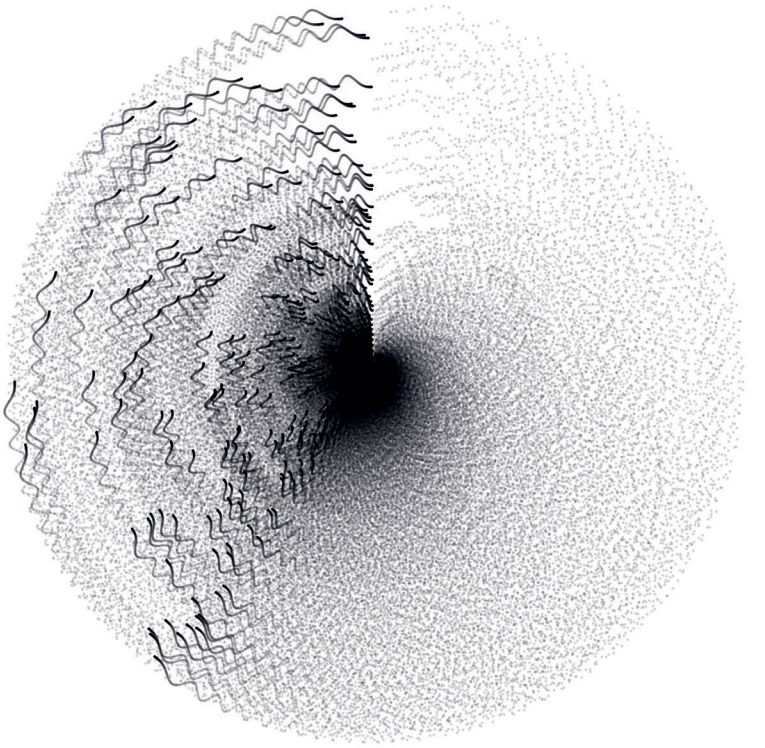
FARMED INSECTS FOR SUSTAINABLE FOOD SYSTEMS



Alejandro Parodi Parodi

Propositions

1. Black soldier fly bioconversion offers a novel opportunity to reduce ammonia emissions from manure.
   (this thesis)

2. There are no environmental benefits if farmed insects used as feed are fed with feedstuffs edible to livestock.
   (this thesis)

3. To stop global warming climate-policy needs carbon emissions reduction targets rather than net-zero carbon emissions goals.

4. The publishing model of high-impact journals poses a dilemma for researchers who care about open access and aim for societal impact.

5. The current "free" social media model leads to illusionary knowledge.

6. Adversities are imperative for good mental health.

Propositions belonging to the thesis, entitled

Farmed insects for sustainable food systems

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Wageningen, 17/10/2022

Farmed insects for sustainable food systems

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This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS)

Farmed insects for sustainable food systems

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Thesis
submitted in fulfilment of the requirements for the degree of doctor at
Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 17 October 2022
at 1:30 p.m. in the Omnia Auditorium.

Alejandro Parodi Parodi
Farmed insects for sustainable food systems
242 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2022)
With references, with summary in English and Spanish

ISBN 978-94-6447-255-4
DOI 10.18174/570891

To Cristina and Flora

Contents

Chapter 1

Introduction

1.1 Context

In a changing climate, global food systems need more than ever to be reconfigured to avoid further environmental degradation and safeguard future food security (Loboguerrero et al., 2020). The environmental impacts of food systems are diverse, but the main ones that require urgent action are climate change and biodiversity loss (Campbell et al., 2017; Kummu et al., 2021; Steffen et al., 2015). The production of animal-source foods (ASF) is a major contributor to these impacts, with most of them being generated during the production of feed, enteric fermentation and manure management (Gerber et al., 2013). TTackling the environmental impact of ASF is imperative to reroute the food system towards new trajectories.

In the search for sustainable food systems, alternative sources of food and feed that could contribute simultaneously to replace ASF or make their production more sustainable are emerging, and farmed insects are one of these. Expectations regarding the potential of farmed insects to contribute to a sustainable food system are high, as in addition to being an alternative source of food and feed, insects fed on residual streams (e.g. from crop production or manure) can upcycle nutrients into valuable macro- and micro-nutrients and thus contribute to the transition towards circular food systems. Despite the high expectations, there is a need for wide-ranging assessments that properly determine if insects can truly bring the environmental benefits that are expected and to identify potential trade-offs associated to their production. This thesis contributes to fill this knowledge gap.

1.2 Insect farming: an emerging sector

Insect farming is an emerging agricultural sector. Although some 2100 insect species have been traditionally consumed for millennia and are still part of the culinary culture of millions of people around the world (Jongema, 2017; Magara et al., 2021; Van Huis et al., 2013), nowadays insects are not only sourced from the wild but also farmed commercially. Insect production systems are diverse and range from high-tech systems with automatized production lines, nutritionally-customized diets and climate-controlled environments, to more rudimentary systems that depend on the seasonal availability of feedstocks, the local climatic conditions and availability of human labor (Figure 1.1). Insect farming is now practiced worldwide (The Insect Industry Map, 2022) and the sector is expected to keep growing steadily in the upcoming decades (Jong and Nikolik, 2021).

The increasing interest in insect farming for food and feed purposes is rooted in its potential to contribute to a food-secure and sustainable future. Insects contain protein, fat, and a diverse set of vitamins and minerals which are all vital for adequate human

Figure 1.1: Feed dispenser in an automatized black soldier fly larvae (BSFL) production facility in the Netherlands (top). Workers separating the remaining BSFL from frass in a semi-mechanized production facility in Uganda (medium). Small-scale BSFL production facility in Colombia. Pictures by: Lisa Zoet (top), Philipp Straub, Marula Proteen Uganda Limited (medium), Karol Barragán (bottom).

and animal nutrition (Rumpold and Schlüter, 2013). The production of these nutrients via insects is expected to bring environmental benefits as compared to conventional livestock since insects have lower feed-conversion ratios (Oonincx et al., 2015), shorter reproduction cycles (Tomberlin et al., 2002) and a lower environmental impact per unit of protein (Halloran et al., 2017; Oonincx and Boer, 2012). In addition, insect farming is expected be environmentally beneficial, especially when practiced in a circular way. In such systems, insects are fed with residual organic streams, and used to upgrade nutrients into a valuable source of macro- and micronutrients for humans and animals. The residual material obtained after insect rearing (i.e., mixture of uneaten feed, insect excreta and exuviae) is a source of nutrients and compounds that can promote plant growth and health (Barragán-Fonseca et al., 2022; Houben et al., 2020; Schmitt and Vries, 2020), and is already used as a fertilizer and soil amendment. Despite that most of the focus is nowadays on producing insects for food and feed applications, alternative uses such as biofuels (Li et al., 2011) pharmaceuticals (Xia et al., 2021) and cosmetics (Almeida et al., 2020) are also envisaged.

Commercialized insect products differ by species, insect life-stage, and degree of processing. The main known farmed insects destined for direct human consumption include the larvae of three beetle species from the family Tenebrionidae, namely *Tenebrio molitor* "yellow mealworm", *Alphitobius diaperinus* "lesser mealworm" and *Zophobas morio* "superworm", multiple cricket and locust species, with *Acheta domesticus* and *Gryllus bimaculatus* and *Locusta migratoria* being the main ones, and the larvae of the moths *Galleria mellonella* and *Bombyx mori* (Van Huis, 2022). These insects are sold whole, either in fresh or dried forms, but recently they are also being processed to be included in the formulation of snacks (Homann et al., 2017), and meat and dairy analogues (Kim et al., 2016; Smetana et al., 2018; Tello et al., 2021). Insects destined for animal feed applications include mostly the larvae of the fly species *Hermetia illucens* "black soldier fly", but also the larvae of *Musca domestica* "housefly", and mealworms. These species are commercialized whole fresh, whole dried and as grounded meal, either with the original fat content or defatted (Liceaga, 2021).

1.2.1 Black soldier fly

Among all farmed insect species, the black soldier fly (BSF) is the one receiving most of the scientific and commercial attention (Tomberlin et al., 2018). This species, now distributed worldwide but with genetic origin in central South America (Kaya et al., 2021), was categorized as a pest decades ago, and is now the main farmed insect (Tomberlin and Van Huis, 2020). Despite that most BSFL production is now destined as feed, its use for food applications has been proposed among scholars (Bessa et al., 2020) and food products are starting to appear in the market (e.g., milk analogues made with BSFL).

The interest in black soldier fly farming is rooted in the ability of its larvae (BSFL) to consume and reduce a wide variety of organic substrates in a relatively short time frame, making it an attractive circular strategy to upgrade the nutrients contained in waste streams into food, feed and crop fertilizers (Figure 1.2). In addition, BSFL bioconversion is considered to have potential for the management and valorization of unsafe organic streams, as it can reduce the presence of pathogenic bacteria (Lalander et al., 2015), degrade pesticides and antibiotics (Cai et al., 2018b; Lalander et al., 2016), and consume contaminated grains with mycotoxins without accumulation in its body mass (Bosch et al., 2017; Purschke et al., 2017).

