect frass may be underestimated. The risk of such unwanted biases is expected to increase with declining soil moisture because the soil water potential declines disproportionally with a unit change in soil water content (note the log-scale on the *y*-axis in Figure 2). Based on this, we could argue that irrigation with an amount over field capacity would install equality in soil water potentials in wet conditions around –6 kPa after excess water has drained. Albeit this is correct and might lead to a conserved plant physiological response, it could introduce a bias in plant nutrient availability by unbalanced leaching from soils because more water would drain from the frass-free soil until –6 kPa is achieved (see Figure 2). This could lead to an overestimation of the plant nutrition effect of insect frass by unintendedly removing more nutrients from frass-free soil.

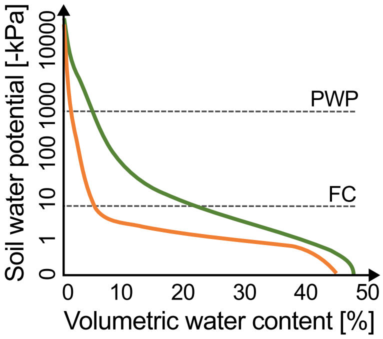

FIGURE 2 Schematic illustration of hypothetical soil water retention in sandy soil without (orange line) and with addition of insect frass (green line). The lower dotted line denotes the field capacity (FC, at –6 kPa), i.e. the threshold at which drainage ceases and water is retained in soils against gravity. The x-values at which the water retention curves cross the FC line indicate the volumetric soil water content after (excess) water from precipitation or irrigation drains. Beyond the FC, soils further dry due to evaporation and/or plant uptake until the permanent wilting point at –1500 kPa (PWP, upper dotted line). Beyond the PWP, soils dry exclusively through evaporation. The soil water potential equals the energy required (by plants) to extract water from the soil (i.e. soil water availability or drought stress). The illustration shows that adding insect frass would lead to a change in plant water availability (water potential) when these soils are irrigated with equal amounts as soils without added frass. In addition, frass addition might also change the amount of plant-available soil water (water content from FC to PWP), e.g. in open pots.

Recommendations for testing insect frass as plant culture component

With a profound understanding of the altered soil hydraulics after application, synergistic or additive effects on plant growth can potentially be achieved with the application of insect frass. More importantly, unintended side effects are avoided. In the following, we propose some measures for planting trials with insect frass that, if applied, can yield better reproducibility of observations and safer assignments of plant growth effects to specific properties of the added insect frass. This section specifically addresses issues related to plant water availability.

A straightforward way to minimise potential side-effects as described in the previous section, is to install equal water potentials in all pots in studies that intend to test the nutritional effects of insect frass amendments on soils. This requires treatment-specific irrigation and a pre-hoc determination of soil water retention. The simplest way to approach the water content related to the soil water holding capacity (at approximately −6 kPa water potential) is to estimate it from the weight differences between the pots filled with substrate after saturating them using a capillary rise method and the weight after subsequent drainage under exclusion of evaporation (Klute, 1986). The weight-based water content between soils with and without insect frass differs and can be used in planting trials as target weights for irrigation. Thus, plants can be maintained under ample moisture conditions, which should largely exclude any growth constraints related to water availability. Depending on the environmental conditions and plant size, the pots can be weighed at time intervals during cultivation to replace the weight loss with water. It is also recommended considering plant growth increments and in particular treatment-specific growth effects. This is because large plants consume water resources from a finite volume faster than small plants and, hence, would grow relatively longer under lower water potentials than smaller plants. For different-sized plants (as can be expected, such as when different dosages of frass are tested in one trial), we would therefore recommend increasing the frequency of irrigation for larger plants rather than replacing more water, for instance, once a day. Increasing the frequency with plant size avoids larger departures from high soil water potential during cultivation.

To test frass amendment effects at specific plant stress levels, such as under drought, the measurement of water retention curves and decision making based on them is desired. This is due to the curvilinear nature of water retention, which is frequently observed in soils or substrates within the plant-available moisture range (Raviv *et al.*, 2005). When unknown, the specific non-linearity of the water content-water potential relationship would introduce errors in prescribed decisions on soil water content that intend to induce equal drought stress across substrates with different frass contents.

3 Assessing the influence of insect frass on plant performance

Similar to other organic amendments, insect frass affects plants in multiple ways, and assessing these effects can be challenging. Frass can be tested under greenhouse conditions using pots as experimental units, applied to soil in open greenhouses (e.g. tunnels), or tested under field conditions, where climatic conditions vary more significantly and impact the obtained results. In addition, frass could be used for elaborating nutrient solutions, applied as fertigation (i.e. delivering nutrients to plants by injecting fertilisers into the irrigation system), or even used in hydroponic cultures. Regardless of how frass is evaluated, one of the most important factors to consider is having adequate controls to infer the correct meaning of the obtained results while enabling replicability of the studies by other parties.

Pot trials

Over the last few years, several studies have evaluated frass as a fertiliser under greenhouse conditions using pots as experimental units. The advantage of conducting pot trials is that they can be conducted under controlled conditions, which can exclude several factors that may affect test results, including temperature, water availability, lighting conditions, and the presence of pathogens, among others. The cultivation of plants in pot trials is often associated with an artificial root-to-shoot ratio, meaning fewer roots compared to natural open-field conditions, factors closely related to a soil's physical characteristics correlated with environmental conditions (Vergani and Graf, 2016). When evaluating the performance of this organic input on plant growth and development, it is important to initially distinguish two ways of using frass: as a soil amendment/growing media that is applied as partial (most commonly) or complete replacement of other commonly used growing media such as peat; or as a fertiliser, in which frass is added to the pots in amounts that are calculated according to the crop's nutrient demand.

