Opportunities and challenges in upcycling agri-food byproducts to generate insect manure (frass): a literature review

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ABSTRACT (250 WORDS)

A range of issues related to sustainability in the agrifood industry have spurred interest in mass production of insects as human food and animal feed alternatives. This rapidly evolving sector address several challenges, including the management of food waste /agrifood by-products and the production of alternative animal proteins demonstrating low environmental impacts that improve sector circularity. The mass production of insects on agrifood processing wastes/ by-products represents an opportunity to address these challenges. While the production of insects offers prospects for sustainable protein production; a major side stream is the production of frass or larval excrement including uneaten feed and chitin-rich exuviae (derived from multiple larval moults). The production of each tonne of edible insects generates 2 to 4 tonnes of frass with an interesting potential in agriculture versus traditional organic amendments (compost, manure, biochar). This review aims to demonstrate the characteristics of frass, its common harvest and conditioning methods, its optimal application rates for planting crops, the mechanisms by which it can protect plants against biotic and abiotic stresses and demystify the risks and potential associated with its application in agriculture. The characteristics of frass are compared with those of conventional fertilizers or other. This report also compiles the Canadian, US and European regulatory frameworks as a novel

plant fertilizer and aims to pave the way for future research necessary for its valorization in plant production.

Key Words

Frass, Edible insects, Insect Manure, Organic Fertilizer, Soil Amendment

1.0 INTRODUCTION

The growing international popularity of edible insects has spurred the growth of this sector around the world (van Huis 2022, Larouche, Campbell et al. 2023). This growth stems from a concern to reduce the ecological footprint and increase the circularity of food systems in the context of climate change and increasing world population (van Huis, Itterbeeck et al. 2013). Insect production systems are an excellent example of sustainable food and feed production. Indeed, the proteins it produces have a very low environmental footprint notably because insects have a very efficient food conversion efficacy (Cortes Ortiz, Ruiz et al. 2016). In addition, insect production could also improve our approaches to managing waste organic residues. Indeed, it is well known that the treatment of organic residues – mainly through landfilling up until recently (Recyc-Québec 2023) harms the environment in many ways (GHG emissions, groundwater pollution, etc.) (El-Fadel, Findikakis and Leckie 1997). In addition, it represents More contemporary methods of recovering residual materials which are gaining traction around the world (centralized composting, industrial biomethanisation) reduce some of the negative impacts compared to landfilling, although these approaches do not fully capture the potential nutrient values coming from food waste. In this context, the valorization of organic residues using insects offers the advantage of directly reintegrating the nutrients into shorter food circuits, rather than approaches downcycling organic resources (composting) or production of biogas intended to be combusted, thus releasing GHG instead of sequestering them in organic matrices. Entotechnologies are nature inspired technologies based on exploiting the ecological services of insects, keystone organic waste recyclers in nature, to develop innovative solutions to current challenges in society including waste management and food production. This approach is often applied to the upcycling or biotransformation of waste organic residues (Hénault-Éthier, Dussault et al. 2017). As a way of example, frass produced by black soldier flies (BSF) can help to recapture N and P from food systems, allowing their reuse as fertilizers, thereby reducing the environmental impacts of conventional fertilizer use (Schmitt and de Vries 2020).

To fully realize the benefits of insect-based organic waste upcycling, not only is it important to integrate insect nutrients into human food and animal feed but also, it is essential to valorize the side streams of this bioprocess. Edible insect farms are booming in Quebec (Hénault-Éthier, Dussault et al. 2017) and around the world (Larouche, Campbell et al. 2023) and as a consequence, the quantities of manure (frass) are also expanding (International Platform for Insects as Food and Feed (IPIFF) 2019). Insects bio convert a fraction of the organic inputs provided to them, resulting in important volumetric reductions (e.g. 54% of input volume reduction for BSF (Kalová and Borkovcová 2013).

The International Platform of Insects for Food and Feed (IPIFF), which brings together producers of insects intended for human and animal food in Europe, estimates that the current production of insect manure already exceeds several thousand tonnes annually, and by 2025 it could reach 1.5 million tonnes per year (International Platform for Insects as Food and Feed (IPIFF) 2019). In North America, estimate of annual frass production and trend projections are just emerging. The production of each tonne of edible insects generates 2 to 4 tonnes of frass (Hénault-Ethier 2020). Other authors suggest that frass production may be as high as 40 times the biomass produced (Poveda 2021). In 2023, the North American Coalition for Insect Agriculture (NACIA) surveyed its members on their frass production. Amongst 16 respondents, 7 commercialized frass in the USA, 5 in Canada, 1 in Mexico and 5 across the three countries (Aaron Hobbs, personal communications, August 2023). While in 2023 most (37%) produced ≤10 tons and 25% produced more than 10k tonnes per year on a dried basis. Production forecast over the next decade predicts a sharp rise in North American production, culminating >5 million tonnes (dry weight) per year by 2033 (Figure 1).

It is therefore necessary to consider the producers of insect above all as producers of an organic by-product which should be valued. Frass is a high-quality by-product, as source-separated organic materials used as inputs for insect rearing typically have minimal contamination such as foreign bodies and metals. Currently, agriculture, horticulture and retail appear as the primary markets for frass applications as a fertilizer (Chavez and Uchanski 2021)(Chavez 2021, Larouche 2022). However, applications of frass as a potential ingredient in animal feed (fish (Yildirim-Aksoy, Eljack et al. 2020, Yildirim-Aksoy, Eljack and Beck 2020, Banavar, Amirkolaei et al. 2022), shrimp (Yildirim-Aksoy, Eljack et al. 2022), rabbits (Radwan, Maggiolino et al. 2023)), and/or secondary processing (feedstock for biomethanisation (Wedwitschka, Gallegos Ibanez and Jáquez 2023), for composting (Song, Ee et al. 2021), or for biochar production (Yang, Chen et al. 2019, Yang, Zhang et al. 2019)) are emerging. Considering the strong growth of the edible insect sector, and the high ratio of frass production per tonne of edible insects harvested, it is therefore important to find value-added opportunities to consolidate the economic model of current and future insect productions.

However, in Canada, producers are currently facing regulatory uncertainty at the provincial and federal levels. The same is true in the United States, while Europe has defined a stricter regulatory approach. At the federal level, new regulations from the Canadian Food Inspection Agency (CFIA) now exclude frass from the definition of manure and requires its registration (Canadian Food Inspection Agency (CFIA) 2020). Insect producers in several European countries face the same exclusion for frass (International Platform for Insects as Food and Feed (IPIFF) 2019). However, biologically, frass really is a type of manure, and shares similar agronomic characteristics as other manures (Hénault-Ethier and Warburton 2023 (in press)). This review aims to demonstrate the characteristics of frass, identify its risks and benefits in applications as a fertilizer and soil conditioner to facilitate its supervision and its marketing.

2.0 MATERIAL AND METHODS

References on frass manufacture, harvest, characterization, use as fertilizer, and regulatory framework were selected for this literature review using databases (Google Scholar and Science Direct). The keywords used include different permutations of French or English terms, singular or plural (frass, fumier d’insectes, insect manure, ténébrions meuniers, mealworm, *Tenebrio molitor*, grillon, cricket, *Acheta domestica*, mouche soldat noire (MSN), black soldier fly (BSF), *Hermetia illucens*, fertilisant, fertilizer, insect agriculture, insect farm).

An inventory of North American frass producers was created by searching Google in both French and English using permutations of insect common and Latin names (see above), product denomination (insect manure, frass), target regions (Canada, USA) and marketing keywords ($, buy, sale, sell, etc.). As the sector is relatively new and constantly evolving, companies and articles have also been identified through discussions with collaborators following the snowball sampling method (Miller 2003)). The networks of the Vitrine Entotechnologique, the Association of Insect Producers and Processors of Quebec (AÉTIQ), the Ministry of Agriculture, Fisheries and Food (MAPAQ) and the North American Coalition for Insect Agriculture (NACIA) were used.

The value of frass was estimated in CAD/Kg, using the retail prices of frass for the formats closest to one kilogram available from each supplier. This method was intended to avoid the confounding effect of declining market value when sales go from small formats (retail) to larger sales volumes (wholesale). The US$-CAD$ conversion rate used is 1.36 based on the rate of August 28th 2023 (Bank of Canada 2023). The average prices of frass per insect species were plotted (non normal distribution) and compared with a *Chi* square (JMP Pro, Version 17, SAS, USA).

A total of 237 references were considered pertinent to the current review (August 2023). 176 were obtained from scientific journals, 21 from governmental sources, 31 from industry reports or websites, and 10 were obtained from personal communication. Due to the rapid evolution of the market, and despite our best efforts, this review may not be exhaustive.

3.0 RESULTS AND DISCUSSION

3.1 Benefits and risks of upcycling agrifood side streams with insects

It is critical to identify the challenges associated with agri-food by-products upcycling with edible insects in order to correctly manage the risk they pose to the environment or to human health during the upcycling process or use of the frass byproducts, notably in agriculture. Broad categories of risk for frass include biological, chemical, and physical risks (Table 1). Human exposure may be occupational or be present in the general population that consume insect products. The following section provides additional details on categories of risk, target populations, and regulatory risk management strategies.

3.1.1 Biological risks

Organic waste upcycling with insects implies potential biological risks which may extend to the ingestion of food crops fertilized with frass. Thus, heat treatment was prescribed by European legislation (70 °C for 60 min). Granulation of frass using heat and pressure creates a densified product with improved safety, but this sterilization may decrease biological functionality (Schmitt and de Vries 2020). Studies have shown that a heat treatment of BSF frass may be efficient to eradicate foodborne pathogens like *Salmonella (Lalander, Fidjeland et al. 2015, Van Looveren, Vandeweyer and Van Campenhout 2021)*.

Others confirm reduction in *Clostridium perfringens* or Enterobacteriaceae, but that despite reduction in total bacterial counts, not all endospore forming bacteria were reduced (Van Looveren, Vandeweyer and Van Campenhout 2021). Studies in mealworms suggest that *Tenebrio molitor*'s gut or excreted frass may not be viable environments for the multiplication or the survival of *E. coli*, perhaps due to microbial interaction with *Lactobacillus* (Cesaro, Mannozzi et al. 2022). More studies on facilitated identification and biological inactivation of potential pathogens in insect farming systems are required to tailor regulatory guidelines.

3.1.2 Chemical risks

More studies are required on the capacity of insects to biodegrade different organic contaminants. Mealworms are known for their ability to degrade some organic contaminants such as polystyrene and polypropylene (Yang, Ding et al. 2021). Biochar made from mealworm frass may adsorb certain pesticides like neonicotinoids (Shi, Wang et al. 2022). Many pesticide studies with insect frass focused on their capacity reduce the use of pesticides through attempts to repel pests from plant production (Sørsdal 2021) or through disease suppressiveness mechanisms. However, few studies measured the capacity of insects to favour biodegradation of pesticides present in organic waste (Lalander, Senecal et al. 2016). A review suggests that organic contaminants like dioxins, PCBs, PAHs, pesticides, pharmaceuticals and mycotoxins are unlikely to accumulate in BSF (Gold, Tomberlin et al. 2018).

However, inorganic contaminants in organic waste may pose the risk of bioaccumulation. Insects like BSF have been shown to bioaccumulate heavy metals and arsenic (Diener, Zurbrügg and Tockner 2015, Mutanekelwa 2022), confirming the risks of feeding heavy metal contaminated organic waste to edible insects (Van der Fels-Klerx, Camenzuli et al. 2016). Nevertheless, the balance between reduction of heavy metal in the substrate (following bioaccumulation in insects) or concentration of heavy metals in the frass (due to substrate mass reduction during biodegradation) needs to be further studied.

3.1.3 Physical risks

Fragments of glass and plastics are increasingly regulated in fertilizing residual material because of the risk they pose to handlers (cuts) or the environment (plastic and microplastic contamination of soil and water bodies). Plastic contamination of organic waste is common especially in mechanically depackaged food waste, but it needs further characterisation (Porterfield, Hobson et al. 2023). The risks of microplastic transfer to agricultural lands following organic waste management with composting and biomethanisation has been reviewed (Porterfield, Hobson et al. 2023), and documentation of this topic upon treatment of organic waste with insects is just emerging. Microplastics have been shown to be ingested and excreted in frass by insects like crickets (Ritchie, Cheslock et al. 2022). BSF has also been shown to ingest microplastics in a way that is proportional to their mouth opening dimension (Lievens, Vervoort et al. 2023). Therefore, plastic contaminated organic waste fed to insects may lead to frass contamination.

3.1.4 Occupational risks

Occupational exposure in farm workers has long been a topic of interest, often drawing links between the presence of insects (as pests in stored grains) and sensitization or allergic reaction of workers (Jeebhay, Baatjies and Lopata 2005). The multiple interactions between insects and occupational health has recently been reviewed (Belluco, Bertola et al. 2023). Frass may be found in grain dust where insect pests are present. Exposure to grain dust has been related to various allergic reactions to grain mites and the presence of *Tenebrio molitor* has been associated with asthma (Jeebhay, Baatjies and Lopata 2005). Occupational exposure to insects has been related to allergies in multiple environment, including amongst edible insect farm workers (Ganseman, Gouwy et al. 2022). Exposure to dust during application of frass may be reduced by creating a pelleted product, relying on high heat and pressure, or pan coating, implying the creation of multilayered pellets at low heat, with or without additives (Caron-Garant, Massenet et al. 2022). While granulation may reduce microbiological risks, pan coating would be preferred where permissible to preserve beneficial microorganisms.

The specific risks associated to upcycling organic waste in insect farms may compound occupational risks for insect farm workers. For example, exposure to atmospheric pathogenic fungal spores like *Fusarium* sp are common in composting facilities (Domingo and Nadal 2009) where moist organic wastes are treated in a process which resembles upcycling with BSF (Domingo and Nadal 2009). The fact that compost packaging areas may contain as much or more fungal spores than the composting platform itself (Gao, Yu et al. 2022) warrants care for frass packaging facilities in organic waste upcycling farms relying on insects. Concerns over exposure of farm workers upcycling organic waste, as well as awareness of appropriate risk mitigation methods, is starting to rise in the edible insect communities (Normandin and Hénault-Ethier 2022).

3.1.5 Risk categories for regulatory purposes

Most of the risk posed by frass produced through upcycling organic waste with insects is similar in nature to that posed by different organic waste (food by-products, manure, etc.) and has already been addressed through regulation targeting other organic waste management techniques such as composting of biomethanisation (ex. (Ministère du développement durable de l'environnement et de la lutte contre les changements climatiques (MDDELCC) 2018) or marketing of fertilizing products (ex. (Canada 1985). A proposed relative risk classification relies on the type of substrate used to feed the insects (Hénault-Ethier and Warburton 2023 (in press)). In summary, frass produced on virgin grain commodities or pre-consumer food waste is considered to have a very low risk for most categories (biological, chemical, physical). Frass produced from post-consumer organic waste, animal manure, or sludges and digestate ranges from low to high risk depending on the categories. Frass which is further composted is likely to represent a lowered biological and organic contamination risk but could need further characterization for inorganic or physical contaminants. Frass produced from potentially contaminated food waste or non putrescible residual matter would generally require further characterisation (more details on risk classifications is presented in the report (Hénault-Ethier and Warburton 2023 (in press)).