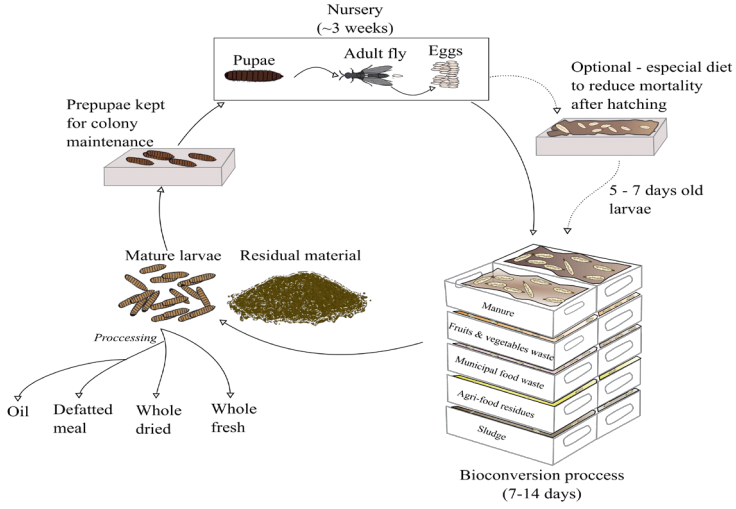

Figure 1.2: A circular black soldier fly (BSF) production system. Some producers apply strict spatial separation between adult nursery and the mass-rearing. While most BSF producers have their own nurseries to maintain their colonies, specialized farms that commercialize only eggs are emerging.

Despite the high dietary flexibility of BSFL, the substrate in which the larvae are reared has a direct effect on the bioconversion efficiency, larval performance, nutrient composition, and environmental impact. Substrate properties that influence the bioconversion efficiency and larval performance include volatile solids and protein content (Lalander et al., 2019), carbohydrate type and abundance (Cammack and Tomberlin, 2017; Cohn et al., 2022), pH (Meneguz et al., 2018), and moisture (Cheng et al., 2017). Furthermore, the substrate has large influence in the larval fat and ash contents, with substrates rich in digestible carbohydrates leading to larvae with higher fat (i.e., specially lauric acid) and ash contents (Barragan-Fonseca et al., 2019; Hoc et al., 2020; Spranghers et al., 2016). Instead, larval protein quantity and quality remains in a narrow range independent of the substrate used (Barragan-Fonseca et al., 2017; Spranghers et al., 2016). Nutrients like omega-3 fatty

acids can be accumulated in the larval biomass if present in the substrate (Oonincx et al., 2019).

Currently, BSFL are used as feed for pets, aquaculture, and monogastrics (i.e., chickens and pigs). In North America and Europe, insect producers have had pet food manufacturers as the main customers (Lähteenmäki-Uutela et al., 2021). However, new feed markets opened up due to recent changes in legislation that allow the use of some farmed insects, including BSFL, as feed for aquaculture, poultry and pigs (i.e., only in Europe) (EU Comission, 2021). Nonetheless, due to safety concerns (see James et al. (2022)), BSFL destined as feed for aquaculture and monogastrics can be fed only with safe feedstocks, which are usually those allowed to be used directly as livestock and aquaculture feed. Instead, in Africa, Asia and recently also Latin-America, BSFL farming is often coupled with organic residual stream management, and as safety regulations are less strict than in the EU, BSFL fed on substrates such as food waste and manure are used to feed fish, poultry and pigs (Tanga et al., 2021; Zhang et al., 2020).

Multiple studies show that BSFL can be included in livestock and fish diets without interfering with the normal growth of the latter. For instance, the dietary inclusion of BSFL did not compromise the growth, feed intake and conversion efficiency in salmonids, although replacement of fishmeal was associated with reduced growth rate and feed intake (Weththasinghe et al., 2021). For other fish species, however, the substitution of fishmeal did not have negative consequences for fish growth and performance (Caimi et al., 2021; Hender et al., 2021; Melenchón et al., 2022; Terova et al., 2019). For poultry, substituting conventional protein sources with BSFL did not reduce growth and performance, as long as the substitution was not higher than 10% (Moula et al., 2018). In addition, recent studies have found that BSFL inclusion in broiler diets improve animal welfare and health-related traits (Ipema et al., 2021; Ipema et al., 2020). Similarly, studies with pigs have showed that BSFL inclusion in pig diets did not affect growth and had indirect benefits for pig health (Biasato et al., 2019; Chia et al., 2021; Crosbie et al., 2020; Jin et al., 2021).

1.3 How could insect farming contribute to sustainable food systems?

1.3.1 Farmed insects as a production strategy

In the context of transforming food systems towards sustainable futures, the so-called production strategies aim to change parts of the production system to reduce the environmental impact of food per unit of product. BSFL are being proposed to be used as a production strategy to reduce ASF environmental impact in two ways. First, as feed

to replace high-impact and human edible feeds in livestock and aquaculture diets, and second, as a manure management strategy.

Farmed insects as feed

Livestock production contributes to the so called 'food-feed competition' by using 40% of the global arable land and consuming one third of the global grain production (Mottet et al., 2017). Similarly aquaculture production consumes 20 million tons of human edible fish every year (Cashion et al., 2017). In addition to being drivers for food-feed competition, monogastric livestock and aquaculture production rely on protein-rich sources such as soybean and fishmeal, which are associated to agricultural expansion and deforestation in tropical areas (Fehlenberg et al., 2017; Gasparri et al., 2013), and overfishing of marine fish stocks respectively (Naylor et al., 2009; Smith et al., 2010). Thus, finding alternative feeds for livestock and aquaculture without promoting food-feed competition and depleting natural resources is required to achieve more sustainable livestock and aquaculture production systems.

In this context, farmed insects such as the BSFL are proposed as a protein-rich and sustainable feed source for aquaculture, poultry and pigs. Life cycle assessments that compared the environmental impact of farmed insects at the product basis with conventional protein-rich feed sources found that, per kg of protein, insects can have a lower environmental impact but only when reared on residual organic streams (Bosch et al., 2019; Smetana et al., 2016; Smetana et al., 2019; Van Zanten et al., 2015b). This is especially the case for land use while the global warming potential of insects tends to be equal or lower than the those of the best performing conventional ASF sources.

Farmed BSFL and manure management

Manure is one of the major sources of nitrogen emissions during ASF production (Uwizeye et al., 2020). Manure-nitrogen emissions are an environmental and public health burden (Leip et al., 2015; Venglovsky et al., 2009), especially in livestock-dense areas with manure surpluses where livestock and feed production are spatially decoupled. Although reducing livestock numbers in livestock-dense regions would reduce the environmental and public problems caused by surplus manure, short term solutions that target the collection and recycling of the nutrients contained in manure are urgently needed.

Feeding farmed insects with manure has emerged as an effective method for manure management. Insects such as the BSFL and housefly larvae are used to reduce manure volumes in a short time (Zhang et al., 2020; Zhang et al., 2012), and recover and upgrade nutrients in manure into larval biomass and fertilizers. While the larval biomass can be used as livestock and aquaculture feed, the presence of parasites and pathogenic microbes

from the family Enterobacteriaceae in the obtained larval biomass generate safety concerns (Khamis et al., 2020; Müller et al., 2019; Van der Fels-Klerx et al., 2018), especially if fed unprocessed to animals. Different interventions have been suggested to minimize risks of disease transmission when manure-fed larvae are used as feed, such as pre-treating manure, processing the larvae (e.g., heat treatment) and feeding other types of livestock or fish, than those that produced the manure in which the larvae were reared (Lalander et al., 2015; Van der Fels-Klerx et al., 2018). However, the effectiveness of those interventions still needs to be tested. Even if not used as feed, manure bioconversion with insects have other additional benefits which makes it an attractive manure management strategy. The residual material after manure bioconversion is a compost with better humification properties than fresh manure (Liu et al., 2019; Wang et al., 2021) and with substantially lower abundance of pathogens (Awasthi et al., 2020; Wu et al., 2021). In addition, manure malodour production is reduced (Beskin et al., 2018), different antibiotics present in manure can be quickly degraded (Cai et al., 2018a), and the probability of selection for antibiotic resistance is reduced (Cai et al., 2018b; Lalander et al., 2016).

1.3.2 Farmed insects as a consumption strategy

Due to the large environmental footprint associated to the production of conventional ASF, consumption strategies have focused on promoting a diet with more plant-source foods, and no or less ASF (Springmann et al., 2016; Willett et al., 2019; Xu et al., 2021). However, ASF are nutrient-dense and key for the provisioning of macro- and micronutrients that are not always as readily available in single plant-source foods. In the search for nutritious and sustainable sources of food, farmed insects have been proposed as an alternative to replace ASF. Insects, being also nutrient-dense ASF, have been acknowledged by the Food and Agriculture Organization of the United Nations for their potential to contribute to healthy and sustainable diets (Van Huis et al., 2013). Insects are generally high in protein, variable in fat, mineral and vitamin contents depending on the species and substrate they fed on, and contain carbohydrates in the form of chitin, sugars and glycogen (Kouřimská and Adámková, 2016). Despite that edible insects are part of the food culture of millions of people in Asia, Africa and Latin-America, their contribution to the nutrition of those populations is largely unknown due to lack of data and information on insect supply and consumption (Roos, 2018). In western societies, consumer, regulatory and production barriers have limited the upscaling of the production of farmed insects for food applications (Belluco et al., 2017), however, efforts to alleviate such barriers are ongoing (Veldkamp et al., 2022). Environmental impact assessments show that farmed insects generally have lower environmental impacts than ASF per kg of protein, even when reared on feedstocks which are also edible for livestock. For instance, mealworms fed on mixed grains have a lower global warming potential and land use, but similar energy use compared with milk, chicken, pork and beef (Oonincx and Boer, 2012). Crickets fed on compound chicken

feed and BSFL fed on agri-food residues have been found to have a lower environmental impact per kg of protein than broilers in many impact categories (Halloran et al., 2017; Smetana et al., 2016; Smetana et al., 2019). Most of the impacts taking place during insect rearing are linked to the production of feed they are reared on, but energy use is also high especially when climate-controlled rearing environments are needed, and when larvae are dried to be sold whole dried or as meal.