As highlighted by Lopes *et al.* (2022), several studies have reported signs of phytotoxicity in plants cultivated with BSF frass (especially when fresh) when it is applied as part of the growing media, with concentrations of >10% of the pot volume being limiting for plant development (Setti *et al.*, 2019). YM frass, which typically shows higher stability than BSF frass (e.g., higher NO_3^-/NH_4^+ ratio, lower self-heating capacity, and higher seed germination), could be applied at higher concentrations, but at the same time, due to its physical nature (dry, fine-grained powder), it is usually not used as a growing medium, but rather as a fertiliser (Poveda, 2021). Pot trials are essential for understanding several aspects of plant growth under certain conditions or fertilisation regimes, under controlled conditions. It is also necessary to study the mechanisms of action of new agricultural inputs, such as the widely unexplored insect frass. Below, we briefly discuss interesting results obtained in experiments conducted in pots and present some recommendations for future studies.

Soils with distinct textures were fertilised with BSF frass and evaluated by Rehan *et al.* (2024) for their fertility levels over time and the productivity of ryegrass (*Lolium multiflorum*) over the course of six months, under semi-controlled conditions. Even though the authors did not evaluate any physical parameters of the tested soils over time (as suggested under 'Recommendations' for testing insect frass as plant culture component), interesting findings were observed, including a higher soil organic matter accumulation and consequent effects on crop productivity in sandy soil in comparison to the other finer-textured soils. Additionally, a positive effect on soil microbiota was detected, measured by the activity of the enzyme dehydrogenase. When evaluating barley (*Hordeum vulgare*) growth fertilised with YM frass in a pot trial, Houben *et al.* (2020) verified similar biomass yield and nutrient accumulation in comparison to a conventional mineral fertiliser, as well as a lower water-soluble phosphorus concentration in the soil when replacing this mineral fertiliser with 50–100% frass. Thus, this might prevent P leaching and increase its fixation in the soil. Both studies demonstrated interesting results that might be translated to field conditions, although such conclusions should be tested accordingly. It is noteworthy that the doses of insect frass applied in pot trials, especially those in which frass is used as an amendment and not as a fertiliser, might sometimes be distant from real application doses, and the translation from pot to field conditions is hampered.

In a study by Asplund *et al.* (2014), six spring wheat varieties were tested for N use efficiency under field and greenhouse conditions. Even though these authors observed a few variety × fertilisation interactions in the field, the results were very similar under both cultivation conditions. Similarly, Loel *et al.* (2014) evaluated the growth and development of sugar beet varieties (*Beta vulgaris* L. subsp. *vulgaris*) under both field and greenhouse conditions. These authors demonstrated that variety and age-specific shifts were observed in the metabolism of plants (including sugar content and CO_2 assimilation), with differences being attributed to the environmental conditions to which the plants were subjected to experimentally. Particularly, the mineralisation rate of N and other nutrients, a major parameter to be considered when evaluating the results obtained in these trials, may be affected by the material that is used for soil amendment. Insect frass contains compounds such as chitin, biogenic amines (e.g. putrescine, cadaverine), small peptides, and phytohormones (e.g. indoleacetic acid, abscisic acid, jasmonic acid) (Green, 2023), which can influence soil-borne processes by affecting microbial activity, organic matter decomposition, and nutrient availability. In this regard, it is noteworthy that proper methods for evaluating N mineralisation must be considered for distinct experimental designs, as thoroughly discussed by Bettiol *et al.* (2022). These considerations are paramount for interpreting results when applying insect frass in pot trials to cultivate distinct plant species and discussing the results from a real-life application perspective.

A great part of the effects observed in pot trials when cultivating plants fertilised with insect frass is mediated by microorganisms. In a pot trial and using *in vitro* tests, Poveda *et al.* (2019) demonstrated microbial-modulated biostimulatory effects of YM frass that were extinguished when the frass was sterilised. Similarly, Praeg and Klammsteiner (2024) demonstrated a great influence on soil microbiota following the application of insect frass from three insect species, and discussed how the shift in the microbial community might affect plant responses. The presence of microbial groups and distinct molecules (e.g. chitin from exuviae, amino acids, small peptides, phytohormones) in insect frass and the mechanisms behind their action still constitute the main knowledge gap on the actions of frass, and unravelling such mechanisms and modes of action should be primarily done in pot trials.

In studies conducted by Barragán-Fonseca *et al.* (2023a,b), it was demonstrated that the presence of insect-derived chitin (exuviae), which when decomposing in the soil, stimulates an increased population of chitinolytic resident microbes, increased the number of flowers, flower reflectance, pollinator attraction, and seed yield in black mustard (*Brassica nigra*). These effects seemed to be determined by a shift in the emission of volatile compounds by the plants, which might have been a microbial-mediated compensation mechanism. Plant defence mechanisms against soil pathogens (e.g. *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum*, among others) were also suggested as being mediated by microorganisms by Arabzadeh *et al.* (2024a). That study evaluated the effect of non-filtered and filtered (using a 0.22 μm filter) BSF frass extracts against the pathogens *in vitro* and revealed that growth inhibition of these plant pathogens was possible when frass extracts were not filtered, i.e. still contained microorganisms. Complementarily, Arabzadeh *et al.* (2024b) assessed the suppressive effect of BSF frass on *F. oxysporum* in a pot trial, confirming the results obtained *in vitro*. Following this perspective, we highlight that evaluating insect frass *in vitro*, followed by a pot trial, and then under field conditions, represents a straightforward pathway to demonstrate the potential effects of insect frass on crop production.

Field trials

As previously discussed, the translation between pot trials into field conditions often does not follow a linear path. First, it is noteworthy that the phytotoxicity that is frequently observed in pot trials using fresh frass, especially from BSFL, seems not to be observed under field conditions, as several studies reported positive growth and no negative effects due to the most probable causes of frass-derived phytotoxicity (e.g. high ammonia volatilisation, phenolic compounds, high EC, self-heating capacity, and other traits suggested by Lopes *et al.* (2024). Beesigamukama *et al.* (2020) applied BSF frass at fertilisation rates of 0–7.5 tons per hectare (equivalent to 0–100 kg N per hectare) and cultivated maize (*Zea mays* L.) in Nairobi, Kenya, during two growing seasons and reported increased nitrogen uptake by the plants in comparison to both a commonly applied organic fertiliser (Safi Organics, Mwea town, Kenya) and urea as a positive control. In the same geographical location, Anyega *et al.* (2021) evaluated the cultivation of three vegetable crops (*Phaseolus vulgaris*, *Solanum lycopersicum*, and *Brassica oleracea* var. *acephala*) in both greenhouse and open-field conditions, demonstrating that even shorter-cycle crops (in relation to maize) developed adequately under BSF frass fertilisation, having improved nutrient use efficiency. When comparing the growth of those crops under both experimental conditions, the authors highlighted the existing differences within the same crop grown in pots or in the field, supporting the hypothesis that some results obtained in one condition are similar to those obtained in another condition, while others are not.