Additional risk factors that were identified include the dispersion of smaller insect farmers over wide territory, the challenge to find perfect timing between by-product discard and insect feed consumption, the need to aggregate organic waste from multiple sources to generate sufficient volumes of feed blurs traceability, the need for adequate hygienization techniques for the organic waste collection containers, preserving waste intact in the long term, managing the risk of contamination for each batch, and also accountability of waste generators (Hénault-Ethier, Fortin et al. 2020).

3.2 Frass and insect manures

The phylum of insects (Hexapoda, or 6-legged animals) is the most species-diverse phylum of the animal kingdom (Mora, Tittensor et al. 2011). Like all animal production, insect production generates a multitude of by-products. The main waste from insect farms is excreta, with or without litter, called frass. This name comes from the German name *raß, frasz or vrāz,* from the root fressen or frezzan, which means "to devour or eat like an animal", and therefore refers more broadly to anything that insects feed on (Thyen, Clark et al. 2005). The first documented use of this term in English dates to 1854 and its definition includes debris as well as excrement produced by insects (Merriam-Webster Dictionary 2021) . This German term has been adopted in English and recommended in French (Hénault-Ethier and Warburton 2023 (in press)). Although this solid waste is biologically equivalent to manure, this definition may differ from regulatory point of view and depending on the harvesting or packaging methods, it may or may not contain insect fragments (exuviae, fragments of dead insects). Biochemically speaking, frass contains molecules from vegetation (ex. cellulose), some from insect exoskeleton (ex. chitin) or gut metabolism (ex. pyranterrone and aminolevulinic acid) (Lopes, Yong and Lalander 2022). In natural environments, insects leave frass everywhere they roam, making it difficult to recover. Other species like black soldier flies (*Hermetia illucens*, BSF) will migrate away from their frass-enriched larval growth environment, when they are ready to pupate. However, under intensive production, BSF and mealworm (*Tenebrio molitor*) larvae live buried in their feed substrate, which gradually turns into frass. Frass from edible insect farms is generally easy to recover by sieving the smaller frass particles from the larger larvae. The sieving equipment, automated or not, in commercial productions must be adapted to the granulometry and moisture content of the frass (rather humid for BSF and dry for mealworms). For the house cricket (*Acheta domestica*), the frass is collected from the bottom of their breeding tank or enclosure using a shovel or other recovery systems such as those by suction. Sieving the harvested frass can additionally help to remove insects' fragments, eggs, and uneaten feed.

Frass can be conditioned to reduce its humidity and ensure its stability during storage. Decreasing humidity and water activity is often recommended for BSF frass due to their higher moisture level which could otherwise lead to spontaneous composting (Caron-Garant, Massenet et al. 2022). However, mealworm frass has a very low moisture content at harvest (8.7%) ensuring stability upon storage (TriCycle Inc. 2020). Drying also decreases microbial loads, concentrates nutrients and decrease offensive odours (Caron-Garant, Massenet et al. 2022). Dried frass is generally a stable material and is easily preserved. According to the Enterra Feed Corporation (in activity from 2007 (Enterra

2023) to 2022 (Forage subordinated debt LP III vs. Enterra Feed Corporation. Court of King's Bench of Alberta(2022)), the BSF frass can be stored for over a year if preserved according to packaging recommendations (sealed container, at room temperature, under low ambient humidity) (Thompson 2021). As an example, Enviroflight (USA) recommends the following: ''Store product in dry area and maintain cool environmental conditions. After opening, ensure proper sealing of packaging to avoid moisture and air. Use within 12 months of manufacturing (see Best Use by Date)'' (Enviroflight 2023).

The study of dissolved organic carbon, humification and stability of frass has been little studied so far (Liang, Zhao et al. 2023). When frass is applied to the soil, through interaction with soil moisture, it become biologically active again. For instance, 35% of the total organic carbon from mealworm frass may be mineralized as rapidly as seven days after soil incorporation (Houben, Daoulas et al. 2020). This is because frass is partially stabilized through insect enzymatic digestion and interaction with gut microbiota but may not be equivalent to a mature compost right after dejection. In the presence of moisture or given sufficient maturation time, frass may reach interesting levels of humification (>50% suggest a promising organic fertilizer). Fluorescent spectra analysis shows that the type of organic waste fed to BSF larvae influences the humification degree of the frass. For instance, fish meat and bone meal digested with BSF may reach higher (61-63%) humification than chicken meat and bone meal (51-55%), while addition of cellulosic waste to this mixture may or may not improve humification (Liang, Zhao et al. 2023). However, despite interesting humification, low germination index values suggest that BSF frass may still be phytotoxic and require further composting to reach maturation (Liang, Zhao et al. 2023). Then again, the low carbon: nitrogen ratio of frass (ex. 13:1 for mealworm (TriCycle Inc. 2020)) may not be optimal to activate a spontaneous process of biodegradation by composting, likely requiring carbon rich inputs. Composting is one of the recommended treatments for manures before use as a fertilizer notably as a means to reduce microbiological and phytotoxicity risks. Beesigamukama, Mochoge et al. (2020) composted BSF frass in a heap for five weeks before use. Adding carbon sources or a pH regulator can also optimize this process (Cortes Ortiz, Ruiz et al. 2016).

3.3 Fertilizing values of frass

Insect frass has a similar fertilizing profile to other organic fertilizers (International Platform for Insects as Food and Feed (IPIFF) 2019). The average nutrient content of different products on the market is presented in Table 2. The nutrient concentration of frass varies depending on its species and diet, with a higher concentration of N (20-130%) and K (17-193%) in BSF, and a higher concentration of P (17-193%) in *Gryllus bimaculatus* (Beesigamukama, Subramanian and Tanga 2022). The macronutrients and micronutrients concentration for different commercial and experimental frass products are summarized in Table 3 and 4, respectively. A diet rich in Nitrogen will yield Nitrogen-rich frass and consequently, a plant fertilized with this frass will have more vegetative biomass than an equivalent plant fertilized with frass obtained from a depleted insect diet. This phenomenon is demonstrated in phytophagous caterpillars (*Mamestra brassica* fed on *Brassica rapa* leaves) (Kagata and Ohgushi 2012) and in BSF fed with variable concentrations of grains (rich in N) and fruit and vegetable residues (poorer in N)

(Hénault-Ethier, Kone et al. 2020). Mealworm frass contains nutrients in an assimilable and rapidly mineralizable form (Houben, Daoulas et al. 2020). BSF frass also seem to be a fast-acting fertilizer, with most N present in an organic form, and a variable ammonia content (Elissen, van der Weide and Gollenbeek 2023). The uptake of frass Nitrogen by plants is affected by the nitrification rate (Watson, Preißing and Wichern 2021). Frass can also affect soil pH, which in turn can influence bacterial activity and bioavailability of nutrients and minerals (Choi and Hassanzadeh 2019).

Post-harvest processing of frass can alter its nutritional value as well. For example, composting can increase the availability of nitrogen from insect exoskeletons. The presence or absence of aeration during composting of frass can also affect its nutrient availability. For instance, BSF frass composted with forced aeration has a greater amount of assimilable N, whereas the absence of aeration is a favorable condition for having a greater amount of assimilable potassium (Song, Ee et al. 2021).

Amending pak choi (*Brassica rapa*) with fresh BSF frass may yield lower biomass than composted BSF frass. However, composting BSF frass may increase GHG emissions. Nevertheless, composting BSF frass with forced aeration yields less GHGs than without forced aeration of the pile (Song, Ee et al. 2021) . Frass which has been composted with aeration can increase the aerial biomass of fertilized plants as well as their seeds germination rate (Song, Ee et al. 2021). Similarly, aqueous mealworm frass extracts may decrease seed germination, but this effect is less important when the liquid extract is aerated (Hénault-Ethier 2021). Higher germination indices were observed with BSF frass while the phytotoxicity of 8 other types of edible insect frass was higher (Beesigamukama, Subramanian and Tanga 2022).

3.4 Microbial diversity of frass

Frass contains a different microbial biodiversity than the feed substrate given to insects (Osimani, Milanović et al. 2018). For example, the sequenced genetic material (V3-4 region of the 16SrRNA gene) of a wheat bran diet is strongly dominated by plant DNA and it contains a low proportion of *Corynebacterium*, *Staphylococcus*, *Enterobacter spp.* and other proteobacteria. By contrast, the bacterial diversity is much greater in the gut of mealworm larvae where there is a strong dominance of *Enterobacter spp.*, *Lactococcus*, *Micrococcus* and *Propionibacterium*, and around fifteen other secondary genera which can be present in varying proportions depending on the samples (Osimani, Milanović et al. 2018). Mealworm frass itself is dominated by the genera *Erwinia*, *Lactococcus*, *Enterococcus* and *Micrococcus.* It contains half as many genera as in the larvae themselves (Gligorescu, Tourancheau et al. 2020). According to other studies on invertebrates upcycling organic matter (*Eisenia foetida*), one can expect a variability of bacterial biodiversity as the feed substrate is transformed into manure, in particular under the influence of the physico-chemical changes occurring in the substrate during the biodegradation process (Hénault-Ethier, Bell et al. 2016, Hénault-Ethier, Martin and Gélinas 2016). Invertebrate manure may contain different microorganisms that could promote plant health either by secreting antibiotic compounds, enzymes, growth hormones, by altering nutrient levels in the soil or by acting as antagonists with potentially pathogenic microorganisms in the soil (Hénault-Éthier, Dussault et al. 2017).

Another plausible microbiota-driven mechanism which could affect plant health is the production of volatile organic compounds (VOC) which can affect the behaviour of insects (repellent or aggregating) (Weaver, McFarlane and Alli 1990). Indeed, while wheat bran and yeast diet contain no trace of these VOCs, 100g of *Tenebrio molitor* L. larvae frass contains butyric acid (0.0889 g), propionic acid (0.0279 g) and valeric acid (0.0175 g).

Frass may contain bacteria capable of fixing atmospheric nitrogen (conversion of N_2 into NH_3). Although approximately 80% of bacterial nitrogen fixation occurs through plant symbiosis (like legume root nodules that provide the required energy for bacterial nitrogen fixation), some free-living microorganisms can significantly contribute to nitrogen fixation, especially in organically managed soils (Orr, James et al. 2011). Frass may also contain other bacteria capable of nitrification (converting NH_3 into NO_2 and NO_3), or even denitrification (reconversion into N_2). Insect guts are also known to be favourable environments for the emission of N_2O, a potent greenhouse gas. Mealworms emit CO_2 (0,45 g eq CO_2/kg BM/day) and N_2O (1,5 g eq CO_2/kg BM/day), but no methane (Oonincx, Van Itterbeeck et al. 2010). Over the whole life cycle assessment of mealworm production, it was observed that the global warming potential is mainly due to CO_2 (79%), N_2O (12%) and CH_4 (8%) (Dreyer, Hörtenhuber et al. 2021). Balancing insect diet by reducing protein content to the minimum required and adjusting pH (techniques applied to cattle farming (Rivera and Chará 2021)), could be tried to decrease N_2O emissions in insect rearing.

Another important pool of N throughout the bioconversion of organic waste with insects may be chitin, an important component of insect exoskeletons. Silkworms contain approximately 45% chitin (Huet, Hadad et al. 2020). In BSF, the larvae contain around 7 to 13% chitin, while the pupae contain around 21 to 33% (Brigode, Hobbi et al. 2020). Mealworms larvae, pupae and adults contain 7.2% to 10.1%, 9.5% and 11.8% chitin respectively (Yu, He and Wang 2021). Thus, it is normal that frass contains an appreciable amount of chitin depending on its insect of origin. Indeed, the exuviae of mealworms remains in the rearing substrate, while those of BSF is biodegraded during in the process of bioconversion. Also, fine sieving upon harvest may remove exuviae and other insect fragments which could enrich the substrate in chitin (Caron-Garant, Massenet et al. 2022). The chitin content of cricket frass was found to be low (1.1 g/Kg) (Caron-Garant, Massenet et al. 2022). BSF supplements in soils can increase the number of chitin-degrading bacteria (such as *Gammaproteobacteria*) and the numbers of fungi that grow rapidly and contain high concentrations of N (such as *Mortierellomycota*) (Nurfikari 2022). Once in the soil, chitin is converted into chitosan by soil microflora and can trigger plant immunity through secondary chemical production which prevent insect attacks, has antibacterial and antifungal activities (El Hadrami, Adam et al. 2010). Substituting 5% of the soil by frass resulted in a significant decrease in the presence of aphids on cabbage plants, as well as a 22% higher growth rate probably due to the fertilizing effect (Coudron, Sprangher et al. 2019). Chitin supplements have been found to increase the *Rhizoctonia* disease suppression in sugar beet (Postma and Schilder 2015). *Fusarium*-induced mortality of cowpea plants was significantly reduced with BSF frass, an effect putatively attributed to chitin content (Quilliam, Nuku-Adeku et al. 2020). However, the pathogen suppressing ability of chitin is questioned by some authors (Watson, Houben and Wichern

2022). Although there are methods for determining chitin in insects such as mealworms [e.g., delipidation (EtOH), demineralisation (1M HCl) and deproteination (1M NaOH) (Mohan, Ganesan et al. 2020) prior to quantification (Fourier-transform infrared spectroscopy - FTIR], chitin determination from complex organic matrices such as frass still requires methodological development (Brigode, Hobbi et al. 2020). The crustacean industry is refining waste pre-treatment and extraction methods to improve yield and purity of derived chitin and chitosan (Trung, Van Tan et al. 2020). These methods could inspire the insect industry to develop chitin extraction or concentration methods to develop added value products from frass. Since the chitin content of frass may be a desirable by-product obtained through insect bioconversion of organic waste, further studies on chitin and chitosan content of frass are required.

Besides the prophylactic effect of chitin, recent work has reported that microbial communities in BSF frass possess significant antifungal activity against a range of phytopathogens *in vitro* and *in vivo* (Arabzadeh, Delisle-Houde et al. 2022). The diet composition significantly affects this activity and heating the frass according to current European regulations (70 °C for 1h) reduces this prophylactic effect.

3.5 Economic value of frass

The retail price of frass per kilogram is quite variable and fluctuates between CAD6.00 and $58.18 (Figure 2, detailed data and references available in Supplementary Table 2). The average value per kilogram of BSF was significantly (X^2, p = 0.0104) lower (CAD 17.51 [range 11.98-29.53]) than that of mealworms (CAD 26.82 [range 13.22-58.18]), crickets (CAD 30.93 [range 17.94-45.05]) or superworms (CAD 32.16 [range 11.98-50.90]). This could be linked to the size of the farms, which are generally larger in the BSF industry, allowing a lower production price depending on the volumes, or even a lower value on the consumers front (Hénault-Ethier, Fortin and Normandin 2021). Finally, pricing does not seem directly related to nutritional content, and based on our analysis the package mass was excluded as a confounding variable (see Supplementary Material Figure 1). This may indicate that production and marketing issues may be more important determinants of free market price determination (Hénault-Ethier, Fortin and Normandin 2021). Sectorial associations like NACIA do not yet have extensive pricing evaluations.