1.4 Knowledge gaps

1.4.1 Nutritional and environmental potential of farmed insects compared to other sources

In the debate of transitioning towards a sustainable and healthy food system, novel sources of food, in addition to farmed insects, have been proposed as alternatives to animal-sourced foods (Post, 2014; Sillman et al., 2019; Van Huis et al., 2013; Wells et al., 2016). Despite the availability of different life cycle assessments that reported the environmental footprints of these sources (e.g., for insects Oonincx and Boer (2012), Salomone et al. (2017), Smetana et al. (2016), and Van Zanten et al. (2015b)), and a vast body of nutritional literature (e.g., see review by Barragan-Fonseca et al. (2017)), the existing evidence has not yet been consistently synthesized and analyzed to determine if farmed insects are a promising source of food that can provide similar nutrition with lower environmental impact than other novel and conventional food sources. One of the limitations for such analyses has been the lack of a common and standardized functional unit that facilitates the comparison among the available studies, and the fact that most environmental impact assessments focused solely on protein and did not include other macro- and micronutrients contained in ASF which are essential for human nutrition. In addition to synthesizing the available evidence to compare the nutritional and environmental performance of farmed insects with other sources, it remains key to identify from the available environmental assessments under which conditions the environmental impact of insect farming was minimized, and what were the methodological limitations faced. This is needed to concentrate future research efforts in overcoming the limitations encountered, and to subsequently assess more accurately the potential of farmed insects as a sustainable source of food and feed.

1.4.2 Nutrient flows and dietary preferences during BSFL rearing

The BSFL are one of the preferred farmed insect species due to their potential to contribute to a circular food system by upgrading the nutrients contained in diverse residual organic streams as food, feed and fertilizers. There has been extensive attention for the bioconversion efficiencies of BSFL when fed on different organic residual streams (see

Bosch et al. (2019) for an overview). However, three topics closely coupled to the streams in which BSFL can grow, have received little attention.

The first are the gaseous losses when BSFL are reared on different substrates. Quantitative insights into nutrient flows and particularly emissions of greenhouse gases (GHG) during rearing of BSFL on different substrates is key for obtaining more accurate estimations of the environmental impacts of BSFL farming, and for understanding the temporal patterns of gaseous emissions and proposing interventions to reduce nutrient losses during the larval rearing phase. Many of the studies that assessed the environmental impact of BSFL using a life-cycle approach omitted the direct GHG emissions occurring during larvae rearing (Salomone et al., 2017; Smetana et al., 2019) or used data generated for other species reared on substrates different than the ones evaluated (Smetana et al., 2016). Only recently, some studies filled this gap (Chen et al., 2019; Ermolaev et al., 2019; Mertenat et al., 2019), but continuous measurements during the larval rearing phase are needed for more accurate measurements.

The second topic is associated to the rearing of BSFL in manure. One of the environmental burdens associated to manure is the loss of nitrogen via ammonia emissions. While it is known that BSFL can uptake nitrogen from manure and store it in their body mass (Oonincx et al., 2015), it remains unknown if part of this nitrogen is sourced from the pool of ammonia-nitrogen. Elucidating the incorporation of ammonia-nitrogen into BSFL proteins is important to understand the potential of BSFL to reduce manure ammonia emissions and upgrade ammonia-nitrogen into a circular protein source for animal feed.

Finally, while BSFL are reared on a wide variety of streams, it remains unknown if larvae prefer to feed on certain substrates over others. Animal welfare is one of the pillars of a sustainable food system, and as insect farming emerges as a new agricultural activity, insect welfare should not be left behind. Exploring dietary preferences is relevant to get insights in BSFL behavior and to enrich the discussion on insect welfare, which is increasingly gaining momentum (Van Hal et al., 2019b).

1.4.3 Environmental performance of BSFL bioconversion in the circular economy

BSFL are envisioned to be fed with organic residual streams to recover and upgrade nutrients into a circular source of food and feed. As in some contexts, the organic residual streams used to feed the insects might already have a use in the circular economy, it is key to assess if BSFL bioconversion can bring environmental benefits compared to these existing uses. For instance, agri-food residues used in Europe to rear insects destined for feed, are ingredients commonly used directly as livestock feed. The existing environmental impact assessments generally concluded that farmed insects fed with such agri-food residues had a lower impact per unit of protein than other feed ingredients (Bosch et al., 2019; Smetana

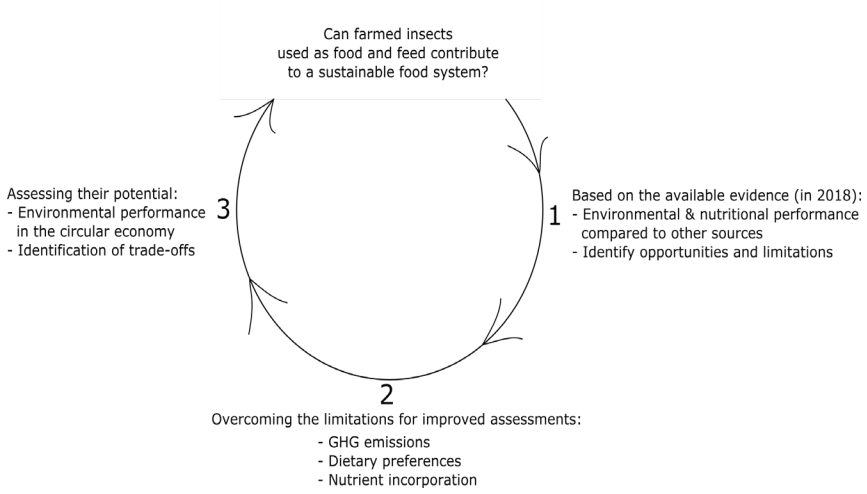

Figure 1.3: Knowledge gaps. The numbers and direction of the arrows indicate the sequence of research steps taken.

et al., 2016; Smetana et al., 2019). This metric, however, does not allow to determine if feeding livestock with such insects will lead to an ASF with less impact, compared to using the agri-residue directly as animal feed. Similarly, manure can be managed using diverse waste management strategies, but so far it is unknown if manure bioconversion with BSFL can bring environmental benefits over current management strategies. Thus, in such contexts, there is a need for new assessments that properly quantify, using the latest experimental evidence and relevant metrics, under which scenarios farmed insects fed on organic residual streams can truly bring environmental benefits.

All nutrient gaps are graphically represented in Figure 1.3.

1.5 Research aim and objectives

The overall research aim of this thesis was to provide a better understanding of the contribution of farmed insects to a sustainable food system when reared on residual organic streams. This research especially focuses on the black soldier fly, one of the main farmed insects worldwide. To achieve this aim, the following specific research objectives (R.O.) were pursued:

R.O.1. Compare the nutrient composition and environmental footprint of farmed insects with other foods.
R.O.2. Quantify the nutrient flows and greenhouse gas emissions during BSFL reared on agri-food residues and manure.

R.O.3. Verify if BSFL displays dietary preferences when exposed to contrasting rearing substrates.
R.O.4. Assess the sustainable use of BSFL as food and feed in the circular economy.

1.6 Structure of the thesis

This thesis is divided in nine chapters. Figure 1.4 shows how all chapters relate to the objectives of this thesis.

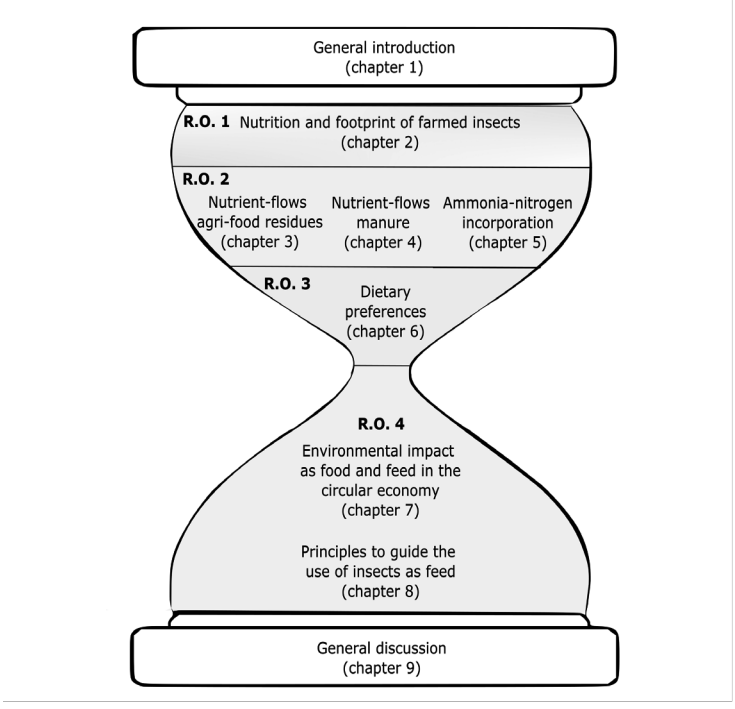

Figure 1.4: Schematic illustration of the structure of this thesis.