Despite the growing number of studies demonstrating a possible translation and interpretation of results from pot- to field-grown crops, one must be thoughtful in this regard. For instance, when evaluating the growth of crops under environmental stress conditions such as drought, one must recognise that water use, root development, and consequently nutrient uptake and water use productivity, will vary greatly in greenhouse and field conditions, as highlighted by Gheysari *et al.* (2021). It is then possible to hypothesise that the microbial interactions observed in both conditions could also be highly distinct, resulting in a cascade of mechanisms and interactions between multiple variables that would result in differential crop development and productivity. As thoroughly discussed by Nelissen *et al.* (2014), scientists must be highly aware when converting basic knowledge (e.g. results from pot experiments) into applications in crops cultivated under real field conditions, to sustainably support food security worldwide. These authors advocated for the adoption of translational research in crop production, stimulating the establishment of field trials across distinct European climates in order to improve the current knowledge, strongly based on pot trials and small experiments, promoting higher sustainability in agriculture.

Potential of frass as hydroponic substrate and for producing liquid nutrient solutions

In hydroponic cultivation systems, plants are cultivated on both liquid and solid media, with the latter both physically and chemically supporting their growth, while allowing proper root gas exchange and sufficient flow (and/or retention) of the nutrient solution applied. The choice of hydroponic substrate can have a strong impact on the economy and environmental costs of the plant production system, underlining the need to explore new materials for this application (Gruda, 2019; Rogers, 2017). Generally, compost can be used as a full or partial component in hydroponic growing media, providing both physical support and nutritional value (Haghighi *et al.*, 2016; Moschou *et al.*, 2022); however, studies on the application of insect frass are lacking. The potential use of insect frass in hydroponic media is a topic for future research.

In addition to standard hydroponic, pot, and field trials, insect frass has been used as a base material for the production of nutrient solutions, which might have endless applications in distinct ways of producing food. Liquid forms of compost (sometimes referred to as "compost tea") have been used in gardening practice for centuries and are often applied as fertigation (Romano *et al.*, 2022a), and insect frass can be explored as such from the perspective of product development for multiple purposes. However, it is noteworthy that while compost tea seems to be appropriate for hydroponic systems, its disadvantage is its reduced nutrient content (especially magnesium and micronutrients) compared to solid frass (Romano *et al.*, 2022b). Nevertheless, this might be an interesting use for insect frass with potential application in the future.

In a study conducted by Tan *et al.* (2021), BSF frass tea has been prepared by adding 200 ml deionised water to every 0.18 g of total N in the frass, in addition to 0.5% (v/v) molasses to boost microbial growth. The tea was fermented for one day with aeration and then applied to lettuce and pak choi by drenching.

Despite the higher concentrations of water-soluble plant nutrients in frass tea, solid frass conferred better plant growth-promoting effects as it provided durable nutrient release. In aquaponic applications, insect frass should typically be directly applied to the system after heat treatment and ground into a fine powder. It appears that BSF frass might be a promising supplement in aquaponic systems, as it has been demonstrated to increase the nutrient content of water and improve the nutritional value of highly produced crops (sweet potato (*Ipomea batatas*), sweet banana chilli (*Capsicum annuum*) and leafy vegetables such as pak choi (*Brassica rapa*) and lettuce (*Lactuca sativa*)) when used as a "frass tea" (Romano *et al.*, 2022b; Tan *et al.*, 2021). The amount of insect frass powder added to the aquaponic system has to be optimised to achieve a balance between boosting plant growth without causing adverse effects on fish (Abdessan *et al.*, 2025). Therefore, it is important to monitor relevant water quality parameters, such as pH, EC, and the concentration of nutrients, such as nitrates.

The use of solid BSF frass compost as a one-time top dressing for the cultivation of sweet potato in aquaponics seems to be at least equally suited as daily inorganic fertilisation during the vegetative stage, as demonstrated by Romano *et al.* (2022a). In that study, solid frass compost resulted in greener and healthier leaves. The authors also performed a benefit-cost analysis, showing the highest benefit-cost ratio for the use of solid frass compost compared to inorganic fertiliser, as well as frass compost tea. Frass extracts, such as those prepared for seed germination tests, are often phytotoxic, as demonstrated by Lopes *et al.* (2024). However, when diluted, this phytotoxicity is reduced (González-Lara *et al.*, 2024). In this sense, considering the lack of knowledge regarding frass extracts and their potential applications, it is proposed that at first, distinct extracts are investigated to achieve high productivity and quality of the extracts, with special attention to the forms of the nutrients present in the extracts; subsequently, its effects should be tested under different fertilisation regimes and experimental designs.

4 Considerations on the safety of insect frass

As briefly mentioned under Pot trials, insect frass without any post-treatment is, in many cases, phytotoxic, especially when used fresh. We advocate this as a topic of significant relevance, as discussed in this section. When applied to the soil, frass-derived phytotoxicity might result in the inhibition of germination, stunted growth, and generally poor development of the plants (Setti *et al.*, 2019). Considering the very rapid bioconversion time of waste by BSFL, frass organic matter is likely to remain unstable (chemically and biologically) and prone to further decomposition, which has been shown to reduce phytotoxicity (Song *et al.*, 2021). Phytotoxicity can arise from several factors, including high concentrations of soluble salts and electrical conductivity (Huang *et al.*, 2024), N in the form of NH_4^+, which due to the generally high pH of frass, is converted to high concentrations of ammonia (Bohm *et al.*, 2023), and undegraded organic matter causing issues such as high self-heating capacity and continuous decomposition in the soil (Wichuk and McCartney, 2010). Thus, it is recommended that the phytotoxicity level of frass should always be minimally verified, with the following suggested measurements as paramount, according to Lopes *et al.* (2024): seed germination test using sensitive plant species (e.g. watercress or lettuce), pH, NH_4^+ concentration, NH_4^+/NO_3^- ratio, EC and, if feasible, tests for self-heating capacity and respiration.