3.6 Environmental benefits of frass

Insect droppings are important for nutrient cycling in natural ecosystems. The incorporation of farmed insect frass as fertilizing amendments in cropping systems is inspired by the natural functioning of ecosystems. The frass that naturally settles on the ground is a rich source of nutrients and labile organic carbon (Lovett and Ruesink 1995)(Frost and Hunter 2004)(Kagata and Ohgushi 2012). Similarly, mealworm frass (75% organic matter) can supply organic carbon to farmed soils. Organic matter is important for the soil ecosystem, its structure and resistance to drought and can act as a sink for carbon sequestration (Food and Agriculture Organization (FAO) 2002).

The use of frass as a fertilizer is also in line with the precepts of circular economy since it valorizes the residues from the production of the growing edible insect sector (Hénault-Éthier, Dussault et al. 2017). This reuse offers an alternative to the linear mode of production that currently dominates, and which leads to disposal in landfill or incineration) or to less recovery by downcycling, such as energy recovery (International Platform for Insects as Food and Feed (IPIFF) 2019). Incineration of organic material releases more GHGs than bioconversion with BSF based on life cycle analyses (Song, Ee et al. 2021). In an open environment, CO_2, NH_3, CH_4 and N_2O emissions may be lower from BSF frass than from compost (Pang, Hou et al. 2020). Organic matter treatment with BSF may lead to 47 times less direct GHG emissions than composting. Over the entire lifecycle, GHG emissions may be halved when organic waste is treated with insects rather than compost (Mertenat, Diener and Zurbrügg 2019).

Therefore, the use of frass offers a sustainable solution for farmers and gardeners. In addition, the substitution of conventional fertilizers with frass could further limit other environmental impacts. Frass may provide other environmental benefits due to its chitin content (Zahn and Quilliam 2017) and its contribution to the enrichment of soil microbiota, including those demonstrating the ability to suppress fungal phytopathogens (Arabzadeh, Delisle-Houde et al. 2022) or general microbial activity (Houben, Daoulas et al. 2020) GHG emissions from composting vary depending on its management methods. However, even in composting piles which are kept under aerobic operating conditions, we can expect trace emissions of CH_4 emissions due to anaerobic conditions in microsites (Mustin 1987)(Boldrin, Andersen et al. 2009)(Environmental Protection Agency (EPA) 2021)

3.7 Comparison of frass with other fertilizers or amendments

Despite the emergence of strong scientific knowledge on the benefits of frass as a fertilizer or soil amendment, knowledge transfer to end-user has only just begun. In an effort to popularize the potential benefits of frass over other better-known fertilizers or soil amendments, Table 6 proposes a comparison with conventional fertilizers, conventional thermophilic composts, vermicomposts, conventional farmyard manures and biochar. The following sections offer more details on the comparison between frass and other fertilizers and other organic amendments, but also provides additional explanations as to how frass can also be used as a substrate in other organic waste treatment technologies like composting or biochar production. While it is relatively easy to differentiate frass from conventional fertilizers, comparisons with other organic fertilizers reveal some similarities and other more subtle differences. A life cycle analysis by Smetana, Schmitt and Mathys (2019) suggests that insect frass is more sustainable than other organic fertilizers. Schmitt and de Vries (2020) nuanced this conclusion by suggesting that environmental impacts need to use comparable fertilizing units as a baseline. In addition, the macronutrient, micronutrient and pathogen contents, as well as the greenhouse gases produced during the process, are highly dependent on the inputs used to produce the fertilizer and the amendment (Schmitt and de Vries 2020)(Walling and Vaneeckhaute 2020). Thus, compost from manure or wastewater treatment plants contains more coliform bacteria than most household composts (Karimi, Mokhtari et al. 2017). Similarly, a high nitrogen diet for insects can lead to frass with elevated levels of this element (Fielding, Trainor and Zhang 2013).

3.7.1 Conventional fertilizers

Frass could totally or partially replace mineral fertilizers with equivalent content of nitrogen, phosphorus and potassium. Frass could help to reduce the use of synthetic fertilizers made from non-renewable materials (International Platform for Insects as Food and Feed (IPIFF) 2019). Decreasing the consumption of non-renewable fertilizers is critical as resources are rarifying (Gilbert 2009). The production of mineral or synthetic fertilizers depends heavily on the exploitation of hydrocarbons which have significant environmental consequences (Hasler, Bröring et al. 2015). Combustion of natural gas is commonly used to generate the heat during the energy intensive production process of nitrogenous fertilizer (Gellings and Parmenter 2016). The high process heat likely limits microbiological risks of synthetic fertilizers. In addition, while conventional fertilizers are still relatively cheap compared to frass, their price have doubled to quadrupled on North American markets between 2020 and 2022 (Crespi, Hart et al. 2022).

Phosphorus leaching is a major environmental issue, especially in phosphorus-saturated soils. By limiting phosphorus emissions into the environment, water quality and ecosystem health are improved (Correll 1998), and more phosphorus is directly available to cultivated plants (Houben, Daoulas et al. 2020). Frass organic amendments have the advantage of being less leachable than inorganic forms of phosphorus (e.g. dialkyl phosphate) (Kleinman, Sharpley et al. 2002). Indeed, for an equivalent total phosphorus content, the soluble phosphorus concentration of frass is five times lower than that of a mineral fertiliser. Despite its organic matrix which reduces the potential for leaching, mealworm frass is nevertheless rapidly mineralized in the soil. It is estimated that 35% of total organic carbon may be mineralized seven days after its incorporation into the soil

(Houben, Daoulas et al. 2020). Thus, frass can also help to rapidly meet the nutritional needs of plants, as a conventional fertilizer would (Houben, Daoulas et al. 2020). Comparing iso-nitrogen concentrations, maize plants grew taller and had more chlorophyll with BSF frass than with mineral fertilizer (urea) (Beesigamukama, Mochoge et al. 2020). Frass can also facilitate the use of potassium by plants compared to mineral fertilizers (Houben, Daoulas et al. 2020). Rosmiati, Nurjanah et al. (2017) mention that frass from BSF fed with coffee grounds increases the utilization of phosphorus and potassium by lettuce plants, which may indicate an impact of frass on the availability of these elements. Similarly, Nicksy (2021) noted 130% higher potassium uptake in nitrogen-rich, fresh, amended ryegrass compared to a control plot. Compared to several conventional fertilizers, frass has an interesting content of diversified micronutrients (Houben, Daoulas et al. 2020). Unlike other organic amendments, conventional fertilizers contain neither phytohormone nor humic acid, they provide few microorganisms and stimulate little soil life, do not improve resistance to antibiotics, do not protect plants against biological pests, are not accepted in organic farming, but they do alter the soil structure detrimentally (Table 5). They can have a greater climate impact than some organic amendments (atmospheric N_2O emissions linked to the use of nitrogen fertilizers (Walling and Vaneeckhaute 2020)) . However, they are stable in storage, easy to spread with conventional farming equipment and have a relatively low price which often justifies their use.

3.7.2 Conventional Manures

Manure from pigs, poultry or ruminants contributes significantly to groundwater pollution, and could in some cases represent a major source of nitrates (Widory, Petelet-Giraud et al. 2005). Nutrient analyses of mealworm frass reveal a similarity to poultry manure (Houben, Daoulas et al. 2020). Similarly, Chavez and Uchanski (2021) suggest that the carbon, nitrogen and phosphorus concentrations of frass and manures are comparable. A difference observed in several studies is the decrease in total nitrogen and an increase in available nitrogen between fresh manure and manure ingested by insects (Chavez and Uchanski 2021).

The conventional production of animal protein (cattle, pigs, poultry) is often associated with a greater environmental impact than the insect production for an equivalent quantity of protein produced (Oonincx and De Boer 2012). This is explained by their higher conversion rate and their lower production of greenhouse gases compared to other livestock (Zahn and Quilliam 2017). Insects also use less soil area and less water and contribute less to the acidification of ecosystems (Schmitt and de Vries 2020). The diversified diet and the potential use of agrifood side streams as an input make the production of insects, and their manure, even more sustainable (Smetana, Schmitt and Mathys 2019). It remains to be seen whether insect frass also has a lower environmental footprint than conventional farm manures.

A study comparing mealworm frass to isonitrogen concentration of chicken manure suggest similar biomass production are achieved in a variety of herbs and vegetables (Hénault-Ethier, Reid et al. 2023). Hen manure fertilization may yield 26% more lettuce than BSF frass (Caron-Garant, Massenet et al. 2022). However, microbially induced soil respiration rate may elevate more rapidly with BSF frass compared to chicken manure, suggesting a more rapid bioavailability of the carbon and nutrients of frass (Caron-Garant, Massenet et al. 2022). Finally, the relative abundance of predominant prokaryote species may differ between BSF frass and hen manure. Firmicutes are by far the most abundant in insect and hen manure, and Proteobacteria may also be relatively abundant in both types of manures. However, Actinobacteria and Bacteroidota may be more abundant in hen manure, while campylobacteria may reach ≥1% in some BSF frass (Caron-Garant, Massenet et al. 2022).

3.7.3 Conventional thermophilic compost

Compost is obtained from a process of bio-oxidation of organic matter called composting (Mustin 1987). Compost contains N, P, K and other oligo-elements in varying concentrations. It is also rich in humic acids and contains phytohormones (such as gibberellin) (Pant, Radovich et al. 2012). Compost undergoes a natural thermophilic phase caused by the elevated metabolic activity of the microbiota it contains. At the height of the thermophilic phase, the biological diversity of the compost drops and heat-tolerant spore-forming bacteria survive. At the end of the process, the microbial diversity of thermophilic compost may be lower than the organic material resulting from mesophilic processes (like vermicomposting or entotechnologies), although it is expected to be richer than conventional inorganic fertilizers. Similarly, to frass, compost improves soil structure, limits nutrient leaching, increases plant resistance to abiotic stresses, and provides protection against various biological pests (Table 5). Depending on the quality of the inputs and the process, both compost and frass may contain various contaminants (pathogens, heavy metals, chemical contaminants, etc.). Like frass, composts may be used in organic farming. The stability of compost in storage varies with its humidity content and the degree of biological maturity of the product generated. Compost stability and maturity are important parameters which are generally regulated in compost quality guidelines (ex. (Canadian Council of Ministers of the Environment (CCME) 2012)). The former coincides with a reduction in the biodegradation process that can be measured as a decrease in respiration rate (Mustin 1987). The latter coincides with more subtle biochemical transformation included extensive humification and lower phytotoxicity of the final product (Mustin 1987). While various levels of stabilization of frass may occur as organic waste transits in the gut of insects, few studies detailed its maturity. Depending on the quality and source of the product, compost's market value may be null (distributed free in bulk to agricultural producers or residents from municipal composting facilities) to low (1-8$/kg at retail). Compost's low N-P-K concentration is within the typical range observed for various insect frass. Thus, to reach equivalent fertilizing units, larger volumes of both composts and frass must be used compared to conventional fertilizers. This can represent significant additional costs for purchasing, storing, and applying fertilizers.

The digestion process by insects is sometimes referred to as entomocomposting (Menino and Murta 2021). Frass and compost have many common characteristics (Hénault-Éthier 2019). Both come from a process of recycling residual organic materials and are rich in trace elements and organic compounds. Their properties vary depending on the inputs and parameters used during the transformation. Notably, conventional composting involves a self-heating phase during which thermophilic bacteria are selected while different pathogens perish (Mustin 1987). Some entotechnologies use a spontaneous thermophilic pre-treatment phase before feeding the insects, which make the nutrients more readily accessible and has the additional advantage of decreasing the concentrations of pathogenic indicator microorganisms. But many feed insects directly with fresh organic material. In some cases, a slight temperature elevation above room temperature is noted. In general, this is not recognized as a significant self-heating phase compared to composting, because insect larvae feeding controls microbial activity. Both compost and frass can stimulate plant growth and improve resistance to biotic and/or abiotic stresses (Hénault-Éthier 2019). Entotechnologies have the additional benefit of rapidly stabilizing organic material, while producing a protein-rich food (Xiao, Mazza et al. 2018).

Trials on vegetable crops suggest that frass (from BSF) and compost enrich the soil and affect plant growth in a similar way (no statistical difference was observed at application rates ranging from 5 to 20 tons per hectare) (Zahn and Quilliam 2017). Adding frass (mealworm) to compost-amended potting soil significantly increases plant growth (Hénault-Ethier, Reid et al. 2023).

The chitin content of compost produced with crustaceans may be significant. For instance, shrimp shells contain 19.3% chitin. However, the chitin concentration is expected to drop during the biodegradation process (Huet, Hadad et al. 2020). Compost produced with other inputs not containing chitin (such as mammalian manure) could still contain chitin due to fungal enrichment during the biodegradation process (Sato, Azama et al. 2010). As chitin (or chitosan) content is associated with plant protection properties, it could be interesting to study whether frass produced with chitin-rich insects contains more chitin than compost.

The stabilization, maturation and humification processes, well defined for composts, remain little studied for frass. However Basri, Azman et al. (2022), compared BSF frass to immature compost, suggesting it may need post-harvest treatment. Frass may also be used as a substrate for composting. For instance, frass that does not meet the regulatory microbiological loads (for coliforms for example), may be further processed through composting, where the temperature elevation will ensure additional sanitation. Composting of frass may lead to carbon (respiration) or nutrient losses (through ammonia volatilization for instance). However, further processing of frass through composting may involve some downsides including additional handling and costs of production. As frass alone typically doesn't have the optimal C/N ratio for composting (e.g. BSF frass C/N ratio ranges from 8 to 27 (Basri, Azman et al. 2022)), composting frass may require the acquisition of carbon-rich bulking agents. Finally, the thermophilic conditions in composting may reduce the microbiological diversity of frass obtained in mesophilic conditions.

3.7.4 Vermicompost

BSF frass is sometimes compared to vermicompost which is produced by earthworms digesting organic substrate (Elissen, van der Weide and Gollenbeek 2023). Vermicompost is a process of bio-oxidation of organic matter activated by the presence of worms, such as *Eisenia fetida* (Mustin 1987). These invertebrates play a major role in the nutrient recycling process and influence the bacterial communities present in composting, (Hénault-Ethier, Martin and Gélinas 2016). Vermicompost has been generally attributed to a high humification rate (Arancon, Edwards et al. 2006), an improvement in the amended soil structure (Ceritoğlu, şahin and Erman 2018), a high phytohormones content (such as gibberellin (Tripathi and Bhardwaj 2004)(Arancon, Edwards et al. 2006)(Pant, Radovich et al. 2012), a rich microbial diversity, increased plant resistance to abiotic stresses (Canellas, Canellas et al. 2019) as well as an increased resistance to pests such as downy mildew or nematodes (Xiao, Liu et al. 2016)(Bahramisharif and Rose 2019). Like frass, it is stable in storage and can be used in organic farming.

However, methane emissions from vermicompost are not negligible due to the anaerobic conditions prevailing in the digestive system of the worms. In addition, the earthworm gut, like that of insects, is known to promote N_2O emissions. The price of vermicompost on the market is generally higher than that of conventional composts, but lower than that of frass.