Chapter 1 provides context and information on the current state of knowledge regarding the use of farmed insects as food and feed, as well as the knowledge gaps and research objectives of this thesis.

Chapter 2 compares the nutritional content and environmental impact of farmed edible insect with different novel sources of food and the main animal- and plant-source foods. This was done by reviewing the available literature on nutritional composition and environmental

impacts, and creating a standardized functional unit that allowed the comparison between foods. The following chapters focused mainly on BSFL as it currently is the main farmed insect species.

Chapter 3 provides the flows of energy and nutrients (i.e., nitrogen, carbon, potassium and phosphorus) and the emissions of carbon dioxide, methane, nitrous oxide, ammonia and total nitrogen, during BSFL rearing on a substrate made of a mix of agri-food residues. This was done by rearing the larvae in climate respiration chambers and determining the chemical composition of inputs and outputs to build complete nutrient balances.

Chapter 4 compares the flows of energy and nutrients (i.e., nitrogen, carbon, potassium and phosphorus) and the emissions of carbon dioxide, methane, nitrous oxide, ammonia and total nitrogen, of BSFL reared on pig manure and of pig manure without larvae. This was done by rearing the larvae on manure in a climate respiration chamber and determining the chemical composition of inputs and outputs to build complete nutrient balances.

Chapter 5 quantifies the incorporation of ammonia-nitrogen into the larval body mass and larval proteins after manure bioconversion with BSFL. This was done by rearing BSFL on manure and using the stable isotope ^{15}N as a tracer.

Chapter 6 determines the dietary preference of BSFL when exposed to agri-food residues and pig manure. This was done by designing and performing a choice-test experiment with BSFL of different ages and previously exposed to different diets.

Chapter 7 quantifies the GHG emissions associated to the production of BSFL reared on agri-foods residues and pig manure, and then compares the GHG emissions resulting from the use of BSFL for food, feed and manure management with existing valorization pathways the same organic residual streams. This was done by modelling the environmental impact using a life-cycle approach.

Chapter 8 provides seven key principles to guide the responsible use of farmed insects as feed. This was done by integrating the information generated in the previous chapters, with the most recent research outcomes on the effects of farmed insect on livestock growth, health and welfare.

Chapter 9 synthetizes the findings of the thesis, propose new lines for research and discusses what changes are needed to create an enabling environment to get the best from insect farming.

Chapter 2

The potential of future foods for sustainable and healthy diets

This chapter is based on:

A. Parodi, A. Leip, I. J. M. De Boer, P. M. Slegers, F. Ziegler, E. H. M. Temme, M. Herrero, H. Tuomisto, H. Valin, C. E. Van Middelaar, J. J. A. Van Loon, and H. H. E. Van Zanten (2018). "The potential of future foods for sustainable and healthy diets". *Nature Sustainability* 1.12, 782–789. DOI: 10.1038/s41893-018-0189-7

Abstract

Altering diets is increasingly acknowledged as an important solution to feed the world's growing population within the planetary boundaries. In our search for a planet-friendly diet, the main focus has been on eating more plant-source foods, and eating no or less animal-source foods, while the potential of future foods, such as insects, seaweed or cultured meat has been underexplored. Here we show that compared to current animal-source foods, future foods have major environmental benefits while safeguarding the intake of essential micronutrients. The complete array of essential nutrients in the mixture of future foods makes them good-quality alternatives for current animal-source foods compared to plant-source foods. Moreover, future foods are land-efficient alternatives for animal-source foods, and if produced with renewable energy, they also offer greenhouse gas benefits. Further research on nutrient bioavailability and digestibility, food safety, production costs and consumer acceptance will determine their role as main food sources in future diets.

Main

Altering diets is increasingly acknowledged as an important step towards achieving several of the Sustainable Development Goals (SDGs). Throughout human history, foods derived from plants, livestock and fish have formed the back- bone of our global diet, however in recent years, other food sources, such as insects, cultured meat or seaweed, are gaining global attention (Post, 2014; Van Huis et al., 2013; Wells et al., 2016). The interest in these so-called 'future foods' has increased as a response to the conflicting contribution of current mainstream foods—especially animal-source foods (ASF)—to securing a nutritious and sustainable diet for a growing human population.

On the one hand, terrestrial and aquatic ASF supply nearly 40% of the world's proteins (FAO, 2017) and have a critical role in reducing malnutrition, especially in low-income countries, by providing essential macro- and micronutrients (Herrero et al., 2017; Van Zanten et al., 2018). Milk, for instance, includes relatively high amounts of calcium, beef is a high-quality source of bioavailable vitamin B12 and zinc, and seafood contains high concentrations of essential omega-3 fatty acids. On the other hand, the high intake of red and processed meat in high-income countries is associated with noncommunicable diseases, such as coronary heart disease and cancer (Pan et al., 2012; Wang et al., 2016). Moreover, global production levels of ASF place severe pressures on the environment through their emissions to air, water and soil, and their use of natural resources. The global livestock sector, for example, releases about 14.5% of all anthropogenic greenhouse gasses (GHG), pollutes ground and surface waters and uses about 40% of all arable land (Gerber et al., 2013; Leip et al., 2015; Mottet et al., 2017). Animals increasingly are fed products from agriculture and fisheries that humans could have consumed directly, causing a so-called food–feed competition. As the demand for ASF is projected to increase further Alexandratos and Bruinsma, 2012, these above described concerns are likely to worsen.

In our search for foods that reduce environmental impacts, we have seen an increasing focus on future foods (Alexander et al., 2017). Although these are often claimed to be nutritious and produced with a lower impact on the environment than most ASF, the existing nutritional and environmental work has not yet been consistently synthesized and analysed. In our study, we combined the nutritional profile with the environmental impacts of future foods under a single framework (also called functional unit). This enabled us to compare them with main conventional plant-source foods (PSF), and aquatic and terrestrial ASF. The aim of this study, therefore, was to assess the environmental potential of future foods as alternatives for ASF compared with conventional protein foods, while maintaining the intake of essential macro- and micronutrients. Our study includes the essential macro- and micronutrients present in ASF which could lead to public health concerns if ASF were to be replaced with other foods in human diets.

Future foods

We define future foods as those foods for which our ability to produce considerable volumes is rapidly developing as a result of technological developments that offer the potential to scale production levels up and/or reduce the production costs out of concern for the environment. On the basis of the currently available data, we selected nine future foods, consisting of terrestrial foods (cultured meat, mycoprotein (*Fusarium venenatum*), black soldier fly larvae (*Hermetia illucens*), housefly larvae (*Musca domestica*), mealworm larvae (*Tenebrio molitor*)) and aquatic foods (chlorella (*Chlorella vulgaris*), spirulina (*Arthrospira platensis*), sugar kelp (*Saccharina latissima*) and mussels (*Mytilus spp.*)) (Figure 2.1). We compiled their nutritional profiles and environmental impacts and compared them with those of important plant-source protein suppliers and with conventional aquatic and terrestrial ASF (Figure2.1).

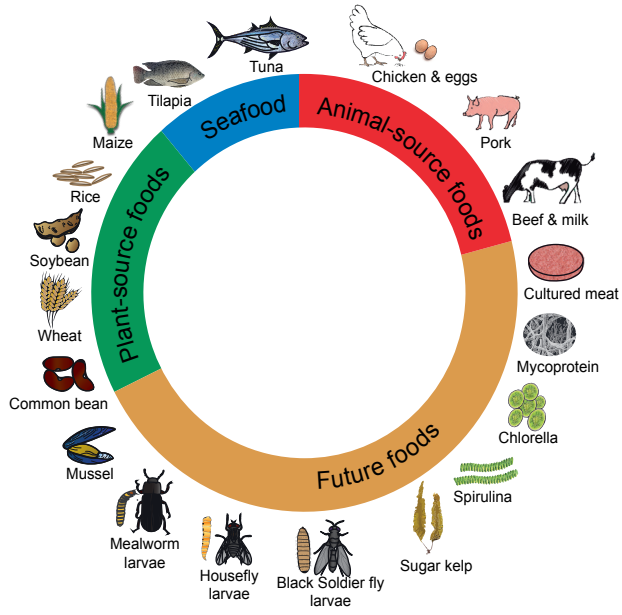

Figure 2.1: The future foods, PSF, seafood and conventional ASF that were included in this study. Future foods include cultured meat, mycoprotein (*F. venenatum*), chlorella (*C. vulgaris*), spirulina (*A. platensis*), sugar kelp (*S. latissima*), black soldier fly (*H. illucens*), housefly (*M. domestica*), mealworm (*T. molitor*) and mussels (*Mytilus spp.*). PSF consisted of common bean (*Phaseolus vulgaris*), wheat (*Triticum aestivum* and *Triticum durum*); soybean (*Glycine max*), rice (*Oryza sativa*); maize (*Zea mays*). Seafood included tilapia (*Oreochromis spp.*), skipjack tuna (*Katsuwonus pelamis).* Terrestrial ASF included chicken (*Gallus gallus*), pig (*Sus scrofa*) and cow (*Bos taurus*).