From a microbiological safety standpoint, the agricultural use of frass obtained from larval bioconversion of contaminated substrates such as manure, faecal sludge, or even contaminated municipal solid waste fractions could facilitate the carryover of pathogens. This risk persists if the frass is not properly treated, although BSFL were previously shown to significantly reduce *Escherichia coli* and *Salmonella enterica* in their substrate, presumably through the excretion of antimicrobial peptides (Lopes *et al.*, 2020). A greenhouse trial showed that after the application of frass containing *E. coli*, the counts of colony forming units in the soil-frass blend were reduced to below the detection limit (Klammsteiner *et al.*, 2020). However, it is not yet clear whether pathogens can reaccumulate in the soil after frass application. Both the reduction of pathogens through larval activity and the diluting effects of its application to soil should not be taken as a guarantee for safe application, and appropriate screening measures have to be implemented. In this sense, insect frass can be subjected to post-treatment (using distinct technologies), to ensure its safety for use.

Frass treatment options

Organic feedstocks suitable for insect-based bioconversion can be highly diverse in composition, similar to the regulations controlling their use. Depending on their origin, the bioburden of these feedstocks can vary significantly depending on their origin. The EU Commission has proposed the use of heat treatment, anaerobic

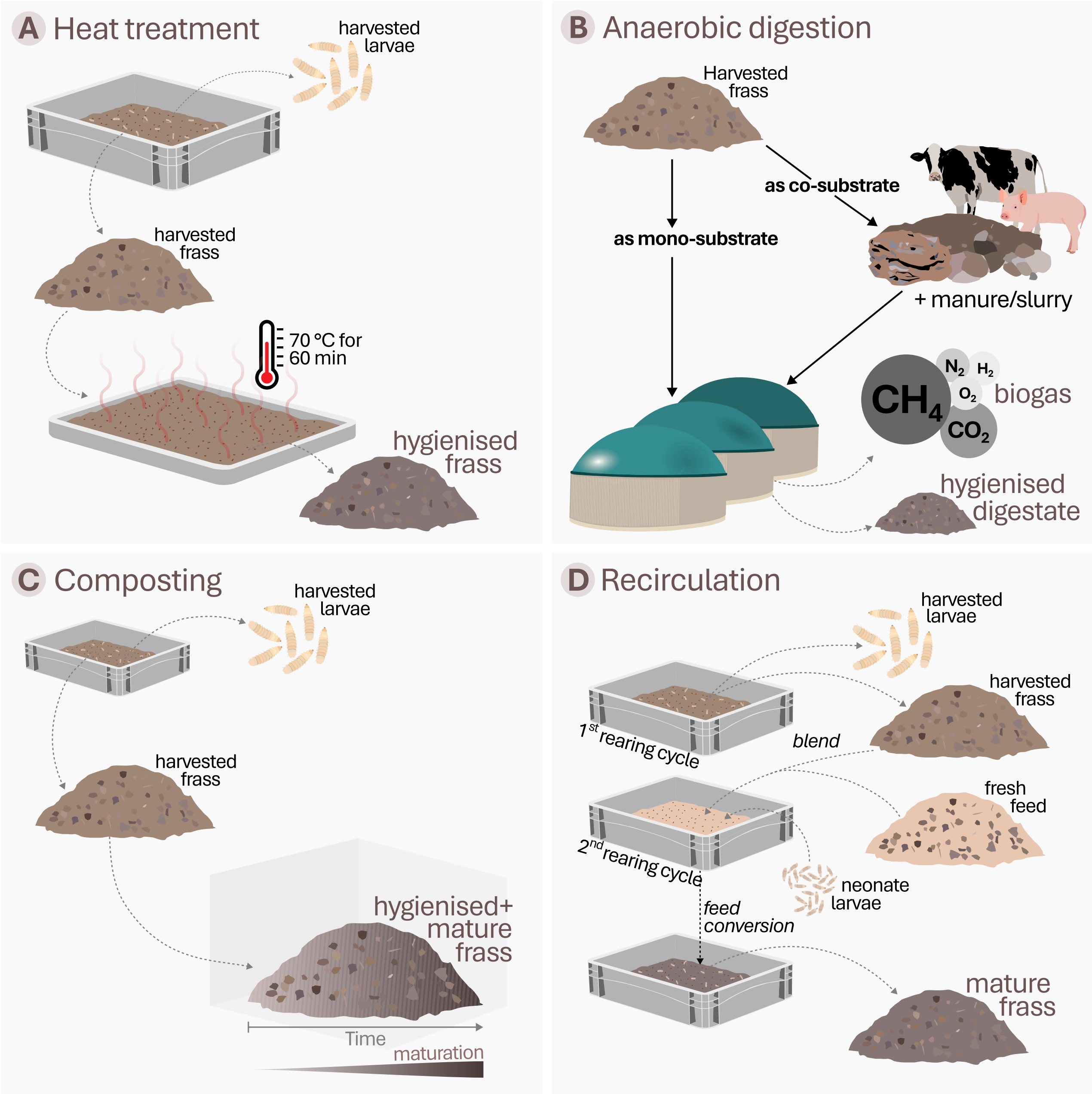

FIGURE 3 Illustration of various treatment options to achieve insect frass hygienisation and/or stabilisation/maturation for safer use. (A) Heat treatment; (B) Anaerobic digestion; (C) Thermophilic composting; (D) Frass recirculation.

digestion, and composting – established methods for treating unprocessed animal manure – to treat insect frass before market placement to ensure its safety (European Commission, 2021) (Figure 3).

Heat treatment

Heat treatment (Figure 3A) of organic fertilisers, including manure, is currently a requirement of many legislations worldwide, such as in the EU (European Commission, 2011), proposed as a means of inactivating pathogenic microorganisms that could pose risks to cropping systems and to the final consumers of the produced crops. Since November 2021, the EU has included insect frass in the aforementioned regulation; thus, this product currently needs to be heat-treated for 1 h at 70 °C before being commercialised (European Commission, 2021). It is noteworthy that such heating does not affect the stability of the compost organic matter, but it might affect other traits.