Compared to conventional compost, vermicompost can support plant growth even more by influencing the microbial fauna, providing more nutrients and creating an environment conducive to their absorption (Arancon, Galvis and Edwards 2005)(Tognetti, Laos et al. 2005). Vermicompost increases the content of plant growth hormones and humic acids (Arancon, Edwards et al. 2006). These humic acids increase the resistance of plants to abiotic stresses (Canellas, Canellas et al. 2019). The presence of earthworms can also stimulate the growth of mycorrhizae (Brown 1995). Similar to vermicompost, frass also provides potentially useful microorganisms for plant growth (Poveda, Jiménez-Gómez et al. 2019)(Houben, Daoulas et al. 2020). A comparison of fungal biomass (measuring extractable ergosterol concentrations) revealed that both frass and vermicompost can increase soil fungal biomass (Watson, Schlösser et al. 2021). However, doubling the application rates of frass (5% v/v instead of 2.5% v/v) induced a disproportional four-fold fungal biomass increase, an intensity which was not observed during doubling vermicompost amendments (Watson, Schlösser et al. 2021).

From a nutritional perspective, commercial vermicompost has been observed to have higher extractable NO_3 and Ca concentrations than BSF, mealworm or buffalo worm frass, whereas all frass types had higher extractable concentrations of all other measured nutrients, including NH_4 concentrations, than vermicompost. Furthermore, frass contained more organic matter, N, and had a smaller C/N ratio, higher pH and higher electrical conductivity than vermicompost. Finally, frass application significantly reduced all extractable metals from soils compared to vermicompost (Watson, Schlösser et al. 2021)

In a study on the growth of cucumbers in the greenhouse, the use of BSF frass resulted in a faster growth rate, taller plants with more leaves and greater production compared to vermicompost treatment (Yaacobi, Shouster-Dagan et al. 2019) In a study comparing mealworm frass and vermicompost aqueous extracts in bok choi hydroponics systems, it was observed that the frass tea yielded more chlorophyll but that the vermicompost tea led to greater plant biomass (Wang 2022). However, both aqueous extracts were outperformed by conventional liquid fertilizers.

The physicochemical stability of vermicomposts varies depending on the management method used, whether continuous, semi-continuous or in series (Hénault-Ethier, Bell et al. 2015). No studies of the stability of frass as a function of operational processes have been identified. However, vermicompost may be more stable upon soil inclusion than frass. Indeed, it has been observed that increasing application rates of frass (from 2,5 to 5% v/v) increases soil microbial respiration while increasing vermicomposting application rates by the same ratio did not lead to an increase in microbial respiration (Watson, Schlösser et al. 2021). Furthermore, it has been suggested that subsequent processing of frass with vermicomposting could increase its stability (Lopes, Yong and Lalander 2022), but this has yet to be demonstrated experimentally.

3.7.5 Biochar

Biochar is a process using pyrolysis at elevated temperatures (300-850°C) used to stabilize organic materials and produce carbon rich soil amendments (Lange, Allaire et al. 2018)(Song, Ee et al. 2021) (compared biochar and BSF frass obtained from horticultural residues. They noted higher content of nitrate and phosphate ions, as well as macronutrients and micronutrients in the frass (Song, Ee et al. 2021). However, the biochar was higher in carbon and had a higher C/N ratio. A combination of biochar and frass could thus have complementary soil amendment properties. Frass used as fertilizer on the biochar-based growing medium can promote 1.6-6.8 times the growth of lettuce, compared to fertilizing with frass tea (Tan, Lee et al. 2021).

Interestingly, the process of converting larval waste in the presence of biochar has been shown to improves the nitrogen retention of frass while increasing the yield of larval biomass (Carroll, Fitzpatrick and Hodge 2023). Conversely, frass can also be used to produce biochar (Yang, Chen et al. 2019). On the one hand, pyrolysis may be an interesting treatment of insect frass-fed inputs which may not enter the animal or human food chain (e.g., biodegradation of plastic wastes; (Wu, Yang et al. 2016)(Brandon, Gao et al. 2018). On the other hand, biochar produced from frass may have interesting properties to adsorb environmental contaminants like industrial dyes (Yang, Chen et al. 2019) or neonicotinoid insecticides (Shi, Wang et al. 2022). The agronomic properties of biochar made from insect frass differ from those of biochar made from chicken manure or other organic waste, and are also influenced by the production temperature (Lataf, Jozefczak et al. 2022). Because it has a high C/N ratio and is a labile carbon source biochar may accelerate the immobilization of labile nitrogen (NH_4^+ and NO_3^-) in the soil solution (Nelissen, Rütting et al. 2012). The conversion of frass into biochar would be an interesting avenue to explore in the agricultural field given the positive effects of biochar on soil health and therefore on plant growth (Government of Canada 2022).

3.8 Resistance to abiotic and biotic stresses

Many organic amendments promote soil and plant health. Mealworm frass has been shown to help plants better resist certain abiotic stresses such as drought, excess water and salinity (Poveda, Jiménez-Gómez et al. 2019). This may be related to the activity of the microorganisms present in the frass. This observation is supported by Houben, Daoulas et al. (2020) who argue that mealworm frass increases soil microbial activity, an activity synergistically stimulated when frass is used in conjunction with mineral fertilizer. Supplementing peat media with 1-2% per volume of BSF frass may increase CO_2 emissions up to 5 times (Caron-Garant, Massenet et al. 2022). Additionally, Xiao, Mazza et al. (2018) observed that the addition of a particular microorganism, *Bacillus subtilis* BSF-CL, improves the germination index and decreases the concentration of potential pathogens in frass. Yet, rapid growth rate and high yield were observed with heat-treated BSF frass containing fewer microorganisms (Yaacobi, Shouster-Dagan et al. 2019). Frass is believed to have a beneficial effect on plant growth as it is rich in microbial biomass, chitin, growth hormones and enzymes.

Frass contains chitin and chitosan. According to the US Environmental Agency, chitin and chitosan can act as plant growth regulators (Emnvironmental Protection Agency (EPA) 2007). Chitin supplements can increase the size of lettuces (Debode, De Tender et al. 2016). Chitin also controls nematodes (Spiegel, Chet and Cohn 1987) and the bacterium *Salmonella enterica* (Debode, De Tender et al. 2016). Plant cells present receptors capable of detecting chitin, leading to a response to curb a fungal infection (Volk, Marton et al. 2019). The antifungal effect may be due to the production of chitinolytic enzymes, stimulated by the addition of chitin, which also degrades the chitin contained in the cell walls of pathogenic fungi (Sato, Azama et al. 2010). That chitin can also promote the immune system of plants to protect them against attacks by insect pests or pathogenic fungi (Kombrink, Sánchez-Vallet and Thomma 2011) or against abiotic stresses such as a high concentration of salt (Ali, Luo et al. 2018). An increase in pathogenic insect predators has been observed following the application of BSF frass (Schmitt and de Vries 2020). As opposed to house crickets or mealworm frass, BSF frass or exuviae reduced the cabbage root fly (*Delia radicum* L.) pest on Brussel sprouts (*Brassica oleracea* L.) (Wantulla, van Zadelhoff et al. 2023). Chitin itself promotes the proliferation of several microorganisms of the phylum: Cellvibrio, *Pedobacter*, *Dyadobacter*, and *Streptomyces* as well as for fungi of the genus *Lecanicillium* and *Mortierella (Debode, De Tender et al. 2016)*.

The chitin present in the shell of insects can be converted into chitosan thanks to the enzymatic activity of common bacteria in soils (i.e., *Serratia marescens*, *Bacillus sp*) (Pelletier 1991). Chitosan is fungicidal and antibacterial (Emnvironmental Protection Agency (EPA) 2007). There are several possibilities for the agricultural application of chitosan, particularly in the context of plant disease control (Orzali, Corsi et al. 2017). Chitin and chitosan can trigger physiological responses in plants allowing them to resist pest attacks. For example, how they might trigger the synthesis of phytoalexins (proteins related to pathogens), protease inhibitors, the synthetis of lignin or the formation of calluses (El Hadrami, Adam et al. 2010). It has been observed that the addition of frass stimulates defense mechanisms against herbivorous insects or against pathogens. However, the effects are variable depending on the species of plant and insect concerned, and the place where the frass is applied (Ray, Basu et al. 2016). It is therefore probable that the use of frass could contribute to reducing the use of pesticides in an agricultural or horticultural context (Schmitt and de Vries 2020), and the effectiveness of this substitution remains to be determined for each use.

On green bean plants (Valentino Green BushBeans), the addition of 1.5% by volume of frass increased plants height by approximately 40%, but the effect was even greater with the joint addition of humic acids in the growing medium (Choi and Hassanzadeh 2019). In the presence of an inoculated fungal pathogen (*Pythium*), bean growth is severely impaired (<85%) compared to control in healthy potting soil. The addition of chemical fertilizer makes it possible to somewhat limit the damage (<15% reduction in the final size after 56 days compared to the control). In contrast, the addition of frass limits losses, with a final size marginally greater than the control. A supplement of frass, humic acid and *Trichoderma* (a biological control agent against Pythium) allows a gain of the around of 30% greater than the growing control in healthy soil (Choi and Hassanzadeh 2019). Similarly, the addition of BSF frass moderated the spread of white mold (*Sclerotinia minor*) on inoculated watercress plants (Setti, Francia et al. 2019).

Antimicrobial and fungicidal compounds can be produced by insects and potentially accumulate in the frass. Elhag, Zhou et al. (2017) identified peptides acting against *Staphylococcus aureus*, *Escherichiacoli*, *Rhizoctoniasolani*, *Sclerotiniasclerotiorum* produced by BSFs. Arabzadeh, Delisle-Houde et al. (2023) observed inhibition in the growth of horticultural fungal pathogens on in vitro growth media (*Tryptic soy agar*) containing 0.1% BSF frass. The inhibition seemed stronger (90%) for the frass from a farm that had consumed a cereal diet (100% Gainesville) than on the frass from other inputs biotransformed by BSF (30-85%). The content of these antimicrobial or antifungal compounds and their usefulness for controlling pests in the context of crop production remains to be developed.

3.9 Impacts of frass on plant growth

Although agricultural producers have reported beneficial effects of frass on vegetable crops (Halloran, Roos et al. 2016), scientific research on its benefits is still in its infancy. In the context of the rapid expansion of edible insect farming, the urgency of finding cost-effective and environmentally friendly substitutes to mineral fertilizers whose production depends on the extraction of hydrocarbons and minerals is critical to increase our knowledge of the benefits of frass in agriculture.

3.9.1 Mealworms frass

Applied at a rate of 10 tonnes per hectare, mealworm frass increases barley (*Hordeum vulgare L.*) biomass as well as nitrogen, phosphorus and potassium uptake (Houben, Daoulas et al. 2020). Mealworm frass may be beneficial to tomato plant growth and support plant health (Blakstad 2021). The seedling phytotoxicity (germination rate) of composted mealworm frass appears to be related to the type of feed given to the larvae (corn straw > wheat bran > rice straw) (He, Zhang et al. 2021). The effect of incorporating wheat bran-fed mealworm frass (0,5% v/v, 3-4-2) or chicken manure (iso-nitrogen) in potting soil amended with municipal compost (5% v/v) was evaluated on 9 vegetable and 3 flowering plants (Hénault-Ethier, Reid et al. 2023). Seedling emergence was inhibited by both manures in beets and carrots (but not corn, radish and arugula), but this did not result in a decrease in edible biomass compared to the control. The height of mealworm-fertilized kale, basil, tomato, and sweet corn plants was higher than the control.

Mealworm frass resulted in a 16-fold increase in edible vegetable biomass compared to the control (results were similar with chicken manure). Finally, the flowers of the plants (dwarf sunflowers, zinnias and nasturtiums) fertilized with frass were larger and more numerous than the control plants. Mealworm frass was also compared to chicken manure in the context of phytoremediation with fast-growing willows (*Salix miyabeana* SX64). It has been noted that while chicken manure contributed to increasing the soil pH, mealworm frass decreased soil pH, which may play a role in metallic ions uptake (Brunette, Bonet et al. 2022). Besides mealworm frass, mealworm larvae meals were also investigated for their fertilizing properties in wheat (Przemieniecki, Kosewska et al. 2021). In this context, it increased the N content of the soil in the same way as to mineral nitrogen fertilizer, but it also increased the concentration of P, K and Mg in the soil concentrations, and enhanced ammonification. Finally, mealworm meal increased *Bacillus* spp. loads, which were correlated with a decrease in *Fusarium* spp. load in the rhizosphere, suggesting potential benefits for plant health (enhanced nutrient availability, prevention in damping off).

3.9.2 BSF frass

Several reviews on the impact of BSF on plant growth, health and soil quality have been published in the recent years (Schmitt and de Vries 2020)(Chavez and Uchanski 2021)(Gärttling and Schulz 2021)(Poveda 2021)(Barragán-Fonseca, Nurfikari et al. 2022)(Basri, Azman et al. 2022)(Lopes, Yong and Lalander 2022)(Watson, Houben and

Wichern 2022)(Elissen, van der Weide and Gollenbeek 2023) so the discussion below is restricted to general conclusions and a few highlights.

The impact of BSF frass on plant growth can be positive, neutral or even negative above a maximum application threshold (Elissen, van der Weide and Gollenbeek 2023). The use of frass from BSF improves the growth of sunflower (*Helianthus annulus*) by helping to enlarge its stem diameter and increase the lipid content of the seeds (Kováčik, Kozánek et al. 2010). On onions, similar final heights were obtained with compost (5 to 15 tonnes/hectare), synthetic fertilizer (0.075 to 0.3 t/ha) and BSF frass (5 to 15 t/ha) (Zahn and Quilliam 2017). Addition of frass equaled or increased the biomass of ryegrass, wheat and alfalfa plants compared to compost, digestate, conventional potassium fertilizer and struvite (Nicksy 2021). BSF frass can also improve grass survival (McTavish, Smenderovac et al. 2019).

BSF frass composition strongly depends on the substrate used as (Hénault-Ethier, Kone et al. 2020), (Elissen, van der Weide and Gollenbeek 2023). The average composition suggests that BSF frass is similar to different manures: C/N and N content like cattle manure, P content similar to pig slurry, dry matter and K content similar to poultry manure and dry matter content similar to compost (Elissen, van der Weide and Gollenbeek 2023). Different parameters can influence the quality of the frass which has repercussions on the yield of fertilized plants. Kagata and Ohgushi (2012) has mentioned the importance of using quality frass as fertilizer. By supplying frass with 3.58% nitrogen to komatsuna plants, their final height was reduced compared to plants that received no supplement. The authors suggested that competition with nitrogen-consuming microorganisms may limit plant nutrient uptake and growth. Similarly, McTavish, Smenderovac et al. (2019) noted lower plant survival and growth rates with frass from insects fed on polluted biomass from degraded environments.