Results

The nutritional profile of future foods

Our results show that the complete array of essential macro- and micronutrients that are present in future foods makes them better alternatives for ASF than PSF. All future foods, except sugar kelp, show a similar or higher dry-matter protein content than PSF and ASF (Figure2.2) and are able to provide essential amino acids (Figure2.5). In addition to protein, most future foods also contain similar amounts of other macro- and micronutrients (Figure2.2b-f). A diet that comprised only PSF could increase the risk of developing a deficiency in vitamin B12 and the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

A mixture of future foods can provide us with all the essential macro- and micronutrients that we need. Calcium, for instance, is currently provided mainly by milk (Van Zanten et al., 2018), but can be provided by sugar kelp or black soldier fly larvae (Figure2.2b). Iron, which is mostly sourced from red meat and eggs, can be found in most future foods, especially in chlorella and spirulina (Figure2.2c) for which the iron content is so high that their intake should be limited to avoid exceeding the upper intake levels for iron. Zinc, which is abundant in all terrestrial ASF and PSF, also appears in future foods, such as sugar kelp, all insect species and mussels, at levels that are comparable to or higher than in beef (Figure2.2d). In terms of vitamins, most future foods contain similar vitamin A concentrations as ASF, except sugar kelp and spirulina, with the latter having concentrations up to 20 times higher than eggs, the ASF that is richest in vitamin A (Figure2.2e). Even though vitamin A is either absent or poorly represented in the evaluated PSF, other PSF rich in β-carotene, such as sweet potatoes, can be used to overcome vitamin-A deficiencies (Low et al., 2007). By contrast, owing to the absence of vitamin B12 in all commonly consumed PSF, those following a vegan diet are advised to take vitamin B12 supplements to avoid health risks (Pawlak et al., 2014). Vitamin B12, however, is found in large amounts in all aquatic future foods and in black soldier fly larvae (Figure2.2f).

Lastly, the two omega-3 fatty acids, EPA and DHA, which in nature are mainly synthesized by microalgae and cyanobacteria and then bioaccumulated through the trophic chain in seafood (Gladyshev et al., 2013; Kainz et al., 2004), are well-represented among aquatic future foods, but are absent in PSF (Figure2.2g). The EPA and DHA content in insects and ASF are either directly linked to dietary levels of these fatty acids or to the low transformation rates of α-linolenic acid into EPA and DHA (Hixson et al., 2016; Hussein et al., 2017; Liland et al., 2017).

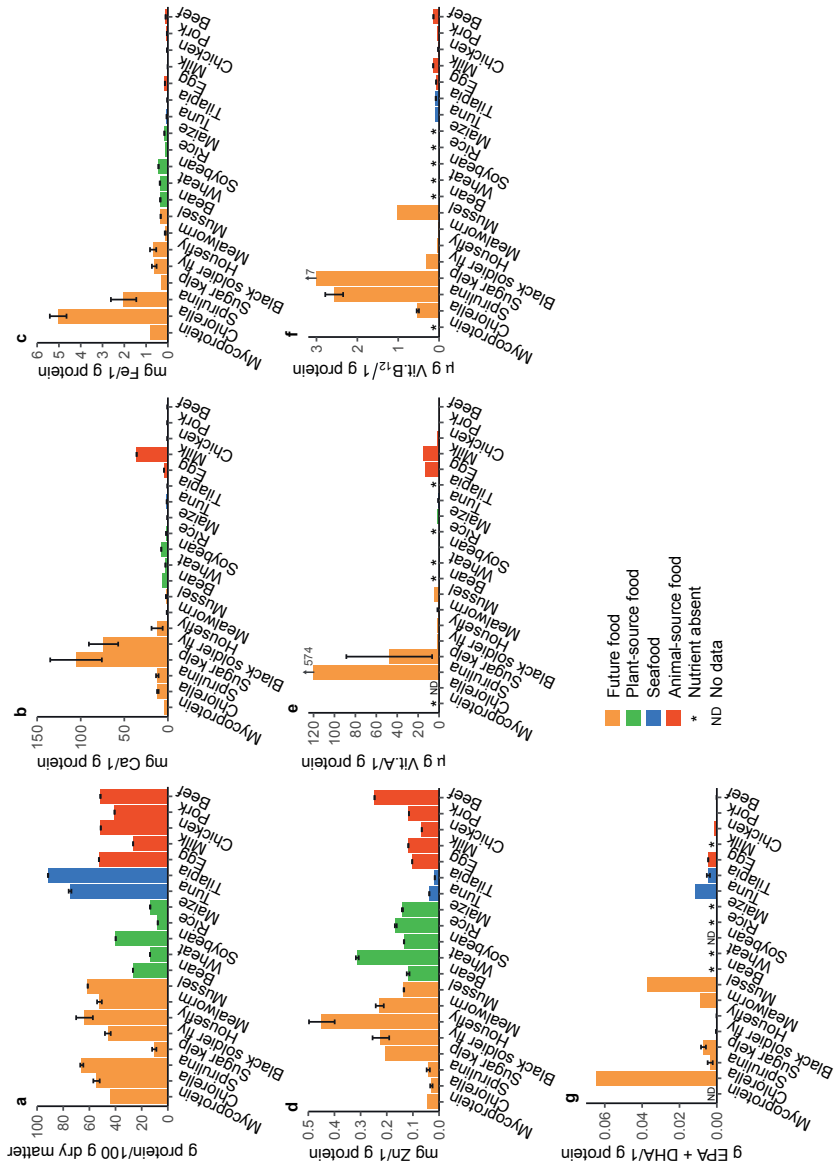

Figure 2.2: Nutritional profiles of future foods. **a**, Protein content in 100 g of dry matter. **b–g**, Nutrient content per gram of protein for each food. Data are mean ± s.e.m. (see Supplementary Tables 1 and 5 for a list of data sources, mean and s.e.m. for each future food and nutrient). Bars without uncertainty levels are based on a single source. High values indicate either high protein or low dry-matter content (**a**) or high nutrient content or low protein content (**b–g**). See Supplementary Figure 2.5 for amino acids.

The environmental impact of future foods

For the production of all essential nutrients, future foods require considerably less land than conventional ASF, except those from fisheries (which are by definition zero), when normalized to equal nutrient intake. Housefly, chlorella, spirulina and mussels have the lowest land use of the future foods (Figure2.3). Compared with the production of PSF, production of future foods requires equal amounts or less land for most essential nutrients (Supplementary Figure2.6). Future foods therefore are land-efficient alternatives for non-fisheries ASF, and can therefore contribute to reducing the competition for land between food, feed, fibre and fuel production. Because land use is centrally coupled to other agricultural environmental impacts (Heck et al., 2018; Leip et al., 2015), a future food system with reduced land use might have the potential to avoid additional land-use change and associated impacts.

The land area required to produce ASF is mainly determined by the amount of land needed to graze animals or produce feed (Mottet et al., 2017). Similarly, land required to produce future foods is mainly determined by the type of 'feed stock' used. For instance, studies that explored a hypothetical large-scale production system showed that under a set of reasonable albeit untested assumptions, the land required to produce cultured meat could be reduced by about 30% if we fed cultured cells with cyanobacteria instead of crops (Tuomisto et al., 2014; Tuomisto and Texteira de Mattos, 2011). Similarly, land required to produce insects is substantially reduced when insects are fed with biomass that humans cannot or do not want to eat (here referred to as leftover streams), instead of with food crops (Salomone et al., 2017; Van Zanten et al., 2015b). Aquatic future foods, such as chlorella and spirulina, have lower land requirements compared to ASF, and can be produced in brackish or saline water areas unsuitable for crop production. Most mussel and seaweed farms, on the other hand, do not require any land, as these activities take place in the sea and nutrients are obtained from the water and—in the case of seaweed—through photosynthesis. This form of non-fed aquaculture makes mussels and seaweed not only a nutritious and low-impact food, but also a production system that can help to reduce excess nutrient loads in eutrophied coastal waters and increase biodiversity (Aubin et al., 2018; Hasselström et al., 2018). It should be highlighted, however, that it is important to locate mussel and seaweed production in clean waters, otherwise they can accumulate water-borne contaminants and pathogens (Lhafi and Kühneb, 2007).

Mycoprotein, sugar kelp, all insects and mussels show similar nutrient GHG intensities (that is GHG emissions per unit of nutrient) to the best performing ASF and seafood (that is, eggs, milk and tuna), and higher nutrient GHG intensities than PSF (Figure 2.4 and Supplementary Figure 2.7). Chlorella and spirulina, show, on average, higher GHG intensities for protein and zinc than most ASF (Figure 2.4). However, studies report large differences in GHG intensities for spirulina and chlorella (see Supplementary Table 7 and Supplementary Methods for a detailed explanation).