When heat-treating insect frass from three different species, BSF, YM and Jamaican field crickets (*Gryllus assimilis*), at 70 °C for 1 h, Praeg and Klammsteiner (2024) observed almost no effects on the nutrient composition of those frass types, including C, N, H and S. The only exception was the concentration of NH_4^+ in the BSF frass, which was reduced by approximately 15.9% compared to fresh frass (prior to heat treatment). Similarly, there was no evidence that a heat treatment for 1 h, as the one foreseen in the EU legislation, would reduce the phytotoxicity of frass or other organic composts, and that such reductions in phytotoxic traits can be achieved only at higher temperatures (Cui *et al.*, 2024). Conversely, the adoption of such a heat treatment is known to affect microbial activity in frass, inactivating some groups of bacteria. Van Looveren *et al.* (2022) demonstrated that *Enterobacteriaceae* counts were reduced to below the detection of 10 colony forming units per g of frass after applying the same heat treatment. In addition, *Salmonella* spp. was absent from the tested frass, and reductions in the counts of *Clostridium perfringens* were also reported. Similarly, Praeg and Klammsteiner (2024) reported a significant reduction in the microbial activity of heat-treated frass, with microbial respiration being reduced by a factor of 23, while microbial biomass carbon was reduced to a third of its initial value in fresh frass. Interestingly, regardless of heat treatment, after the YM and cricket frass were applied to the soil, both fresh and heat-treated frass stimulated the soil's microbial activity in a similar way, which demonstrates that even though heat treatment affects frass microbial activity, once it is applied to the soil, its benefits in relation to stimulating soil microbiota are the same.

Considering the similarities that insect frass types have with other manures and the placement of frass in the current national legislations across the world, it is likely that the requirement of heat-treating frass at 70 °C for 1 h will continue to be a part of the requirements for commercialising frass in the future. Therefore, an in-depth understanding of how such heat treatment affects the microbial community of frass from different insects and how this affects the soil-plant system is a major challenge to be addressed in future research. In addition, it is noteworthy that studies should also focus on the effects of this treatment on the production and concentration of bioactive substances in frass (e.g. phytohormones, amino acids, and small peptides), especially considering that their presence and functionality are closely related to microbial groups.

Anaerobic digestion

Anaerobic digestion (Figure 3B) is a microbial process that breaks down organic matter in the absence of oxygen, generating biogas (mainly methane and carbon dioxide) and a nutrient-rich digestate often used as a fertiliser (Ward *et al.*, 2008). It occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where complex organic molecules are sequentially converted into methane, H_2, and CO_2 (Yin and Wu, 2024).

Biomethanisation is a promising but underexplored option for treating frass from insect mass-rearing facilities, offering a way to valorise waste and address frass storage challenges. Similar to its use as fertiliser, the biomethane yield of frass varies significantly depending on the composition of the rearing substrate, with biogas production potentials ranging from 44 m^3/ton to 668 m^3/ton and methane production from 26 m^3/ton and 502 m^3/ton of volatile solids (Magro *et al.*, 2024). The co-digestion of frass with conventional anaerobic digestion substrates may further enhance biogas production through synergistic effects (Elissen *et al.*, 2019). While anaerobic digestion can be operated with low-tech solutions, building biogas plants for efficient and safe waste treatment often requires significant investment (Breitenmoser *et al.*, 2019). Key benefits include the hygienisation of frass, microbiological stabilisation of digestate, generation of exhaust heat that can be reused in rearing and production, and sustainable waste treatment (Magro *et al.*, 2024; Wedwitschka *et al.*, 2023). To date, research primarily focused on the digestion of BSF frass, with some studies on YM, cricket, and silkworm frass as mono-substrates (Bulak *et al.*, 2020; Łochyńska and Frankowski, 2018; Wedwitschka *et al.*, 2023). However, co-digestion with other low-value substrates, such as manure, with subsequent use of the digestate as biofertiliser, remains underexplored, but could enhance process stability and improve economic feasibility (Lamolinara *et al.*, 2022; Magro *et al.*, 2024).

Composting and vermicomposting

Thermophilic composting (Figure 3C) is one of the most well-known methods for organic matter decomposition and stabilisation. Due to its undigested organic matter fraction and typically low C/N ratio (<20), insect frass (especially from BSF larvae) is a good candidate for thermophilic composting (Hénault-Ethier *et al.*, 2024). However, the input of additional materials to a composting pile with frass is necessary, which might result in extra costs and infrastructure. Adopting this method for fresh frass can improve its quality and safety of use, as demonstrated by Song *et al.* (2021) considering that pathogens are inactivated due to thermophilic temperatures and also that nutrients are made more available for plants throughout the composting process. As process temperatures rise, thermophilic and thermotolerant organisms outcompete mesophiles, thereby reducing bacterial diversity, accelerating decomposition, and inhibiting fungal growth, with pathogens being most efficiently suppressed at temperatures of around 55 °C, but higher temperatures exceeding 80 °C favouring undesired odours and microbiological inhibition (Insam *et al.*, 2023). Then, after a proper maturation stage, the resulting material is safer and more adequate for being applied in the soil.

In a study by Wu *et al.* (2023), the authors demonstrated that straw addition followed by composting further increased the degree of humification of the fresh frass (collected directly after bioconversion of waste by BSFL). Subsequent composting has also been proven to significantly inactivate pathogenic bacteria, while favouring cellulose-degrading bacteria and cellulase activities in composted frass fertilisers in that study. This may confer frass composts with the ability to degrade complex organic substances faster than natural composts, thereby improving the composting efficiency. Using metagenome imputation and functional profiling of bacterial communities, the latter authors found that nitrogen-related routes such as N_2 fixation, which are key for maintaining crop productivity, were strengthened during the bioconversion of pig and chicken manure by BSFL and the subsequent composting process. These findings point towards plausible microbial-based effects that may explain the potential usefulness of composted frass fertilisers as soil conditioners and/or plant growth promoters.