3.10 Phytotoxicity potential of frass

The phytotoxicity of frass has been tested in various studies (Zahn and Quilliam 2017)(Setti, Francia et al. 2019)(Wei, Li et al. 2020)(Song, Ee et al. 2021). On BSF frass, Song, Ee et al. (2021) observed a marked decrease in the germination rate of pakchoi (B*rassica rapa*) compared to the control when 100g/L of the extract was used. On the other hand, Setti, Francia et al. (2019) observed an increase with 50g/L of the extract on watercress plants (*Lepidium sativum*). Different effective concentrations may explain the apparent contradictions in the results (Zahn and Quilliam 2017). Indeed, the phytotoxic effect of mealworm frass on radish and watercress germination varies with concentration as demonstrated in an experiment using a stock solution with a frass: distilled water ratio of 1:10 and with aqueous extracts of 25, 50, 75 and 100% (Hénault-Ethier 2020) as per the protocol of Ali, Luo et al. (2018). All concentrations inhibited radish germination and there was a positive correlation with increasing concentration (Hénault-Ethier 2020). The addition of frass rather delayed the germination of watercress and similar germination rates were observed after 96 hours. In another experiment, the germination and growth of the same plants subjected to three frass teas were tested in soil (Pro-Mix®, Premier Tech, Canada) (Hénault-Ethier 2020). Under these conditions, the germination rate of the watercress with the addition of tea was similar to that of the control, whereas it was reduced for radishes. However, the growth of the watercress was more affected by the addition of tea than that of radishes. Frass phytotoxicity appeared to decrease when a frass tea was aerated longer before seed exposure and when the aqueous extract was applied to a growing medium (Pro-Mix) rather than a paper towel. This suggests that phytotoxicity could be due to oxidizable compounds, such as NH_4, and/or adsorbable on organic matrices (Hénault-Ethier 2020). Higher concentrations of mealworm frass (3% w/w) were associated with the inhibition of ryegrass seedling germination, possibly due to ammonia or salinity (Watson, Preißing and Wichern 2021).

The influence of oxygenation on phytotoxicity for seedlings is observed not only in the aqueous extracts but also in the solid matrix. It has been observed that composting frass with aeration increases the germination rate, while it is decreased by composting without aeration. This effect could be due to the decrease in phytotoxins that may be present in fresh BSF frass (Song, Ee et al. 2021). Similarly, composting of the mushroom bed substrate under aerobic conditions (by beetle larvae *Protaetia brevitarsis*) reduced the initial phytotoxicity of the substrate (Wei, Li et al. 2020).

3.11 Applications of frass as a fertilizer

For fertilizer use, frass may be applied fresh or after a conditioning pre-treatment (sifting, drying, granulation, pelletization in a tumbler, mixing with other materials, or creating aqueous extracts through infusion or maceration (Caron-Garant, Massenet et al. 2022). Currently, insect frass is mainly used as a fertilizer in nurseries, in viticulture, by professional or amateur horticulturists as well as by farmers (International Platform for

Insects as Food and Feed (IPIFF) 2019). There is little scientific literature to guide frass-producing companies and consumers in the application of frass as a fertilizer. Table 6 illustrates the great variability in fertilization recommendations.

In a greenhouse potting soil experiment, Houben, Daoulas et al. (2020) observed equivalent growth in barley (*Hordeum vulgare* L.) using 10 Mg/ha of *Tenebrio molitor* frass or N-P-K equivalent mineral fertilizer. When comparing mineral N-P-K fertilizer to 10 or 20 Mg/ha mealworm frass (5-2-1.7), Poveda, Jiménez-Gómez et al. (2019) noted that organic amendments increased the microbial metabolic activity of the soil, but only the highest concentration increased soil microbial functional diversity. Kagata and Ohgushi (2012) suggest a rate higher than 0.5 g of frass per 110 g of soil for the growth of komatsuna plants.

The optimal rate and timing of application depend on insect diet, rate of decomposition and crop (Fielding, Trainor and Zhang 2013). For example, nitrogen-rich dust decomposes faster, influencing the period during which nutrients are available to plants (Kagata and Ohgushi 2012). Lovett and Ruesink (1995) attribute this rapid decomposition to the high labile carbon content of frass, which stimulates soil microbiota and promotes nitrogen fixation. Recommended application rates for frass also vary based on plant type or culture systems. Enterra suggests adding three times more BSF frass for greenhouse production compared to fertilizing lawns, mature trees and outdoor gardens (Enterra 2020). Similarly, Obie's Worms recommends four times more frass for gardens than lawns (Obie's Worms 2020). Down to Earth recommends application rates based on plant size (Down to Earth 2020). Other companies recommend different dosages if the frass is spread on the surface, mixed with the substrate, applied as a solution to the leaves, or used for the preparation of tea (Entomo Farms 2016, Boogie Brew 2020, Down to Earth 2020, Organic Nutrients 2020, Grow Daddy 2021, Midwest Mealworms 2021, Vermont Mealworm Farm 2021). Application frequencies are also variable: every week (Organic Nutrients 2020), every 2 weeks (Midwest Mealworms 2021), (Vermont Mealworm Farm 2021), up to every 8 weeks (Boogie Brew 2020). The application of frass at inappropriate rates can greatly affect crops. However, in the industry, one can easily find misleading claims about its application. For example, some manufacturers suggest that it is impossible for the frass to burn the plants. As with other mineral or organic fertilizers, the rate of application of frass should be controlled according to its fertilizing value. Too low, it can lead to nutritional deficiencies. Too high, it can have a deleterious impact on plant growth. For example, the application of BSF frass at a frass: soil ratio of 1:2 had a phytotoxic effect on corn plants (Alattar, Alattar and Popa 2016). Similarly, 20 tonnes per hectare of BSF frass significantly reduces the height of onion plants compared to an application rate of 5, 10 or 15 tonnes per hectare (Zahn and Quilliam 2017). (Zahn and Quilliam 2017) suspect that the considerable proportion of nitrogen in the form of ammonium in the BSF frass may stunt plant growth. Alattar, Alattar and Popa (2016) support this hypothesis and add a potential decrease in drainage due to the low porosity of the frass. Other signs of ammonium toxicity include discoloration of leaves, production 15-60% below usual levels, decrease in mycorrhizae and in the rate of germination and survival of seedlings (Britto and Kronzucker 2002).

Higher frass application rates increase soil electrical conductivity. Unless an excess of frass is used at the start of an experiment, plant growth generally decreases soil electrical conductivity, suggesting nutrient uptake from frass by plants (Zahn and Quilliam 2017). The organic matter content of frass-fertilized soil also increases significantly with plant growth, due to root and microbial development (Zahn and Quilliam 2017).

In Canada, application rates of frass as fertilizer should also meet the Canadian Food Inspection Agency (CFIA) Safety Standards for Fertilizers and Supplements (T-4-93). Due to health and environmental concerns, certain plant essenztial micronutrients (such as copper, molybdenum and zinc), may have maximum application thresholds. The concentration of other contaminants (other metals, dioxins, furans, *Salmonella* and faecal coliforms) is also subject to maximum thresholds (Canadian Food Inspection Agency (CFIA) 2020).

3.12 N mineralization rates for frass

The nitrogen mineralization rate determines the amount and the appropriate period for plant nutrient uptake. The nitrogen concentration in frass is directly proportional to the nitrogen content in insect food (Fielding, Trainor and Zhang 2013). Indeed, the availability of nitrogen and the microbial activity of the amended soil vary significantly depending on the raw material used to raise the insects (Gebremikael, Wickeren et al. 2022). The rate of nitrogen mineralization from frass can therefore directly influence plant growth. Results of a study comparing frass to commercial fertilizers showed that the corn crop had higher nitrogen uptake in plots treated with BFS frass than in plots treated with commercial fertilizers or urea (Beesigamukama, Mochoge et al. 2020). Other researchers have also shown that nitrogen is available mainly in the form of ammonium in soils amended with BSF frass, whereas soils treated with commercial fertilizers have a higher concentration of nitrates. Moreover, periods of net nitrogen immobilization are shorter (30 to 60 days) in soils treated with frass compared to soils treated with commercial fertilizers (60 to 95 days). The mineralization rate is 3 to 10 times higher for soils treated with frass. Nitrification is also 2 to 4 times greater in these soils. Nitrogen release increases 3-fold in the soil with repeated applications of frass. Therefore, frass has a very positive effect on the rate of nitrogen mineralization and the growth of plants accordingly (Beesigamukama, Mochoge et al. 2020). Nevertheless, BSF frass was found to reduce N total leaching by 87% compared to isonitrogen conventional fertilizer application (0.3 kg/m^2) in potted coleus (*Plectranthus scutellarioides*) plants (Beasley, Kuehny et al. 2023). Though organic fertilizer may have slower N release rate (through decomposition of complex biolomecules) compared to synthetic fertilizers, perhaps limiting plant production speed, many producers are moving towards slow release forms to minimize N losses, and frass may have interesting qualities in this respect (Beasley, Kuehny et al. 2023). The rate of mineralization would therefore have an impact on the use of frass as a slow-release fertilizer (Beasley, Kuehny et al. 2023).

Research shows that chitin also provides mineral N after the mineralization of organic N by the decomposition of chitin. For example, insects reared on a diet low in protein consume their exuviae (rich in chitin) and produce frass with a high nitrogen content (more than 58% of the nitrogen present in the exuviae was recycled into the frass). This demonstrates that insects digest the nitrogenous compounds contained in chitin (Mira

2000). In addition, extracellular fungal hydrolytic enzymes, such as proteases and chitinases, present in frass also play a key role in the release of organic nitrogen. Since they are able to decompose organic nitrogen so that it can be assimilated by plants (Sánchez 2009).

4.0 History of frass

4.1 Documented history of frass use in agriculture in North America

In North America, the use of frass in agriculture has been documented in the popular literature for over a decade, but there are few references to this history in scientific publications. In the United States, the harvesting of cricket frass from live bait farms was the subject of an episode of the mythical television series Dirty Jobs (Purinton 2010). It has not been possible for the authors to trace the exact timing of the first sales of frass in Canada. The British Columbia company Enterra, founded in 2007 and closed in 2023, marketed frass as of 2015 and obtained product approval in 2021 (Thompson 2021). The EntomoFarms company in Ontario, founded in 2014, has been giving its frass since its founding and began selling it in 2021 with a large banner of retail stores (Goldin 2021). On the European side, the first marketing approval was obtained in 2020 for mealworm frass by the company Ynsect (FarmŸng 2020). Under the Canadian Fertilizers Act, frass was marketed as a special fertilizer (without any particular need for certification or registration) until October 2023. At the time of writing, only 10% of the Québec (Canada) insect farmers had submitted frass registration applications to CFIA according to novel regulatory requirements (Table filière des insectes comestibles du Québec 2022).

The regulatory framework allowing the sale and use of frass which varies between different regions is reviewed in the following sections. In North America, the Canadian framework (section 4.2) is more restrictive than in the USA (section 4.3), and both countries have lower regulatory constraints to frass commercialisation than the European Union (section 4.4). Finally, as upcycling food waste with edible insects and the production and use frass evolves rapidly, a case study on the dynamics and stakeholders involved in province of Québec (Canada) is proposed (section 4.5).

4.2 Canadian Regulatory Framework

In Canada, the Fertilizers Act includes the Fertilizers Regulations which are enforced by the CFIA. A fertilizer is defined as a "Substance or mixture of substances, containing nitrogen, phosphorus, potassium and any other plant nutrient, manufactured or sold as such or represented as such.''(Agence canadienne d'inspection des aliments (ACIA) 2020) A supplement is defined as a "Substance or mixture of substances, other than a fertilizer, manufactured or sold to enrich soils or promote plant growth or the productivity of crops, or represented as being suitable for such purposes''(Agence canadienne d'inspection des aliments (ACIA) 2020).

Before October 26, 2020 (and during a 3-year transition period expiring in 2023), frass could be sold as a specialty fertilizer and did not require registration with the CFIA. As defined in section 1.16 of Schedule II to the former Fertilizers Regulations, manure is "the excrement of birds or other animals, dried and ground, with or without litter, containing at least 50% organic matter" (Agence canadienne d'inspection des aliments (ACIA) 2020). In its new version, there is no longer a registration exclusion for special fertilizers.

Additionally, the Fertilizers and Supplements Basic Ingredients List now includes a more restrictive definition of manure (list modification date 2020-11-13) targeting birds and mammals only. The CFIA considers that the presence of insect body fragments in insect droppings renders frass non-compliant with the definition of manure (Duvernay 2018). In addition, the agency considers the mention of insect manure to be misleading about the identity of the product and in contravention of section 19(2) of the Fertilizer Regulations (Saulnier 2020) . However, the presence of hair and feathers in manure is a common contaminant of cattle or poultry manure (Adhikari, Nam and Chakraborty 2018) and manufacturers of commercial products based on hen manure, for example, indicate that they have no constraints about the level of feather fragments present in their product (ActiSol 2020) . Frass being a new product, it seems that no representation by insect producers was made during the public consultation phase leading to the adoption of the new regulation. This text is therefore intended as a tool to raise awareness about frass, presenting its characteristics which, in our opinion, allow it to be considered as animal manure which should be excluded from the registration process.

In Canada, any fertilizer or supplement imported or sold must be safe for human, animal, plant and environmental health. Regardless of the level of evaluation (I, II or III), all products must at least provide a compliant label and a list of characteristics to be registered. The label must present a description of the product, the guaranteed analyses, the instructions for use and the warnings. Additional features to include are analysis of heavy metals and physico-chemical parameters (pH, organic matter, moisture content) (Agence canadienne d’inspection des aliments (ACIA) 2020). Most registrations are valid for 60 months after issuance. All products may be subject to surveillance (inspection, sampling, testing, verification of labels) and non-compliances are sanctioned by regulatory measures which may include stoppage of sale or prosecution. The price of the basic registration ($357.70 in 2020) can be increased if an evaluation of safety ($511.00) or efficacy ($255.50) is required, and the price minimum for the study of an application is $1022.00 (Agence canadienne d'inspection des aliments (ACIA) 2020).

The definition of an "animal metabolite" is not provided in the Fertilizers Act or Regulations, nor in the CFIA Registration Application Guide. From a biological point of view, a metabolite is any product formed for the maintenance of life, which could include feces(Teunissen, Verheul and Willemse 2018). However, its use is often reserved for small molecules secreted by cellular metabolism, rather than complex macromolecules excreted by living organisms, such as manure. In this sense, a manure may contain metabolites, but should not be classified as a metabolite per se.

4.3 US regulatory framework

In the United States, the regulations surrounding fertilizers are under territorial jurisdiction. Thus, despite the definitions of fertilizers provided by the federal agriculture (United States Department of Agriculture, USDA) and environment (EPA) agencies, and the regulatory standardization project initiated by several states (Association of American Plan Food Control Officials), each state can freely manage fertilizers (TSG Consulting 2021). The regulations are therefore heterogeneous across the country. The marketing of frass does not seem to be prohibited there or governed by special certification requirements (Hershiser 2020).

The Federal Agricultural Definition of Fertilizers refers to formulated or processed products that provide plant growth nutrients or beneficial bacteria capable of converting nutrients to plant-available forms (Subtitle B. Regulations of the Department of Agriculture, CFR 3201.22) (USDA). Organic fertilizers (biobased fertilizers) must contain in the finished product a minimum of 71% carbon (mass) from authorized organic matter. Fertilizers are also subject to regulation by the United States Environmental Protection Agency (U.S. Environmental Protection Agency, Resource Conservation and Recovery Act of 1976, section 6002). In addition, frass, which may result from the recycling of organic materials, can be favored by the federal regulations surrounding procurement (Comprehensive Procurement Guideline, 40 CFR 247.15) since it supports the use of products containing recycled materials by federal agencies.