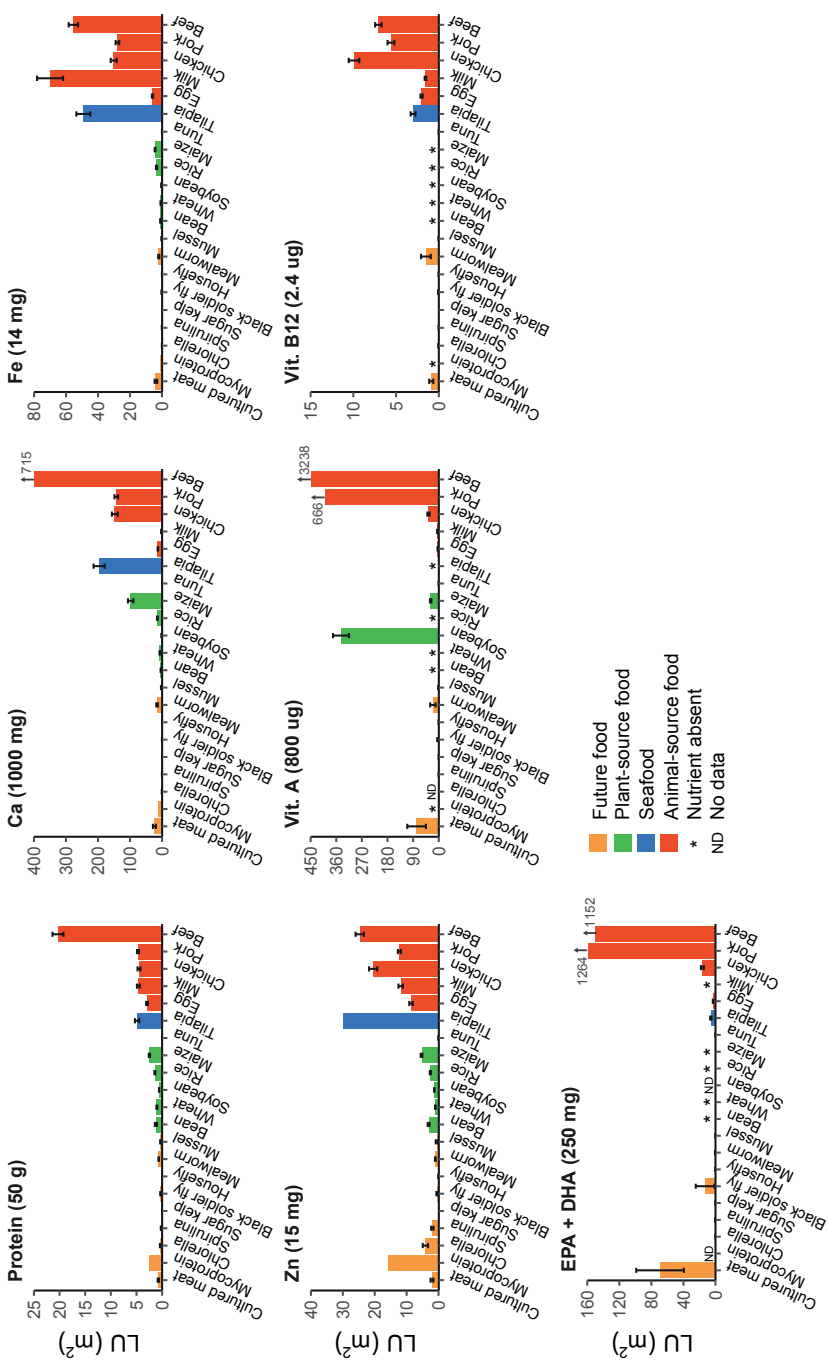

Figure 2.3: Land use of various produce expressed per daily recommended intake of each essential nutrient.For cultured meat, we assumed a nutritional profile similar to that of beef, and pork or chicken. Expressing land use (LU) per unit of daily recommended intake does not suggest that the daily nutrient requirements should be fulfilled by one type of food. Data are mean ± s.e.m. (see Supplementary Tables 7 and 8 for a list of data sources, mean and s.e.m. for each food and nutrient). High values indicate either high land use or low nutrient content. See Supplementary Figure 2.8 for amino acids.

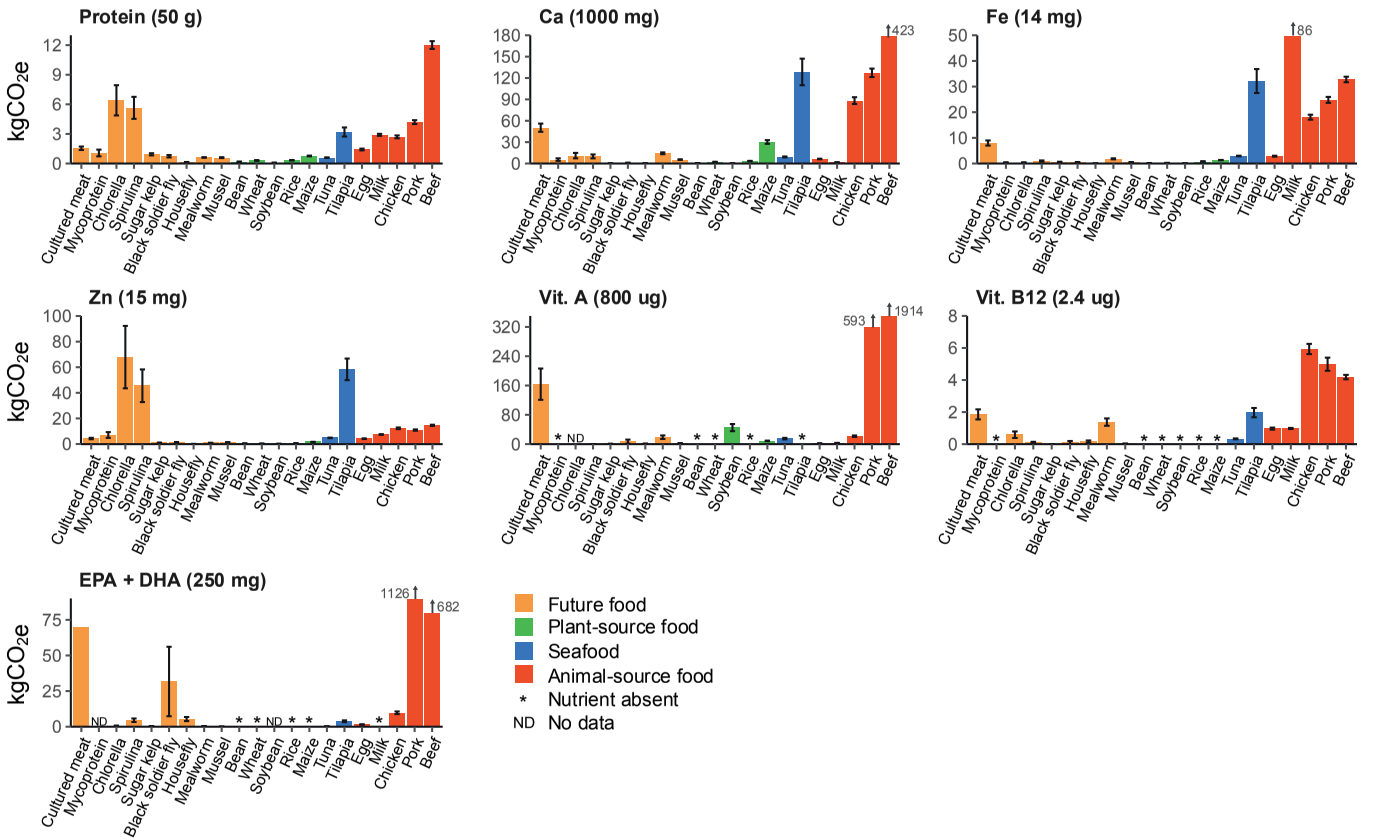

Figure 2.4: GHG emissions resulting from producing the daily recommended amount of each nutrient with each protein source. For cultured meat, we assumed a nutritional profile similar to that of beef, and pork or chicken. GHG emissions are expressed as CO_2 equivalent emissions in kg ($kgCO_2e$). Expressing GHG emissions per unit of daily recommended intake does not suggest that the daily nutrient requirements should be fulfilled by one type of food. Data are mean ± s.e.m. (see Supplementary Tables 7 and 8 present for a list of data sources, mean and s.e.m. for each food and nutrient). High values indicate either high GHGs or low nutrient content. See Supplementary Figure 2.9 for amino acids

The sources of GHG emissions differ among future foods, PSF, seafood and ASF. For terrestrial ASF, enteric fermentation (methane (CH_4)), feed production (carbon dioxide (CO_2) and nitrous oxide (N_2O)), and manure management (CH_4 and N_2O) are the main sources of emissions (Gerber et al., 2013). In wild fisheries, the level of GHG emissions mainly depends on fuel consumption of fishing vessels per unit of fish landed. This in turn depends on the fishing method used and the status of the fished stock (Ziegler et al., 2016). For an intensive tilapia farm, however, about 87% of the GHG emissions relate to feed production (Henriksson et al., 2018).

Conversely, GHG emissions of future foods mainly originate from high energy-consuming processes and the current use of fossil energy sources. To produce mycoprotein, for example, energy is required to maintain constant temperatures during the fermentation process, as well as for heat treatments and centrifugation (Wiebe, 2004). Similarly, most of the GHG emissions and energy use of cultured meat occurs during the cultivation process, which requires constant temperatures (Tuomisto and Texteira de Mattos, 2011). Chlorella and spirulina require high energy-consuming processes for cultivation, dewatering and drying to make these foods marketable. In insect production systems, GHG emissions are mainly caused by the use of electricity for heating the rearing environment in temperate climates, drying the larvae and feed production. GHG emissions associated with the production of insects, however, can be minimized by feeding them nutritious leftover streams (Smetana et al., 2016). As in traditional livestock rearing, insect rearing results in direct GHG emissions of CH_4 and N_2O. Expressed per kg of body weight gain, however, mealworms emit 20 times less CH_4 and 50 times less N_2O emissions than pigs (Oonincx et al., 2010). Unlike insects, bivalves, such as mussels, do not require feed inputs during farming because as filterfeeders, they feed on planktonic organisms that are found in the water that flows through the farm. They, however, produce direct GHG emissions through the release of CO_2 during shell production (Ray et al., 2018). These emissions are generally not accounted for in life cycle assessment (LCA) studies, and could potentially increase GHG emissions from mussel farming (Ray et al., 2018). If mussel shells, on the other hand are accounted as carbon sink (Aubin et al., 2018), the CO_2 emissions from shell production could be compensated. The role of mussels in the oceans' carbon cycle is currently in need of more research.