Unlike composting, vermicomposting does not involve a thermophilic phase and relies on the combined action of earthworms and microorganisms to accomplish the breakdown of organic matter. Earthworms facilitate the aeration and fragmentation of organic material, enhancing its turnover and increasing the rate of decomposition (Edwards and Arancon, 2022). Dulaurent *et al.* (2020) observed in a pot trial that earthworm activity was accompanied by increased nutrient concentration in barley in the presence of mealworm frass. This points towards a synergistic effect between earthworms and insect frass on soil fertility, favouring short-term recycling of nutrients from frass. Bearing this in mind, the subsequent processing of fresh frass through vermicomposting also appears to be a viable option for increasing its stability and safety of use. However, the feasibility of vermicomposting as a posterior treatment for frass still requires further validation.

Frass recirculation

A novel method for frass stabilisation and improved safety was recently proposed by Lopes *et al.* (2024), in which fresh frass was recirculated back into the bioconversion process (Figure 3D), being provided to the larvae as part of their feed substrate. This may be interpreted as a process similar to vermicomposting. Fresh frass still contains a high proportion of undigested organic matter, and young larvae can still benefit from its nutrients, as it is known to contain most of its N in organic form, and the minimal fraction of inorganic N is predominantly in the form of N-NH_4^+ (Lopes *et al.*, 2022; Setti *et al.*, 2019). In that study by Lopes *et al.* (2024), the authors evaluated frass recirculation in a food waste bioconversion setup, highlighting that this process might not be as effective for other waste substrates, such as high-fibre components like spent grains or plant waste (e.g. plant trimmings after harvesting the fruits such as cucumbers or tomatoes). Because post-consumer food waste is a highly diverse substrate, it appears that frass recirculation leads to better results in terms of larval yield per box, waste-to-biomass bioconversion efficiency, and frass stability and maturity degree (including higher stability of organic matter, lower self-heating capacity, reduced C/N ratio, increased NO_3^-/NH_4^+ ratio, and lower NH_3 and CO_2 emissions from frass). Therefore, this novel method is proposed as a way of increasing frass quality, and future studies should evaluate the long-term effects of frass recirculation within an industrial setting, as well as the effects of frass stabilised by this method in the soil and plants. It is noteworthy that this technology should also be tested for other insect species in addition to BSF.

5 Plant protection potential of insect frass

Similar to other organic amendments and fertilisers, the benefits of insect frass for agriculture appear to go beyond the simple provision of nutrients. Due to the action of biostimulants including beneficial microorganisms, amino acids, small peptides, and phytohormones, frass has been demonstrated to have additional effects on plant growth. Current evidence has demonstrated the potential antimicrobial activity of insect frass, against several pathogens, including soil-borne microorganisms and plant pathogens. Unravelling these potential benefits could stimulate the development of a new research area for the insect industry, increasing its potential beneficial actions on the sustainability and circularity of food systems.

Antimicrobial activity of insect frass

It is evident that BSF and YM modulate the microbial community of the substrate on which they feed on (Kuznetsova *et al.*, 2022; Osimani *et al.*, 2018). This modulation could be caused by indirect effects, including the depletion of nutrients that enhance microbial competition for nutrients (Ghoul and Mitri, 2016), thus increasing the temperature of the substrate (Yakti *et al.*, 2022) or the excretion of compounds with antimicrobial activity (Müller *et al.*, 2017). The presence of these compounds in the produced insect biomass has high potential for application in agriculture and pharmacology.

Antimicrobial resistance (AMR) has become a public health priority worldwide representing a threat under the One Health framework (White and Hughes, 2019). To overcome the rise of AMR, there has been increasing interest in exploring alternative natural niches, such as insects, as potential sources of active molecules with antibiotic potential (Manniello *et al.*, 2021; Mantravadi *et al.*, 2019; Van Moll *et al.*, 2021). In addition to being one of the most abundant groups of living organisms in terms of both species' richness and abundance (Jankielsohn, 2018), insects are highly adaptive to all types of organic matter (Eggleton, 2020). Moreover, they are capable of displaying a rapid and highly effective immune response against pathogens, making them remarkably resilient to microbial infections (Sheehan *et al.*, 2020). Beyond their exoskeleton barrier, insects display a wide range of antimicrobial defence tactics, including melanisation, phagocytosis, and production of antimicrobial peptides (AMPs).

Antimicrobial peptides are a class of small peptides (usually less than 10 kDa) that have some advantages over conventional antibiotics, such as their fast killing time and ability to act on multiple targets simultaneously (Erdem Büyükkiraz and Kesmen, 2022; Fahmy *et al.*, 2024; Zharkova *et al.*, 2019). Currently, 364 AMPs of insect origin have been identified (Wang *et al.*, 2022), and the BSF has the second largest number of AMP encoding genes, as elucidated by Vogel *et al.* (2018) and Moretta *et al.* (2020). Among them, defensins (44%), cecropins and lysozymes (18%), attacins (7%), and other AMPs (<5%) have been identified in BSF larval and adult transcriptomes (Moretta *et al.*, 2020). For BSF, both feeding and oviposition occur on substrates with a high microbial load, such as animal manure or decomposing plant matter, which may have triggered the diversification and expansion of its AMP repertoire. Exposure to pathogens in their environmental niche constitutes one of the main driving factors responsible for the evolutionary adaptation of an insect's AMP repertoire (Zhou *et al.*, 2024). Although the exact mechanisms by which insect AMPs exert their antimicrobial activities require further investigation (Beesigamukama *et al.*, 2023), the secretion of AMPs by BSFL could contribute to the hygienisation of the feeding substrates by lowering their pathogenic load during the rearing process, and hence, in the insect biomass and the leftover frass (Mudalungu *et al.*, 2021; Surendra *et al.*, 2020). In sum, considering the potential of insect-derived AMPs to perform multiple antibacterial, antifungal and antiviral activities, their applicability as additives in agriculture, food, and feed industries appears as a powerful and promising alternative (Dho *et al.*, 2023; Nazeer *et al.*, 2021; Wang *et al.*, 2016), that could be of use in insect farming. However, the stability of AMPs requires further validation when employed as usable additives (Hwang *et al.*, 2022).