In Canada, all residual organic materials used and sold as fertilizer, such as residues from the production of insects or frass, are governed both by a provincial and a federal framework. At the federal level, the Fertilizers Act and its implementing regulations aim to regulate their sale and marketing in Canada. While it has not been possible to identify specific regulations on frass in other Canadian provinces, the province of Québec is currently actively evaluating regulatory changes specifically for frass (section 4.5).

4.4 European regulatory framework

Prior to November 2021, the European legislation governing animal by-products (EU animal by-products) governed animal manure in general (Commission des Communautés Européennes (CCE) 2011). It defined manure as excrement including or not the urine of livestock (other than fish), with or without bedding (Article 3.20). Although frass is not explicitly referred to in this definition, several competent national authorities regulated frass as a generic category of “manure” (International Platform for Insects as Food and Feed (IPIFF) 2019). The conditions of use of frass therefore had to conform to those of other types of farm animal manure. This classification allows spreading on agricultural land without pre-treatment but required heat treatment at 70°C for 60 minutes for any other use (Annex XI, section 2, EC Regulation No 142/2011). This regulation also allows the use of frass by organic farmers (Commission des Communautés Européennes (CCE) 2008). Farmers wishing to apply it directly to their land must meet appropriate safety standards for biological and microbiological contaminants. Contrary to the generally favorable provisions of the European regulatory framework described above, several European countries very strictly regulated the conditions for the marketing of frass on national markets. Some even classified frass under “category 2” which excluded manures because frass may contain animal matter that is not strictly fecal in the frass, such as living or dead insects, feed residues and exoskeletons. This classification made the valuation of frass in these countries more uncertain. It required, for example, sterilization under pressure or at high temperature (133°C for at least 20 minutes, or 70°C for at least 60 minutes), if a competent authority demonstrated that these treatments reduce biological risks in a satisfactory manner. (Annex XI, section 2, EC Regulation No 142/2011)

(Commission des Communautés Européennes (CCE) 2008). This heterogeneous regulatory framework limited the possibility of upcycling this high-quality product and its commercialization on the European market (International Platform for Insects as Food and Feed (IPIFF) 2019). Thus, a large part of the frass produced in Europe could not be reused in agriculture and was intended for disposal or energy recovery (International Platform for Insects as Food and Feed (IPIFF) 2019). IPIFF had recommended the establishment of recognized standards to produce insect fertilizers at European level (2019). Amongst their recommendations were risk assessment for disease transmission allowing direct application to agricultural land, without conditioning. In addition to generating additional income for insect producers, agricultural valuation would increase the competitiveness and growth of the agricultural sector as well as increase its potential for circularity (2019).

After November 2021, insect frass was explicitly defined under Regulation (EU) 2021/1925 as ‘’a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5% in volume and not more than 3% in weight. Any product registered under different national guidelines had until November 2022 to comply to the new dead insects threshold (by mass or volume) as well as the heat treatment (1 hours at 70C) to reach microbiological thresholds when seeking authorisation from national competent authorities allowing the use of frass as organic fertiliser (International Platform for Insects as Food and Feed (IPIFF) 2023).

4.5 Dynamics and stakeholders driving the evolution of the Quebec provincial framework: a case study

The province of Québec (Canada) has been a pioneer in the North American edible insect industry and a fertile ground for upcycling organic waste with insects (Hénault-Éthier, Dussault et al. 2017). This sector is still expanding rapidly (Larouche 2022), and is likely the largest concentration of producers in a single territory in North America. In 2020, the 23 edible insect producers operating in the province of Quebec (Canada) were estimated to produce between 184 tonnes and 375 tonnes of insects (median of 279 tonnes) per year (Hénault-Ethier 2020). Considering that each ton of edible insects generates 2 to 4 tonnes of frass, multiplying insect by this factor, places frass production estimates between 368 and 1,500 tonnes per year in Quebec in 2020 (Hénault-Ethier 2020). A single new BSF farm opened in 2023 in Drummondville (Entosystem, Québec Canada) is expected to produce 50 tonnes of frass per day (Caron-Garant, Massenet et al. 2022). Admittedly, the production of frass in Quebec is still far from the total tonnages produced by conventional methods of recycling organic materials (215,000 tonnes of compost, 18,000 tonnes of digestate), but it seems important to bet on the development of outlets for this by-product before production expands. Composting was first and foremost developed as an organic materials management technology and initially little emphasis was placed on generating high-quality, value-added fertilizer products. Hence, only 47% of the compost produced in Quebec corresponds to category A or AA recognized as the best quality categories with unrestricted use (Recyc-Québec 2023). The main outlets for compost are the manufacture of potting media (39%) and landscaping and green spaces

(18%), far ahead of agriculture (10%), horticulture (11%) and retail (6%) (Recyc-Québec 2023). In Québec, the most likely market for frass is agriculture (Larouche 2022). However, sectorial associations like the *Association des éleveurs et transformateurs d'insectes du Québec* do not yet have a complete portrait of frass marketing and pricing. However, many regulatory uncertainties persist in Canada. In the province of Quebec, frass can be regulated as manure or fertilizer depending on the format in which it is sold and used ((Hébert 2015, Ministère du développement durable de l'environnement et de la lutte contre les changements climatiques (MDDELCC) 2018)).

A proactive working group made up of multi-sectorial stakeholders have identified particular geopolitical or marketing constraints and opportunities in this territory. In an effort to develop more specific frass regulation aligned scientific knowledge, the Frass Working Group from the Edible insect sector board in Québec (Canada) proposed relative risk intensity classification for frass which depends on the type of organic substrate used to feed the insects (Hénault-Ethier and Warburton 2023 (in press)). Beyond understanding the intrinsic risks that the frass material may pose, understanding the broader risk factors in the emerging insect production sector relying on organic waste inputs was addressed by multiple stakeholders in a work session from the Vitrine Entotechnologique (Hénault-Ethier, Fortin et al. 2020).

Since Quebec's environmental regulations have often spread to the rest of Canada and inspired other nations, a case study focusing on Québec's regulatory framework evolution is pertinent.

In the province of Quebec, the Environment Quality Act (EQA) and associated regulations more specifically govern organic matter recovery activities, including the processing, use and storage processes of these materials. However, recently at both the provincial and federal levels, the application of new regulations has changed the regulatory interpretation for the use and marketing of frass as a fertilizer, leaving some regulatory uncertainty. As regulations are constantly changing, the following paragraphs are intended to provide the current picture of the regulatory status of frass (as of March 2023), more particularly for the production, processing, marketing and use in the province of Quebec. To simplify the understanding of the applicable regulatory framework, we assume that the frass was produced from i) edible insects intended for the agri-food sector; and ii) residual organic materials used for insect diets are source-separated, free of contaminants, and have exclusively material from plant origin and from agrifood processing industries and/or unsold groceries.

At the provincial level, frass corresponds to a residual material within the meaning of section 1 of the EQA, whether it is defined as animal waste or not, whether it is composted or not, on the farm or not. It remains a residual material throughout its processing chain until it is legally recovered, recycled (spreading for example), or eliminated.

The Environment Quality Act (LQE, art. 20) specifies that activities for the recovery, elimination, treatment and storage of residual materials must not harm the quality of the environment, either by polluting surface or underground water, air or soil (Québec 2009). All activities likely to result in the release of contaminants into the environment (including the recovery of residual materials) must be subject to ministerial authorizations. Applications for authorization, a process still unknown to Quebec insect breeders, must be submitted to the regional offices of environment ministry (*Ministère de l'environnement et de la Lutte aux Changement Climatiques*, MELCC) depending on the location of the future breeding or place where the frass will be stored, treated and valued. However, certain activities may be exempt from authorization or be eligible for a declaration of compliance. According to the framework in force, the activities exempted from authorization are either by regulation or through administrative exemptions listed in guides and guidelines published by the MELCC.

Currently, the MELCC does not specifically name the frass as such in its frameworks or in the guides that accompany them. Also, the obligations for authorization requests and the main exemptions will depend on the recognition of frass in the definitions of residual materials found through the various frameworks. From our understanding, as long as this material is not directly named, it could be subject to interpretation depending on the case and the context. The MELCC is currently processing requests for information from insect breeders on a case-by-case basis and different scenarios remain applicable. Following various exchanges with the competent authorities, the two most likely scenarios are presented here. However, in view of the complexity of the application of the current framework and considering that the evolution of the regulations could modify these scenarios, it is recommended that all producers validate their understanding of the framework with the regional management of the MELCC attached to its place of production or valuation of frass.

Currently, two scenarios for the definition of frass are identified through the regulatory framework. The frass could therefore be recognized as:

a) **Animal manure** within the meaning of the Regulation respecting agricultural operations (*Règlement sur les exploitations agricoles*, REA) (MELCC 2021);

b) **Residual material containing animal waste**, governed by the Guide to the recovery of fertilizing residual materials (*Guide de valorisation des matières résiduelles fertilisantes*, MRF) (Hébert 2015).

The list of authorization request obligations and exemptions identified for frass-related activities according to these definitions are listed below.

A. **Frass assimilated to the definition of animal waste**

The regulatory recognition of frass as animal waste within the meaning of the REA or as a material assimilated to this definition, is surely the most facilitating regulatory scenario for the sector. Indeed, under the Regulation respecting the supervision of activities according to their impact on the environment (*Règlement sur l'encadrement d'activités en fonction de leur impact sur l'environnement*, REAFIE), this recognition would imply authorization exemptions for the direct spreading of non-composted frass (art. 274), their composting (art. 279) and the spreading of the resulting compost (art. 274) (MELCC 2020). Should frass be recognized as an animal manure, it would be useful to determine the terms of references for volumes generated and nutrient concentrations per unit of production, a value commonly used for other livestock to facilitate environmental management (Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ) 2020).

However, even if they are exempted from the authorization request, these activities are still subject to certain restrictions in relation to their nature of animal waste and the associated risks (dissemination of pathogens for example). These restrictions are listed in the REA (art. 29.1, 30, 31 for spreading) and the regulation on water abstraction and protection – (*Règlement sur le prélèvement des eaux et leur protection,* RPEP, art. 63, 64, 71 for spreading and art. 59, 60, 62 and 71. for composting (MELCC 2021)).

Obviously, this definition could only apply to edible insect farms integrating non-contaminated residual materials of plant origin into their recipes in order to be as close as possible to the scenarios currently found in other agricultural farms.

A. Frass not defined as animal waste within the meaning of the REA

In the event that the frass would be considered as a residual material containing animal waste, and not only as animal waste, but the application of authorization exemptions becomes more limited.

Thus, for storing and using uncomposted frass as a fertilizing residual material (FRM) would be governed by the *Guide sur les matières résiduelles fertilisantes(Hébert 2015)*. Because frass is not explicitly defined as a FRM in this guide, it would be considered as all “other residues”. Thus, before obtaining an authorization for its use as a fertilizing material, it would theoretically be necessary to demonstrate its fertilizing properties (Table 7.1 of the Guide). However, since various studies have already established a fertilizing profile for frass (see the section on fertilizing value), this demonstration may be simple.

Once the FRM status has been confirmed for frass, the environmental impact and acceptability of incorporating it into the ground must be assessed. Thus, the content of chemical contaminants (C), pathogens (P), foreign bodies (E) and its category of odors (O) must be evaluated by the producer submitting an authorization request to the ministry (see table 6.1 of the Guide for the analyzes required). The quality 'rating' for each criterion (C, P, E and O) takes into account the content values characterized, but also the risk factors related to its storage and use. This categorization will determine which storage and use conditions are acceptable for each product with respect to the RFM Guide, but also according to the the RPEP and the REAif spreading on agricultural land is intended.

In compliance with the regulation in place, another authorization would also be required to compost frass (see section 14 of RFM Guide). Compost on a farm may allow treatment of farm products and exogenous materials (produced off the farm) if the maximal volumetric capacity of 1000 m^3 is always respected (including for the material stored, under active composting and the finished products). In this scenario, the frass could however be assimilated to the definition of farm product if necessary.

For industrial composting, above 1000m^3 or not on a farm site material not exempted from an authorization, another guideline for the supervision of composting activities must be followed (Ministère du développement durable de l'environnement et de la lutte contre les changements climatiques (MDDELCC) 2018). Once composted, the status of frass is clarified at the regulatory level: it becomes compost. Because compost is a designated FRM whose fertilization potential has already been demonstrated (FRM Guide, tables 7.1 and 6.1), it is no longer necessary to demonstrate the exact fertilizing properties of composted frass. However, its use is still governed by the MRF Guide and is defined by the compost quality criteria (see MRF Guide, 2015, sect.4; sub-section 4.3.2 and 4.3.3.

The regulatory framework allowing the use of frass is strongly dependent on its definition. If frass is not considered as animal waste (under REA), it could be defined as an RFM and listed (like it is already the case for composts). This scenario would make it possible to allocate by default certain exemptions or declaration of conformity according to the environmental quality of the frass (classification C, P, O and E) thus simplifying the process of activity and valuation for breeders.

B. Valorization of frass exempted from authorization: the exemption of small volumes of 'fertiliser'.

Until very recently, one of the provincial exemptions used by Quebec insect producers for the marketing of frass as a fertilizer was the administrative exemption associated with the marketing of frass sold in small containers (<50 L) in accordance Federal Fertilizers Act (Agence canadienne d'inspection des aliments (ACIA) 2020). In fact, the use of an RFM marketed in small volumes in accordance with the regulatory requirements of this law (composition and labelling) is exempt from authorization at the provincial level (FRM Guide, Table 4.3). However, this exemption requires bagging of frass (50L and less) which can be limiting in the context of the production of large volumes of frass. Finally, the recent reform of the Fertilizers Act and associated regulations complicates the registration procedures at the federal level for frass as of October 2023 (see next section) which could limit the use of this exemption for the smallest producers.

5.1. Tolerances of whole or fragmented insects in frass

The presence of fragments or whole insects should not be an eligibility criterion to restrict the use of frass or its categorization as animal manure.

The presence of whole insects or fragments is inevitable in the frass. Indeed, it has been verbalized that the fragments of insects contained in the frass could make it non-compliant with the definition of manure both in Europe (Regulation (EC) 1069/2009) and in Canada (Lyette 2020). For this purpose, it has been proposed that frass should not be defined as a mixture containing excrements and parts of dead insects and feeding substrate, but rather more simply refer to it as the substrate in which edible insects live (European Commission (EU) 2021). In Europe, tolerances for dead insects and eggs are limited at 5% by volume or 3% by weight. This intolerance to insect fragments in their manure is asymmetrical to their tolerance in the food sector, where they are quantified in number of identifiable fragments per mass, rather than percent per volume or per mass. Significant amounts of insect fragments are tolerated in foods directly intended for human consumption (US Food and Drug Administration (FDA) 2019). Thresholds vary depending on the type of food but can reach up to 10 insect fragments per gram of powdered dry food (i.e., ground thyme) and up to 250 whole insects per gram of vegetables (i.e., hops) depending on the FDA (2018). The thresholds for different products are presented in Supplementary Table 3. Moreover, manures and composts are also generally naturally colonized by several insects, and their use is welcome in agriculture (Mustin 1987) .