Because GHG emissions associated with producing future foods mainly result from using fossil-intensive energy sources, a transition towards renewable energy sources would reduce their GHG intensity. Even though this argument also holds for ASF, non-CO_2 GHG emissions associated with ASF production, such as enteric CH_4 emissions; CH_4 and N_2O emissions from manure management; and N_2O emissions from fertilizer application (Gerber et al., 2013) , cannot be mitigated by using renewable energies. A reduction in CH_4 and N_2O emissions will require additional innovations, such as feeding animals with safe leftover streams, innovative manure management systems or precision fertilization. Well-managed grazing livestock can potentially offer GHG benefits through the process of soil carbon

sequestration but, so far, the overall effect on livestock emissions seem negligible and time-limited (see Garnett et al. (2017), Smith (2014) and Supplementary Discussion). For these reasons, we hypothesize that the GHG mitigation potential of future foods in a renewable energy society is likely to be higher than that of ASF.

Discussion

We show that essential nutrients are present in raw future foods, but to what level these nutrients will be conserved after processing remains unknown for most minerals and vitamins. Moreover, the extent to which these nutrients are bioavailable and digestible is only known for specific foods and nutrients. In vitro models have shown, for example, that protein digestibility of different insects (Marono et al., 2015; Ramos-Elorduy et al., 1997; Yang et al., 2014) ranges from 67% to 98% and that bioavailability of micronutrients such as iron, calcium and zinc in edible insects is similar to or higher than those in beef (Latunde-Dada et al., 2016). Similarly, the in vitro digestibility of seaweed protein (Latunde-Dada et al., 2016) ranges from 56% to 90%. Protein digestibility of mycoprotein, spirulina and chlorella was found to be 15, 25 and 30% lower than that of milk casein, respectively Edwards and Cummings, 2010; Mišurcová et al., 2010. Resistant cell walls together with the presence of specific compounds (see Supplementary Discussion) might limit the digestibility of both seaweed and microalgae, but efficient and non-costly cell-disruption techniques (for example, heat and mechanical treatments or enzymatic lysis) provide options for making algal proteins more digestible (Kose et al., 2017; Maehre et al., 2016). Spirulina production is supported by the WHO (World Health Organization) in the fight against malnutrition, and studies, which show that chlorella and spirulina can help to ameliorate iron and folate deficiencies (Nakano et al., 2010; Selmi et al., 2011) or increase the total-body vitamin A reserves (Li et al., 2012), confirm that these nutrients can be absorbed in the human body. Vitamin B12, which is only synthesized by certain bacteria and archaea, is found in bioavailable forms in mussels, seaweed species and chlorella (Watanabe and Bito, 2018), but not in spirulina, which contains an inactive vitamin B12 analogue that cannot be absorbed by the human gut (Watanabe et al., 1999). Further research, therefore, is needed to assess and improve the concentration of bioavailable nutrients in future foods as well as the digestibility of these nutrients. In addition to bioavailability, future foods need to be further explored in relation to food safety (see Supplementary Discussion) and allergies, as there is evidence that suggests that people who are allergic to shrimp are at risk when eating mealworms or other edible insects (Broekman et al., 2017). It is therefore important to emphasize that future foods should be consumed as part of a diverse diet, ensuring that specific nutrient requirements are fulfilled and upper intake limits of nutrients are not exceeded. This can be achieved by rationing their amounts in diets and by using adequate preparation methods (Lüning and Mortensen, 2015; Maehre et al., 2016) or processing technologies (Bußler et al., 2016; Ursu

et al., 2014) to improve the availability and digestibility of nutrients. More information on bioavailability, digestibility, allergies and food safety is crucial to help us to better understand the potential role of future foods in human diets.

Overall, we show that the environmental benefits of future foods are associated with high nutrient-use efficiencies, use of green technologies and the use of leftover streams. Even though some of those arguments can also be applied to the current production of ASF, future foods have potential characteristics that can lead to substantially lower environmental impact. Insects, for example, fed on leftover streams that have sufficiently high nutrient contents, have higher reproduction rates, shorter maturation periods, lower energy investment for growth and higher protein-use efficiencies, than conventional production animals (Oonincx et al., 2015; Oonincx and Boer, 2012). In addition, as the whole insect larva is edible, there are no losses associated with non-edible biomass—such as bones, feathers and skin. Rearing insects on nutritious leftover streams has been shown to have especially high environmental benefits (Salomone et al., 2017; Smetana et al., 2016). Some of these residual streams, however, could also be fed to livestock and markedly reduce the environmental impact of livestock (Van Zanten et al., 2018; Van Zanten et al., 2016). Owing to the relatively higher growth rate of insects, the environmental impact of livestock nevertheless will remain higher in most situations. Cultured meat and mycoprotein also offer the possibility to produce edible biomass and, considering that their production takes place in controlled environments, there are numerous opportunities for using technology to achieve higher efficiencies and to minimize losses through recycling mechanisms and precise input–supply (Post, 2012). For cultured meat, however, challenges such as the development of serum-free nutrition medium and the design of large-scale bioreactors should be solved first. Spirulina and chlorella are primary producers that, in contrast to crops, can be produced on marginal lands, while other aquatic future foods, such as seaweed and mussels, have the capacity to absorb excess nutrients from coastal areas that are otherwise not accessible for food production. Farming in the oceans is much less optimized than on land, and even though current mussel and seaweed farms are efficient, they could be considerably improved by, for example, breeding and adjusting production technologies to local conditions to increase productivity and quality. Exploiting these characteristics, in combination with renewable energy systems that operate in the same production areas where future foods are produced may, therefore, help the transition towards a more sustainable food system. We are only in the very early phases of finding applications for these new raw materials, either as main foods or food components.

Despite the importance of our findings, the selection of future foods and their environmental impact was constrained by the availability of LCA studies. Different species of insects, microalgae and cyanobacteria, seaweeds or bacteria, with a more promising nutritional and environmental performance than the future foods that are included here may be even better candidates for future diets. Moreover, our analysis has only covered the impact categories of land use and climate change. The impact of future foods on other environmental issues,

such as water pollution, eutrophication, acidification, biodiversity and air quality, should be further explored.

With the exception of cultured meat, all future foods are currently commercially available. Crucial factors to scale up these foods from their traditional production regions to other regions of the world include the control of food safety hazards, the development of innovations that enable increasing the production scale and the concomitant reduction of production costs (as these are currently high compared to ASF) as well as making these foods attractive and affordable to present and coming generations. Future foods have the potential to become an important element of future sustainable healthy diets. To make this happen, private and public interventions will be required to foster their adoption and help in the transformation towards sustainable food systems.

Supporting information

The supporting information of this chapter includes:

Appendix A - Methods

Appendix B - Suppementary Figures (S1-S5)

Appendix C - Supplementary Discussion

The original publication also contained sections of Supplementary Methods and Supplementary Tables. Along the chapter there are references to items that belong to these sections, however these were not included in this thesis due to space constraints. Both sections can be accessed through the online version of the publication.

Appendix A - Methods

Selection of future foods

We searched the available literature for environmental impact assessment—or LCA—studies that enabled us to recalculate the environmental impact of both conventional and future foods per kilogram of dry matter product, assuming a cradle-to-factory gate approach. The search resulted in the selection of the following terrestrial future foods: cultured meat, mycoprotein (*F. venenatum*) commercially available as "Quorn", the larvae of three insects (black soldier fly, housefly and yellow mealworm (*H. illucens, M. domestica and T. molitor*, respectively)); and aquatic future foods: the cyanobacteria spirulina (*A. platensis*), the microalgae chlorella (*C. vulgaris*), one brown seaweed (*S. latissima*) and blue mussels (*Mytilus spp.*).

Five traditional plant species that are considered to be important sources of proteins in current diets were selected and included in the analysis to put the nutritional and environmental impacts of future foods in perspective. The selection of these species was based on different criteria: common beans for being the pulse with the highest production volume, wheat, rice and maize for being the crops that supply the highest amounts of plant protein globally and soybean for its high protein content (see Supplementary Methods).

The selection of terrestrial ASF was based on the most consumed animal products on a global scale: beef, pork, chicken, eggs and milk (see Supplementary Methods). For aquatic ASF, we selected tilapia (*Oreochromis niloticus*), which is the farmed fish produced in the largest volumes and for which LCA data are available, and skipjack tuna (*K. pelamis*), which is the wild-caught fish species with the highest volume used for direct human consumption for which LCA data are available (FAO, 2016).

Nutritional composition

The nutritional composition of all future foods, except mussels, was obtained from the available literature (Supplementary Table 1). For blue mussels, we used the nutrient database of the US Department of Agriculture (USDA and United States Department of Agriculture, 2017). Because the nutritional composition of cultured meat is unavailable, we assumed that cultured meat had the same nutritional content as beef, chicken and pork, and only used these data for the environmental impact section. This assumption is justified, because various cultured meat developers across the world are currently investing in the culturing of cells of cattle, pigs and poultry (Post, 2018) and because cultured meat can be tailored, as it is possible to decide the quality and quantity of fat and micronutrients. However, it is important to highlight that certain nutrients present in conventional meats that are synthetized by gut microorganisms (for example, vitamin B12 and omega-3 fatty acids) (Jenkins et al., 2008; Moll and Davis, 2017) are likely to be absent in cultured meat unless supplemented. The supplementation of such nutrients is not accounted for in this study. For PSF, seafood and terrestrial ASF, the nutritional composition was obtained from the USDA National Food Composition Databases (USDA and United States Department of Agriculture, 2017) (see Supplementary Table 2 for nutrient database (NDB) numbers). The nutrient content of all foods corresponds to the edible portion of raw samples.