Apart from their own immune system, insects can cope with environmental threats by making use of the molecular defences provided by microbial symbionts that live within their gut, or in specialised cells called bacteriocytes, as well as on the insect′s exterior surface or in their surrounding habitat, including their nest or food provisions (Van Arnam *et al.*, 2018). In particular, this defensive strategy based on insect-microbe symbiosis can help the insect combat colonisation by entomopathogens or other invaders competing for nutrients and space (Pickard *et al.*, 2017). Supporting this, there is increasing evidence regarding the antimicrobial capacity of insect-associated bacteria and fungi (Chevrette *et al.*, 2019; Correa *et al.*, 2019; Gorrens *et al.*, 2021b; Van Arnam *et al.*, 2018; Varotto Boccazzi *et al.*, 2017). For instance, these latter authors demonstrated a significant *in vitro* inhibitory effect of *Trichosporon* spp. isolates, which have already been found in industrially reared insects such as the BSF, against the foodborne pathogenic bacterium *Staphylococcus aureus*. Moreover, they established a strain collection of dominant aerobic bacteria from the gut of BSFL reared on chicken feed and fibre-rich substrates (Gorrens *et al.*, 2021a). As a consequence of larval growth and substrate consumption, it can be assumed that microbes with antagonistic activities or bioactive compounds derived from these microbes or insects are present in the frass. In a recent study, Suraporn *et al.* (2024) isolated lactic acid bacteria with probiotic qualities and antagonistic activity against major foodborne pathogens from silkworm (*Bombyx mori*) larval faeces.

The antimicrobial activity of frass might also rely on the presence of non-gene-encoded compounds, such as lipids and chitin (Koutsos *et al*., 2022). On the one hand, insect lipids are promising candidates as antimicrobial agents owing to their ability to potentially damage and destabilise the bacterial cell membrane (Borrelli *et al*., 2021; Franco *et al*., 2024; Marusich *et al*., 2020). These latter authors demonstrated an effective antimicrobial inhibition against *Micrococcus flavus* and *E. coli* cultures, used as Gram-positive and Gram-negative reference strains, by lipids extracted from BSFL. The degree of this effect varied depending on the rearing substrate which implies that the BSFL fatty acid (FA) profile could be modulated through different diet administrations. However, as pointed out by Franco *et al*. (2024), additional factors, including the stage of larval development and rearing conditions, must also be considered to fully understand the biochemical pathways involved in FA synthesis in order to pave the way towards the use of insect lipids as antibacterial agents.

Chitin is also of interest because it is one of the most abundant natural polysaccharides, second only to cellulose, and a major structural component of the insect exoskeleton (Koutsos *et al*., 2022). In BSF, chitin content has been reported for all developmental stages (Wang *et al*., 2020), as well as in the sheddings left after pupal emergence and in the frass (Jasso *et al*., 2024). Chitosan, the fully deacetylated form of chitin, is typically the focus of a wide range of applications owing to its high solubility and nontoxicity. The benefits of chitin and chitosan rely on their properties (Jia *et al*., 2024), such as those of antimicrobial agents (Guarnieri *et al*., 2022; Hadj Saadoun *et al*., 2022), plant growth promoters, and elicitors of plant resistance against biotic and abiotic stresses (Li *et al*., 2020). Particularly the extraction of chitosan from BSFL cuticles using enzymatic instead of thermochemical approaches has the potential to enhance the value chain of insect mass rearing while generating a highly efficient fungicidal product capable of inhibiting plant pathogens (Escobar Rodríguez *et al*., 2025). Guarnieri *et al*. (2022) reported that chitosan extracted from different development stages of the BSF (larvae, pupal exuviae and dead adults) had antibacterial effects against *E. coli* and *M. flavus*. Likewise, Lagat *et al*. (2021) found that application of 2–5% chitosan from BSF pupal exuviae led to significant growth inhibition of resistant strains of bacteria, especially *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *S. aureus* and *Candida albicans*. Antibacterial activity has also been demonstrated in chitosan isolated from YM, leading to growth inhibition in *Bacillus cereus*, *Listeria monocytogenes*, *E. coli* and *S. aureus* (Shin *et al*., 2019).

Antimicrobial assays using insect frass: direct antibiosis and induced systemic resistance

Numerous studies have shown the efficiency of organic amendments such as compost and vermicompost, as well as their liquid derivatives, known as compost and vermicompost teas, in inhibiting the growth or inactivation of soil-borne plant pathogens caused by pathogenic fungi, including *Pythium*, *Rhizoctonia*, *Verticillium*, and *Fusarium* species (Jiang *et al*., 2023; Rehman *et al*., 2023). A wide variety of bacterial species with strong antifungal activity (*Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Streptomyces* spp.) have been identified in vermicomposts of different origins (De Corato, 2020; Vambe *et al*., 2023). Recent evidence from *in vitro* experiments has also shown that frass obtained from BSFL has antifungal/anti-oomycete activity against the plant pathogens *Alternaria solani*, *Fusarium oxysporum*, *Phytophthora capsici*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and to a lesser extent, *Rhizoctonia solani* (Arabzadeh *et al*., 2022, 2023, 2024a,b). From a soil perspective, (Setti *et al*., 2019) also reported that BSF frass was unable to suppress *R. solani* damping-off disease in garden cress, whereas it was more effective with regard to *S. minor*.

To assess antimicrobial activity, Arabzadeh *et al*. (2022) employed a dual-culture overlay assay by mixing different concentrations of fresh frass, ranging from 0% (referred to as the control) to 1% with sterile physiological saline solution. After precipitation, the frass extracts (filtered and non-filtered) were poured into Petri dishes and incubated in the dark for 48 h to allow microorganisms to grow. For each phytopathogen, 10-mm diameter potato dextrose agar covered with actively growing mycelium was then placed in the centre of the agar plate and incubated at room temperature in the dark, for seven days. The Percentage Inhibition of Radial Growth (PIRG, %) was estimated by measuring the colony radii of the tested fungal pathogens and computing them into the equation of PIRG (%) = $((R_1–R_2)/R_1) \times 100$, where R_1 and R_2 correspond to the control and frass treatment plates, respectively. Mycelial radial growth was calculated as the average of four perpendicular radii from the inoculation point towards the direction of the antagonist colony (frass treatment) or the supposed area (control).