In addition to tolerances for contaminants of an entomological nature, there are also tolerances in foods for contaminants originating from rodents (e.g., hair count) which are indices of contamination by well-known pathogen vectors. While the presence of animal fragments is framed in food, it does not appear to be in farm animal manure. The presence of hair is indeed inevitable in cattle manure or pig manure, just as the presence of feathers is in chicken manure. According to chicken manure merchants (ActiSol 2020), no threshold or eligibility criteria have been established by the government for the presence of feathers in their products. Insect producers therefore seem to be faced with a different argument from the authorities, possibly because frass is a relatively new product on the market.

Drawing inspiration from the standards applicable to food, standards could be set concerning the permitted content of fragments or whole insects, living or dead, in the frass. For live larvae that can disperse in the environment or insect eggs, we should aim for their total eradication. Since dead larvae are difficult to remove completely with the mechanized sieving methods currently available on the market, a maximum tolerance threshold could be set, for example at less than 2% of the total mass of the frass (International Platform for Insects as Food and Feed (IPIFF) 2019). Dead insects, their fragments and their exoskeletons, being present in large quantities in the frass and containing appreciable levels of nutrients, can be useful for plant production and soil health. It would be wise to assess whether their presence entails more advantages or risks, particularly in terms of biosecurity.

5.2 Optimization of methods for eliminating whole insects or fragments

IPIFF recommends mechanized sieving and removal of live larvae that may remain in the frass (International Platform for Insects as Food and Feed (IPIFF) 2019). The frass obtained by fine sieving (420 µm) should contain mostly excrement, with no residue of insect fragments or eggs. Mealworm eggs are 1.7–1.8 mm long and 0.6–0.7 mm wide (Ghaly and Alkoaik 2009). Fine sieving would therefore remove the smallest life stage of the mealworm from the manure, thus ensuring the absence of viable insects in the frass.

Some Quebec insect producers finely grind frass to incorporate insect fragments (Vitrine Entotechnologique 2020). This practice reduces the quantity of rejects by making it possible to recover these by-products directly from the frass. Grinding could also help eliminate eggs or live insects that can disperse in nature during outdoor spreading, especially in mealworm farms. According to the production methods for BSF, spawning takes place in different aviaries than the feeding tanks intended for the fattening of the larvae from which the frass comes. It is therefore unlikely that BSF eggs will be found in the frass harvested there.

Each producer should be able to choose the most appropriate techniques for his production to achieve the objectives set in terms of allowable contaminants.

5.3 Rigorous safety monitoring and risk reduction treatments

Rigorous monitoring of the chemical and microbiological parameters of frass is recommended to ensure product safety (International Platform for Insects as Food and Feed (IPIFF) 2019). The evaluation criteria could be based on those set for fertilizing residual materials or composts.

In Canada's *Fertilizer Regulations*, manure must have been subjected to "a physical, chemical, or biological treatment, including composting, or a combination thereof, capable of mitigating the presence and effect of harmful or generally harmful substances that may be associated with unprocessed forms of these materials” (Canadian Food Inspection Agency (CFIA) 2020). These treatments are intended to increase the safety and stability of the amendments, but they can also alter the nutrient content.

The amount of information required during registration depends on the risk profile of the product. A product that may present disparities in its characteristics from one sample to another is classified as a level II risk and requires analysis results. One whose risks are unknown is classified at level III and evidence of safety and additional data must be provided in the application for registration (Canadian Food Inspection Agency (CFIA) 2020). Manure is *de facto* excluded from a registration obligation because there is no mention of manure in the requirements specific to ingredients to demonstrate their harmlessness. This absence obliges the interpretation of the regulation by referring to ingredients which could have common properties (ie derived from a living organism or from the transformation of residual matter, rich in organic matter), but which are not in all stitches consistent with the properties and characteristics of frass. Considering the fertilizer function of frass, it can be classified as a level II risk because the potential impacts and control mechanisms of similar materials (composts, flours or organic waste) are known. Thus, similar requirements could be applied, such as screening for metal contaminants (As, Cd, Co, Cu, Cr, Hg, Mo, Ni, Pb, Se and Zn) and pathogenic organisms (*Salmonella* spp. and faecal coliforms). If the frass rather corresponded to the definition of an organism metabolite, it would be classified at level III and would require an additional assessment including the characterization of the toxicological hazards such as the hazard profile for mammals (acute, chronic toxicity, carcinogenicity, endocrine disruption, etc.) (Canadian Food Inspection Agency (CFIA) 2020).

European studies (International Platform for Insects as Food and Feed (IPIFF) 2019) suggest that the microbiological contamination of the frass of certain insects, particularly that of mealworms, is relatively low. For example, levels of *E. coli*, *Salmonella* spp. nor *Clostridia* spp. exceeded the detection limit of 10 CFU g^{-1} in MSN frass (Setti, Francia et al. 2019). Contamination is often below the normal values observed in manures from other farm animals and composts. However, the frass has already been recognized as a potential vector of pathogens. *Erwinia* bacteria *tracheiphila*, a plant pest, could be detected in striped cucumber beetle frass from 24 to 96 h after consumption of inoculated food(Mitchell and Hanks 2009). Coliform bacteria have also been detected in frass obtained from insects fed cereal diets (Setti, Francia et al. 2019) .

Bacteria typically regulated in North America include *E. coli* (<1000 colony forming units-CFU/g) and *Salmonella* spp. (absence in 25g), while in Europe, *Enterococcus* spp . is also standardized (< 1000 CFU/g) (Commission des Communautés Européennes (CCE) 2011). Non-compliance with microbiological thresholds could lead to the impossibility of applying or marketing the product or requiring subsequent heat treatment to achieve satisfactory microbiological criteria depending on the desired use. A heat treatment of 70 °C for 60 min is proposed to reduce harmful organisms in insect frass, based on EU regulations ((EU) No. 2021/1925). However, heat treatments reduce the presence of microorganisms that can be beneficial to plant (Arabzadeh, Delisle-Houde et al. 2022) and soil health. They thus represent significant financial and environmental costs but associated with limited benefits. Heat treatments can also alter the fertilizing value of the product. The establishment of treatment parameters specific to insect frass, including an evaluation of conditioning temperatures, appears necessary.

6. CONCLUSION

Insect frass is a circular economy product with considerable potential for agricultural, horticultural or soil regeneration applications. It can also be used in organic farming. By its fertilizing value and its synergetic action, it can be used as a partial or even total replacement of mineral and synthetic fertilizers whose ecological footprint is important. Its content of micronutrients and various organic compounds, including chitin, could also make it a potential pesticide while improving the resistance of plants to abiotic stresses, the structure of the soil and its microbiota.

Biologically, frass corresponds to the definition of manures and although this is recognized in European regulations, recent changes in Canadian regulations exclude frass from the definition of manures, which seems biologically inadequate. Regulations still need to be developed and harmonized to oversee its use and minimize biosecurity and health risks. Several companies are already marketing the frass of different insects, and the tonnages produced are expected to grow in the years to come with the expected growth of edible insect farms in both North America and Europe. Despite studies carried out by private companies, few data on fertilizing values, application rates, microbiological profiles, impacts on plants and soils and acceptability for different applications have been published in the scientific literature. It is therefore recommended that governments invest heavily in research projects to better understand these properties. Research should also enable the development of packaging methods and standards to preserve the nutritional value and beneficial microorganisms of crops. The synergies with mineral fertilizers, humic acids and biological control agents, as well as their potential to reduce the use of different pesticides, represent a strategic interest for sustainable agriculture.

Declaration of competing interest

The authors declare the following competing financial interest(s): Louise Hénault-Ethier, Alexis Fortin, Étienne Normandin, Guillaume de La Rochelle Renaud are co-founders, and Noémie Hotte was affiliated with TriCycle, an enterprise that produces and markets mealworm frass and edible insect protein. However, all authors confirm that this scientific paper conducted in collaboration with different academic institutions respected the ethical and academic integrity standards. Other authors declare that they have no conflicts of interest regarding this study.

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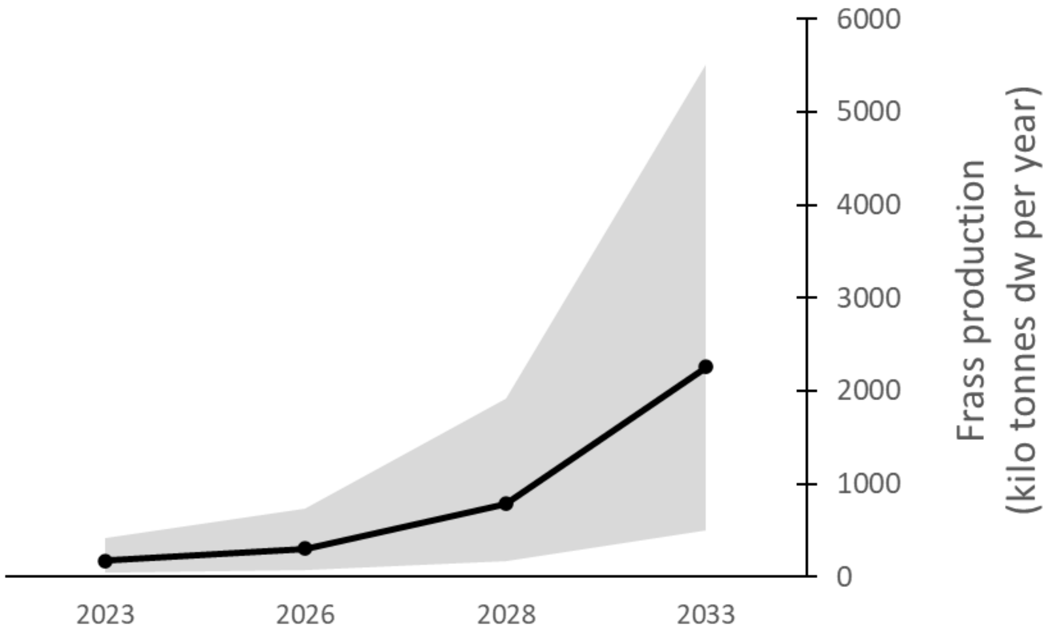

Figure 1 1: Projected growth of frass production on North American markets based on a 2023 NACIA member survey (n = 16). The grey zone delimitates the minimal and maximal production, while the dotted black line is based on the median of numerical classes proposed in the survey answers. Figure produced by the authors based on values obtained through a personal communication with Aaron Hobbs, NACIA coordinator, august 2023.

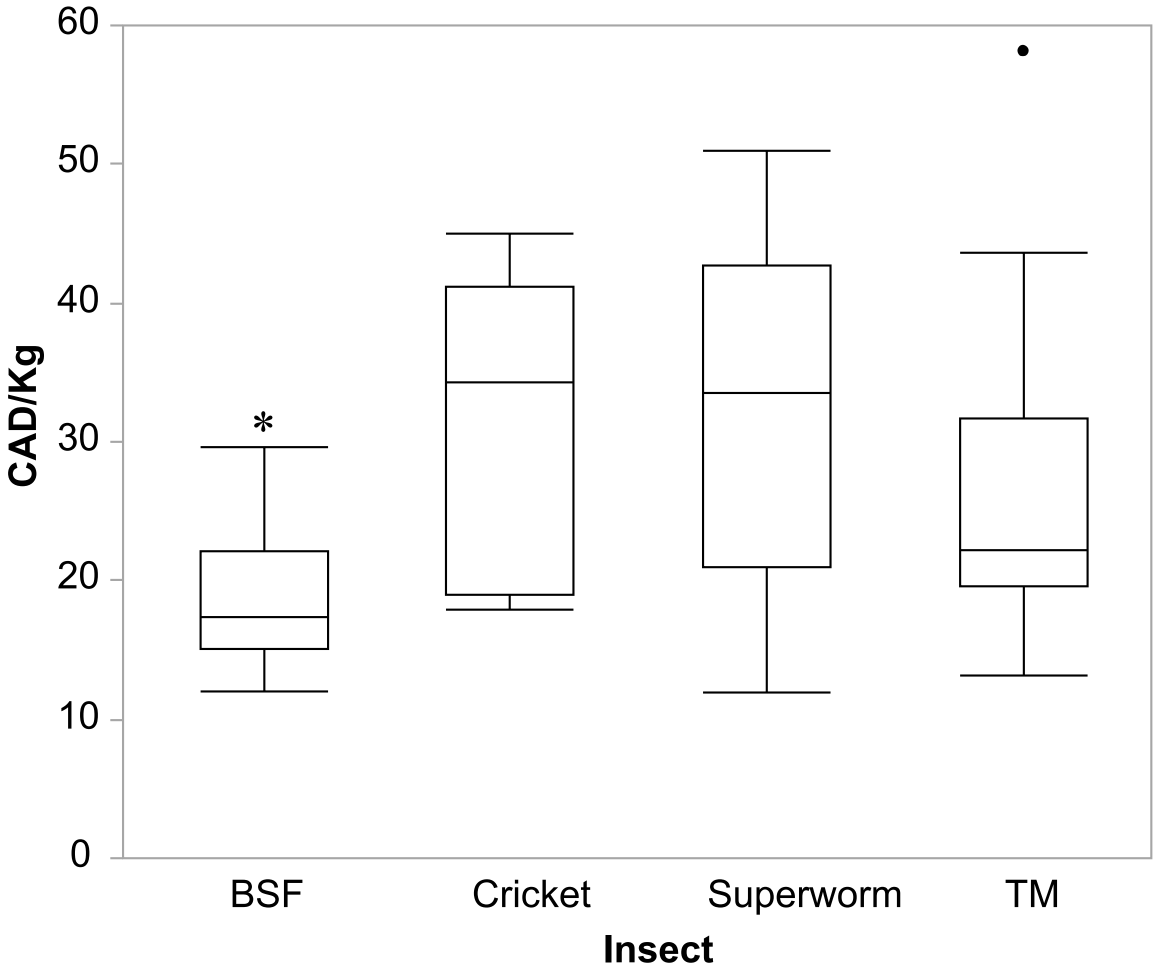

Figure 2: Average retail price of frass from different insect species sold in North America. For BSF n = 20, Cricket n = 5, Superworm n = 5 and Tenebrio molitor (TM) n = 16. Insect species significantly influence frass prices (X^2, p = 0.0104).

Table 1: Intrinsic risk categories or target exposure groups for frass produced through upcycling organic waste with insects.

Category	Examples
Biological	• Bacteria like *E. coli*, *Salmonella*, Coliforms • Viruses • Parasites
Chemical	• Organic contaminants like pesticides, antibiotics, persistent organic pollutants • Inorganic contaminants including trace metal elements • Odours
Physical	• Sharp or cutting material like glass • Fragments of plastic or microplastics
Occupational	• Ingestion of unprocessed organic waste or frass leading to biosafety concerns • Dermal contact triggering local irritation or sensitization • Ocular contact leading to infection • Inhalation contact leading to respiratory difficulties or secondary bacterial or fungal infection

Table 2: Minimum and maximum nutrient content of frass according to values reported by studies and commercial labels. Complete references are available in Supplementary Table 1.