By applying whole-genome sequencing, Arabzadeh *et al*. (2023) identified *Bacillus velezensis*, a bacterium used as a biocontrol agent, as the only bacterium that showed inhibition against the tested phytopathogens in both the initial diet and the resulting BSF frass. Interestingly, these authors stated that such inhibitory effects varied with the type of feedstock, and, except for *B. cinerea*, it was neutralised following filtration of the frass extracts. Similarly, sterilisation of compost teas was previously found to cause complete or partial loss of mycelial growth inhibition of several phytopathogens (Dionne *et al.*, 2012). The sterilisation or filtration of an environmental sample is expected to affect its microbial load and community composition (Lees *et al.*, 2018), thereby influencing its effectiveness in disease suppression. Although it is difficult to discern the exact suppression mechanism, it is plausible that the microbiota present in the insect frass may compete for space, nutrients, or other available resources against the pathogen, as has been reported for vermicompost (De Corato, 2020).

Other plausible strategies could rely on the production of antimicrobial compounds (Vambe *et al.*, 2023), and/or the release of defence substances such as phenolic compounds or siderophores that may confer systemic resistance to the plant (Beesigamukama *et al.*, 2023). Plant defence against phytopathogens can be characterised by three distinct steps, starting with the recognition of pathogens, followed by signal transduction events that lead to counter-systematic plant defence responses (reviewed in Vlot *et al.* (2021)). Among the microbe-derived elicitors that can prime plant defences, chitin has been known for centuries to induce the expression of pathogenesis-related (PR) genes in plants (Hedrick *et al.*, 1988). Recent advancements have shed light on the molecular mechanisms by which chitin and chitosan induce plant defence and promote plant growth, which can guide the efficient production of chitin-based plant stimulants (Li *et al.*, 2020b).

Given that exoskeleton fragments of insects can be found in frass, it can be assumed that they undergo degradation processes that generate chitin-elicitor molecules in the soil. This degradation may require microbial activity for the release of these molecules (Nayak *et al.*, 2020). A study by (Blakstad *et al.*, 2023) showed that a frass-soil mixture incubated for two weeks was able to prime the defence of *Arabidopsis thaliana*, particularly when combined with *B. cinerea* infection. The induced systemic resistance was confirmed by observing higher mRNA accumulation of defence genes mainly associated with the jasmonic acid signalling pathway. A growth promotion effect has been observed in tomato plants when their substrate was mixed with 0.1% house cricket (*Acheta domesticus*) frass, while an inclusion rate of 0.5% and 1% displayed elicitor effects with lower growth and high activity of antioxidant enzymes, such as catalase and phenylalanine ammonium lyase, which are characteristic of plant stress elevation (Ferruzca-Campos *et al.*, 2023).

In addition to the antimicrobial activity of frass and its microbiota, nematicidal effects against the root-knot nematode (*Meloidogyne incognita*) has been observed. Extracts from BSF frass were prepared by mixing it with exuviae to enrich the chitin content, and effective microorganisms (such as *Lactobacillus* spp. and yeasts) and molasses were added to the mixture which was fermented for six weeks with daily stirring. A 10% suspension was applied directly to root-knot nematode eggs or juveniles, and the emerging juveniles and survival of juveniles were quantified. The prepared frass suspension led to juvenile paralysis and mortality (Kisaakye *et al.*, 2024).

The frass and exuviae of BSF have been used as soil amendments in a greenhouse experiment in which Brussel sprouts were grown for five weeks before being infected with cabbage root fly larvae (*Delia radicum*). Soil amended with BSF frass and exuviae suppressed the growth of pest larvae and reduced the population, which was not observed when using the same residual streams from house crickets and YM (Wantulla *et al.*, 2023). The authors indicated that the most likely mechanism for suppressing cabbage root fly larvae is the modulation of the soil microbiome by the frass and chitin of BSF. In addition to chitin, studies on plant-pest interactions have revealed the presence of other compounds in the frass of insect pests (e.g. proteins), which can induce or suppress plant immune responses (Ray *et al.*, 2016). A better understanding of the molecular triggers in frass that enhance plant defence can enable the optimal downstream processing of insect frass in order to potentially develop effective frass-based plant defence promoters.

6 Future perspectives for insect frass research

Insect frass is a highly variable product influenced by several factors, including the insect species that generated it, the feed substrate provided, and the post-treatment methods applied. It has been proven to function similarly to other organic amendments, such as compost and vermicompost, by boosting soil fertility and resilience, promoting plant growth, and improving resistance to biotic and abiotic stressors. Beyond its role as a source of plant nutrients, frass contains different biostimulants, including microorganisms, amino acids, and phytohormones, exerting metabolic effects in distinct plant species. However, the underlying mechanisms remain a critical knowledge gap. It is important to note that frass is not a 'complete fertiliser' from a nutrient perspective, as it frequently lacks essential nutrients. In addition, positive effects observed in certain experimental designs may not be linearly translated to other realities, highlighting the need for translational research to cover these gaps.

We highlight topics that should be focused on in future frass-related research: (i) frass should be blended with other sources of nutrients and bioactive compounds, aiming to develop tailored fertilisers for specific crops; (ii) frass composition should always be disclosed considering at least the essential and highly recommended parameters described in Section 2; (iii) the mechanisms behind frass effects should be investigated, considering the presence of microorganisms, chitin, and phytohormones; (iv) stabilisation of insect frass, especially from BSFL, should be carried out before applying it, aiming at improving its safety and enabling the use of frass as a growing medium for short-cycle crops or even as hydroponic media; (v) the interactions between distinct factors related to frass (e.g. microorganisms, volatile compound emissions by plants, hormones, among others) should be assessed. Attending these and other critical topics outlined in this chapter will enable the widespread inclusion of insect frass in global food systems, supported by rigorous, reproducible, and high-quality research efforts.

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Conflict of interest

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.

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