Insect	**n**	**N (%)**			**P (%)**			**K (%)**		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
BSF	30	3,2	2,0	5,6	2,1	0,7	8,7	2,2	0,2	8,1
TM	21	3,3	1,6	5,0	3,2	0,4	5,0	2,2	0,7	3,5
Cricket	4	5,2	3,0	7,0	3,5	2,0	4,0	2,6	2,0	3,2
Superworm	2	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0

Table 33: *Physicochemical characteristics of frass from different insect types on different diets based on experimental data or commercial products available in North America. Insect diets marked with an asterisk were obtained from agri-food sidestreams. Control feed and virgin grain diets examples are provided as a reference.*

Insect	Insect diet	Origin of the analyzed frass	N (total) (%)	P (P_2O_5)	K (K_2O)	pH	Density (g/L = kg/m^3)	Residual moisture (%)	References
	Chicken feed	Experimental, Unpublished data	3.30	2.65	1.67	5.95	561	7.3	(Pappuciyan 2020)
	Wheat bran*	Experimental, Montreal Biodome, QC, CAN	2.89	2.63	1.56	n/a	319	8.7	(Hénault-Ethier, Marquis et al. 2018)
	80% wheat bran* + 20% mycelium*	Experimental, Montreal Biodome, QC, CAN	2.40	1.87	1.25	n/a	320	8.8	(Hénault-Ethier, Marquis et al. 2018)
Mealworms	40% wheat bran* + 60% mycelium*	Experimental, Montreal Biodome, QC, CAN	1.59	0.77	0.70	n/a	319	8.5	(Hénault-Ethier, Marquis et al. 2018)
	Wheat bran* + mix of fruits & vegetables*	Commercial, FertiFrass, TriCycle Inc. Montreal, QC, CAN	3.17	3.65	2.21	5.84	358	8.7	(TriCycle Inc. 2020)
	Wheat bran* + mix of grains + mix of fruits & vegetables*	Commercial, FertiFrass, TriCycle Inc. Montreal, QC, CAN	3.3	4.3	3.0	6.14	427	15.22	(TriCycle Inc. 2020)
	Unknown	Les engrais Ofrass, Insectes Intrinsekt Stoneham, QC, CAN	4.0	4.7	2.7	5.92	526	8.81	(TriCycle Inc. 2020)

	Unknown	Ofrass Fertilizers, Intrinsekt Insects Stoneham, QC, CAN	n/a	n/a	2	5.6	533.3	10	
	Wheat bran + organic carrots	Commercial, Space Coast Mealworm Palm Bay, Florida, USA	4.17	2.70	1.64	7.24	n/a	n/a	(Space Coast Mealworms 2021)
	Wheat bran + potatoes	Commercial, Vermont Mealworm Farm Braintree, VT, USA	3.22	4.40	2.88	n/a	n/a	n/a	(Vermont Mealworm Farm 2021)
	Unknown	Commercial, Chubby Frass WA, USA	2.0	3.0	2.0	n/a	n/a	n/a	(Chubby Mealworms 2021)
	Unknown	Commercial, Grow Frass, Quebec, QC, CAN	5	3	2	5.7	n/a	<< 10	(Grow frass 2021)
house cricket	Unknown	Commercial, Frass Forward Entomo Farms, Norwood, ON, CAN	6.97	4	3.2		n/a	n/a	(Entomo Farms 2016)
	Pre-consumer food waste from grocery stores*	Experimental, Laval University, Quebec, QC, CAN	2.7	0.7	4.1	n/a	n/a	n/a	
BSF	50% Cereals Gainsville Diet – 50% Pre-consumer food waste from grocery stores*	Experimental, Laval University, Quebec, QC, CAN	2.4	1.7	3.4	n/a	n/a	n/a	(Kone 2020)
	100% Cereals Gainsville Diet	Experimental, Laval University, Quebec, QC, CAN	5.6	8.7	8.1	n/a	n/a	n/a	(Kone 2020)

Mixed Cereal Residues*	Commercial, Entosystem Sherbrooke, QC, CAN	3.4	1.1	2.3	n/a	n/a	n/a	(Entosystem 2021)
Unknown	Commercial, Build A Soil Premium Insect Frass, Montrose, CO, USA	3	4	2	7.87	n/a	n/a	(Build a Soil 2021)
Unknown	Commercial, Boogie Black, Boogie Brew Rohnert Park, CA, USA	4	2	2.5	n/a	n/a	n/a	(Boogie Brew 2020)
Pre-consumer food waste from grocery stores*	Commercial, Down-to-Earth, Insect Frass Eugene, OR, USA	3.0	1.0	2.5	n/a	n/a	n/a	(Down to Earth 2020)
Unknown	Commercial, BSFL Frass, Obie's Worms, Halifax, NS, Can	2.5	0.7	1.3	5.5-7.0	n/a	n/a	(Obie's Worms 2020)
Unknown	Commercial, BSFL Frass, Enterra, Maple Ridge, BC, Can	2	1	1	6.5	460	<13	(Enterra 2020)
50% wheat bran, 30% alfalfa, 20% cornmeal	Experimental, Untreated	4.4	5.2	4.1	8.8	n/a	51.4	(Setti, Francia et al. 2019)
Brewery spent grain*	Experimental, Untreated	2.1	1.16	0.17	7.7	1350	30.1	(Beesigamukama, Mochoge et al. 2020)
Distillery stillage and spent grains*	Commercial, Enviroflight, Enviropro,	3	2	1	6.2 – 6.7	n/a	n/a	

n/a : not defined

Table 44: Micronutrient content of frass from different insects reared on different diets based on experimental data or commercial products available in North America.

Insect	Diet	Ca (%)	Mg (%)	B ppm	Cu ppm	Fe ppm	Mn ppm	Zn ppm	Reference
	40% wheat bran + 60% mycelium	3.54	0.24	n/a	14	228	78	40	(TriCycle Inc. 2021)
	80% wheat bran + 20% mycelium	1.59	0.3	n/a	16	217	130	79	
	100% wheat bran	0.12	0.34	n/a	14	158	162	103	
	Wheat bran + mix of grains + mix of fruits & vegetables	2.722	0.995	14.1	146.7	366.5	215.1	234	
	Wheat bran + mix of fruits & vegetables	0.340	0.790	2.6	21.2	160.9	185.4	118.6	
Mealworm	Bran 50%, Corn 50%	3.55	7.53	n/a	n/a	n/a	n/a	n/a	(Intrinsekt 2021)
	Bran 75%, Corn 25%	0.21	0.48	n/a	13	174	72	99	
	Bran 75%, Corn 25%	n/a	n/a	n/a	10	160	50	80	
	wheat groats	0.21	0.49	n/a	13	174	72	99	
	Cereals and vegetables organic	0.31	0.76	< 10	33	251	n/a	194	(Biotechnology 2021)
	Unknown	0.18	0.5	n/a	n/a	1000	n/a	n/a	(Grow frass 2021)
	Carrots and wheat substrate	3.55	n/a	n/a	10	150	190	90	(Critters and Crawlers 2021)
Cricket	Carrots, wheat substrate and source of protein	3.55	0.66	n/a	n/a	120	100	130	(Critters and Crawlers 2021)
BSF	Mix of grains, fruits & vegetables	0.36	0.45	10	20	1000	120	100	(Kone 2020)
	50% wheat bran, 30% alfalfa, 20% cornmeal	4.5	0.8	n/a	46.1	600	n/a	140	(Setti, Francia et al. 2019)

n/a : not defined

Table 55: *Comparison of characteristics between frass, conventional fertilizers and other organic amendments. For the sake of simplicity, parameters are presented in their simplest expression with a high-medium-low-none scale, but additional details and references are presented in the text. Adapted from Hénault-Éthier (2019).*

	Conventional Fertilizer	**Conventional Manure**	**Conventional Thermophilic Compost**	**Frass**	**Vermi-compost**
Humic acid content	None	Medium[a]	Medium[a]	Variable[b]	High[c]
Soil structure improvement	None[d-e]	Medium[f]	High[f]	High[g]	High[h]
Limits nutrient leaching	None [e]	Low[i]	High[i]	High[j]	High[k]
Storage Stability	High[l]	Low[m]	Medium [m-l]	High[n]	High[m]
Improved resistance to abiotic stresses	Low[e]	Medium[f]	High[f]	High[o]	High[p]
Protection against biological pests	Low[e]	Low[q-r]	High[q-r]	High[g]	High [s-t]
Phytohormone content	None	High [a]	High[a]	High [o,u]	High[v,c,a]
Microbial diversity	Low[d]	High	Medium[w]	High[o]	High[w]
Potential contaminants	- Heavy metals - inorganic acids - organic pollutants[y, e, x]	- Pathogens - Chemical contaminants [o,z]	- Pathogens - Heavy metals - Chemical contaminants [o, z]	- Pathogens - Heavy metals[aa]	- Zinc - Pathogens [o,z]
Pathogen control mechanisms in the product	-Thermal[bb]	- Thermal sanitation - Microbial interactions[m]	- Thermal sanitation - Microbial interactions[m]	- Thermal sanitation [cc] - Microbial interactions [dd]	-Microbial interactions[w]

Applicable in organic farming	No[z]	Yes[z]	Yes[z]	Yes[z,ee]	Yes[z]
Production of GHG (CO_2, CH_4, and N_2O)	High[ff]	Medium[gg, hh]	Medium[gg, hh]	Low[ii]	Medium[w]
Market price	Low[jj]	Low	Low	High[b]	High

References : a. (Pant, Radovich et al. 2012); b. Current paper and (Chen, Liu et al. 2023) c. (Arancon, Edwards et al. 2006); d. (Pahalvi, Majeed et al. 2021); e. (Pahalvi, Rafiya et al. 2021); f. (De Corato 2020); g. (Houben, Daoulas et al. 2020); h. (Ceritoğlu, şahin and Erman 2018) ; i. (Epelde, Jauregi et al. 2018); j. (Grey and Henry 1999); k. (Bagheri, Zare Abyaneh and Izady 2021); l. (Brar, Sarma and Chaabouni 2012); m. (Hénault-Ethier, Bell et al. 2015) ; n. (Beesigamukama, Mochoge et al. 2020); o. (Poveda, Jiménez-Gómez et al. 2019); p. (Canellas, Canellas et al. 2019); q. (Hénault-Ethier 2007); r. (De Corato 2020); s. (Bahramisharif and Rose 2019); t. (Xiao, Liu et al. 2016); u. (Zahn and Quilliam 2017); v. (Tripathi and Bhardwaj 2004); w. (Hénault-Ethier 2007); x. (Li and Wu 2008); y. (Environmental Protection Agency (EPA) 2021); z. (Office des normes générales du Canada 2020); aa. (Basri, Azman et al. 2022); bb. (Gellings and Parmenter 2016); cc. (Annex XI, section 2, EC Regulation No 142/2011); dd. (Liu, Tomberlin et al. 2008); ee. (Legault 2018); ff (Hasler, Bröring et al. 2015); gg (Boldrin, Andersen et al. 2009); hh (Walling and Vaneeckhaute 2020); ii (Pang, Hou et al. 2020); jj (Crespi, Hart et al. 2022).

Table 66: Recommended application rates for commercial insect frass.

Insect	Product name	Company	NPK	Per surface area (g/m²)		Per volume (Kg/m³)		For frass tea (ml/L)	Density (g/L)	Average equivalent kg N/ha		Reference
				min	max	min	max			min	max	
Cricket	Frass Forward	EntomoFarms	7-4-3	n/a	n/a	45.36[a]	68.04[a] –	20	n/a	n/a	11906.79	(Entomo Farms 2016)
Cricket	GrowFrasst	Ferme Grevio	4-3-3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(Grevio 2021)
Cricket	n/a	Les Grillonettes	5-4-3	176	269	n/a	n/a	n/a	n/a	111.25	n/a	(Charlot 2021)
Cricket	n/a	Alimentomo Inc.		179.404	269.107	n/a	n/a	n/a	n/a	0	n/a	(Alimentomo 2021)
Cricket	Ento Frass	Ferme Gryllus	5-3.2-2.2	n/a	n/a	22.68[b] –	45.36[b] –	20	n/a	n/a	5102.91	(Ferme Gryllus 2021)
n/a	Insect Frass	Organic Nutrients	2-2-2	n/a	244.128	1.33	2.67	33.02	n/a	48.83	120.12	(Organic Nutrients 2020)
BSF	Boogie Black	Boogie Brew	4-2-2.5	n/a	244.128	4.79	7.19	13.21	n/a	97.65	718.89	(Boogie Brew 2020)
BSF	Premium Insect Frass	Build a soil	3-2-4	n/a	n/a	0.67	1.33	n/a	453.592	n/a	90.09	(Build a Soil 2021)
BSF	Insect Frass	Down to Earth	3-1-2	48.826	97.651	1.20	2.40	n/a	283.5-302.4	21.97	161.80	(Down to Earth 2020)
BSF	n/a	EntoSystem	3-2-2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(Charbonneau 2020)
BSF	EnterraFrass	Enterra	2-1-1	n/a	250	4.79	7.19	n/a	460	50	359.44	(Enterra 2020)
BSF	BSFL Frass	Obie's Worms	3-1-1	100	400	n/a	n/a	75	n/a	75	0	(Obie's Worms 2020)

BSF	EnviroFrass	EnviroFlight	3-2-1									(EnviroFlight 2021)
mealworm	Ferti-Frass	TriCycle	3-3-2	n/a	600	2.67[c] –	5.33[c] –	15	533.333	180	360.00	(TriCycle Inc. 2020)
mealworm	AllNatural Insect Frass	Space Coast Mealworms	4.1-2.7-1.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0	(Space Coast Mealworms 2021)
mealworm	Grow Frass	Hexapoda	5-3-2	n/a	n/a	4.71	n/a	n/a	n/a	n/a	353.35	(Grow frass 2021)
mealworm	All pure Vermont mealworm frass	Vermont Mealworm Farm	3.22-4.40-2.88	n/a	n/a	2.36	4.71	16.51-33.02	n/a	n/a	341.34	(Vermont Mealworm Farm 2021)
mealworm	Frass Fertilizer	Chubby Mealworms	2.0-3.0-2.0	n/a	122.064	n/a	4.71	n/a	n/a	24.41	141.34	(Chubby Mealworms 2021)
mealworm	Frass & Furious	Grow Daddy		n/a	195.302	80.00 [d]	n/a	37.5 [f]	n/a	0	0	(Grow Daddy 2021)
mealworm	Insect Frass	Midwest Mealworms		n/a	244.128	4.71	5.33 [e]	n/a	n/a	0	0	(Midwest Mealworms 2021)
mealworm	Ofrass	Insects Intrinsekt	4-2.5-2	n/a	640	n/a	n/a	n/a	n/a	256	n/a	(Intrinsekt 2020)
mealworm	P-Frass™	Pholoho Biotechnology	4-4-3	30	80	2	n/a	n/a	320	22	2667	(Biotechnology 2021)

n/a : not available

Notes: Manufacturers recommended frass application [a.] from 10 to 15% frass by volume; [b.] 5-10% frass by volume; [c.] 0.5 to 1% frass by volume; [d.] 15% frass by volume; [e.] 1% frass by volume; [f.] 20g/L. For comparison purposes, the applications recommended by the manufacturers in % by volume are converted into g/m^3 using an average density of ρ = 453.6 g/L for crickets (Build a Soil 2021) and 533.3 g/L for mealworm (TriCycle Inc. 2020). Similarly, manufacturers' recommendations for gross weight of frass by volume or area were converted to equivalent units of nitrogen per hectare using the fertilizer value provided by the manufacturer and a standard soil depth for tillage of 30 cm (Gosselin, Lapointe and Tousignant 2000).