Because the nutritional contribution of ASF, such as beef, pork and chicken, varies between different parts of the animal (for example ham, shoulder, loin and so on), equation (1) was applied to calculate the average nutritional content per kg of product:

$$T = \sum_i n_i P_i \tag{2.1}$$

where T is a specific nutrient content for a whole animal, n_i is the concentration of a nutrient in part i (for example, wing, breaks, leg, and so on), P_i is the proportion of part i in the total edible weight of the animal (see Supplementary Table 3 for values) and $\sum_i P_i = 1$.

Per study and per food type, we expressed the concentration of each nutrient in 100 g of dry matter product and subsequently, we expressed the nutrient content present in 1 g of dry matter protein of each food. This enabled us to compare how much of other macro- and micronutrients are supplied when each food is used as a protein source. We calculated the mean ± s.e.m. values per nutrient and per food, based on the total number of nutritional values collected (Supplementary Tables 1 and 5).

Environmental impact

We used 27 LCA studies to calculate the environmental impact of all future foods. We included two environmental impact categories for which quantitative data was available and for the attention paid to these two impacts in the discussion on livestock production and the environment: climate change expressed in kg CO_2 equivalent and land use expressed in m^2 per year. To make the multiple studies comparable under the same functional unit, the results of the LCA studies were first recalculated to express the environmental impacts per kg of product on a dry matter basis, with a system boundary from cradle-to-factory gate (see Supplementary Table 7). To avoid the influence of any methodological effect (for example, different types of allocation used in different studies) in our analysis and conclusions, we tried to minimize the effect of allocation. For future foods, no allocation between final co-products was needed as the production of future food does not result in multiple outputs. Insects, for example, can be consumed as a whole, whereas grains need to be processed and therefore yield multiple outputs (for example, flour and wheat middling). During the production of future foods, inputs are used. When possible, we used data that allocated 100% of the impact from feed production to the main feed product, thus considering possible other products (that is, straw) as by-products; such data were available in one of the cultured meat studies (Tuomisto and Texteira de Mattos, 2011). Some studies used allocation of environmental impacts of specific inputs (that is, feed ingredients); these data were therefore used without recalculation. Assumptions for all LCA studies can be found in the Supplementary Methods. The recalculated units per kg of dry matter product can be found in the Supplementary Table 7.

The environmental impacts of ASF and PSF were derived from previous studies (Leip et al., 2014; Leip et al., 2015) and are based on the common agricultural policy regional impact analysis (CAPRI) model. For PSF, allocation was applied for cereals, allocating about 3% of the emissions to straw. For ASF, allocation was based on the nitrogen content of the final products. In CAPRI, meat and milk are produced by different activities. Calve raising and heifers produce the meat; milk cows no longer grow and emissions are almost fully allocated to milk, except for a small part allocated to calves (meat). The same principle is true for laying hens and fattening chickens. Therefore, the effect of the allocation method related animal products (the end product) is low. For some feeds (cereals, oil cakes), allocation is used; this is similar to the future foods discussed above.

We used the direct and indirect GHG emissions of all countries in the European Union. GHG emissions of PSF corresponded to direct and indirect N_2O emissions associated with manure and fertilizer application on soils, crop-grazing, crop residues and indirect N_2O emissions associated with leaching and ammonia volatilization. In addition, we included CO_2 emissions that result from fertilizer production, seed production, plant protection, use of machinery and electricity consumption on the farm. Emission estimates of PSF include further emissions from land use (cultivated histosols), but exclude emissions of carbon

sequestration in permanent or managed grasslands (Weiss and Leip, 2012). For ASF, we accounted for the following emission sources: all those described for PSF for the required feed; N_2O emissions associated with manure management (housing and storage) and land use change for feed production; CH_4 emissions associated with enteric fermentation, manure management and land use change for feed production; CO_2 emissions associated with feed transport and feed processing; and GHG emissions from land use change for feed production (that is, carbon losses from aboveground biomass and organic soils). Emissions from feed production are not limited to production within the European Union, but emissions from imported feeds are included (Leip et al., 2010; Weiss and Leip, 2012).

The impacts of ASF were transformed from 1 kg of fresh carcass weight to 1 kg of dry matter edible product using the conversion factors listed in Supplementary Table 6. The impacts of PSF were transformed to 1 kg of dry matter edible product. Supplementary Table 7 shows the recalculated impacts for both PSF and ASF.

The environmental impact of fished skipjack tuna and farmed tilapia was obtained from the LCA literature. For assumptions and sources, see Supplementary Methods.

Using equations (2) and (3), we calculated the environmental impact of each food source for a given nutrient:

$$A_{s,n} = \frac{B_n \times 100}{C_{s,n}} \tag{2.2}$$

$$Y_{n,i} = \frac{A_{s,n} \times E_{s,i}}{1000} \tag{2.3}$$

where $A_{s,n}$ is the amount (in grams) of a food source s that is needed to satisfy the daily requirement for nutrient n, B_n is the daily requirement for nutrient n and $C_{s,n}$ is the concentration of nutrient n in 100 g dry matter of a food. With the value of $A_{s,n}$, equation (3) was used to calculate $Y_{n,i}$, the environmental impact i of a food to satisfy the daily requirement of nutrient n, where $A_{s,n}$ is the amount of a food source that is needed to satisfy the daily requirement for nutrient n and $E_{s,i}$ is the environmental impact for the different impact categories i (GHG emissions and land use) for 1 kg of dry matter of a protein source s.

$A_{s,n}$ and $Y_{n,i}$ were calculated for all values reported in the literature. Thus, if two studies found different calcium and protein content for the same food, we calculated the $A_{s,n}$ for each study. If a study did not report the protein content, we used an averaged protein content based on other studies. Subsequently, the $Y_{n,i}$ was calculated for all the land use and GHG emissions reported in the literature and then summarized by the mean ± s.e.m. values per food and nutrient (for values see Supplementary Table 8).

The daily requirements were obtained from the Nutrient Reference Values-Requirements given by the Codex Alimentarius for labelling purposes (FAO and WHO, 1985) (see Supplementary Table 4 for specific values). As the Codex Alimentarius does not include the daily requirements of omega-3 fatty acids, we used a value of 250 mg for EPA plus DHA for adults, indicated by the European Food Safety Authority as an adequate intake of these nutrients (EFSA, 2010).

Appendix 2B - Supplementary Figures

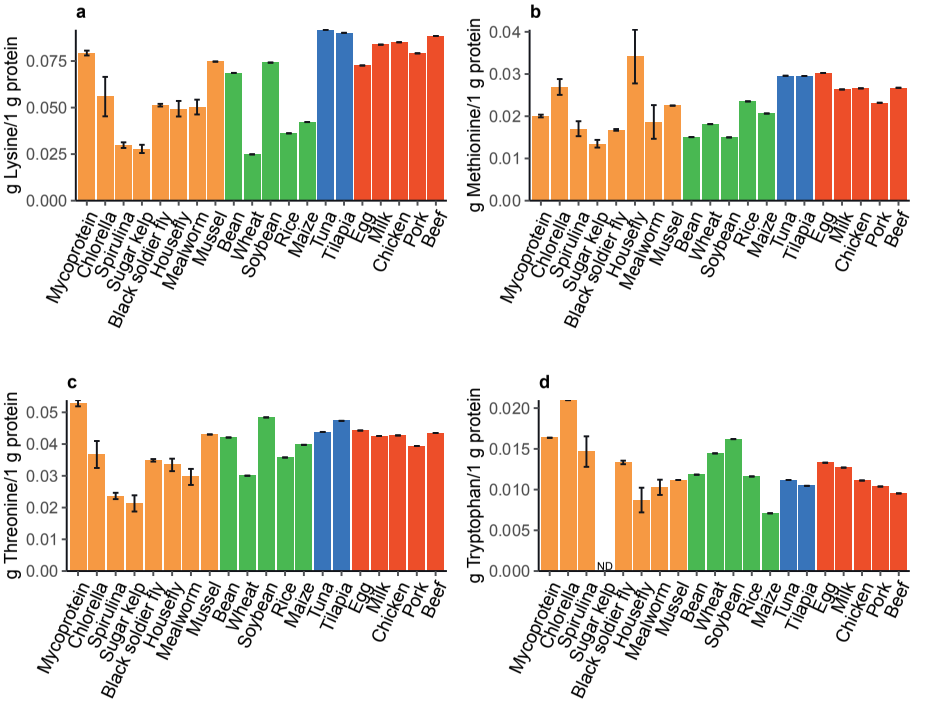

Figure 2.5: Amino acid content per gram of protein for each food. The mean and standard error of the mean (s.e.m.) are shown for each case (Supplementary Tables 1 and 5 contain the list of data sources, mean and s.e.m. for each future food and nutrient and can be accessed through the online version of the publication). Bars without uncertainty levels are based on a single source. We selected these four amino acids for their limited availability in certain poor-quality proteins.

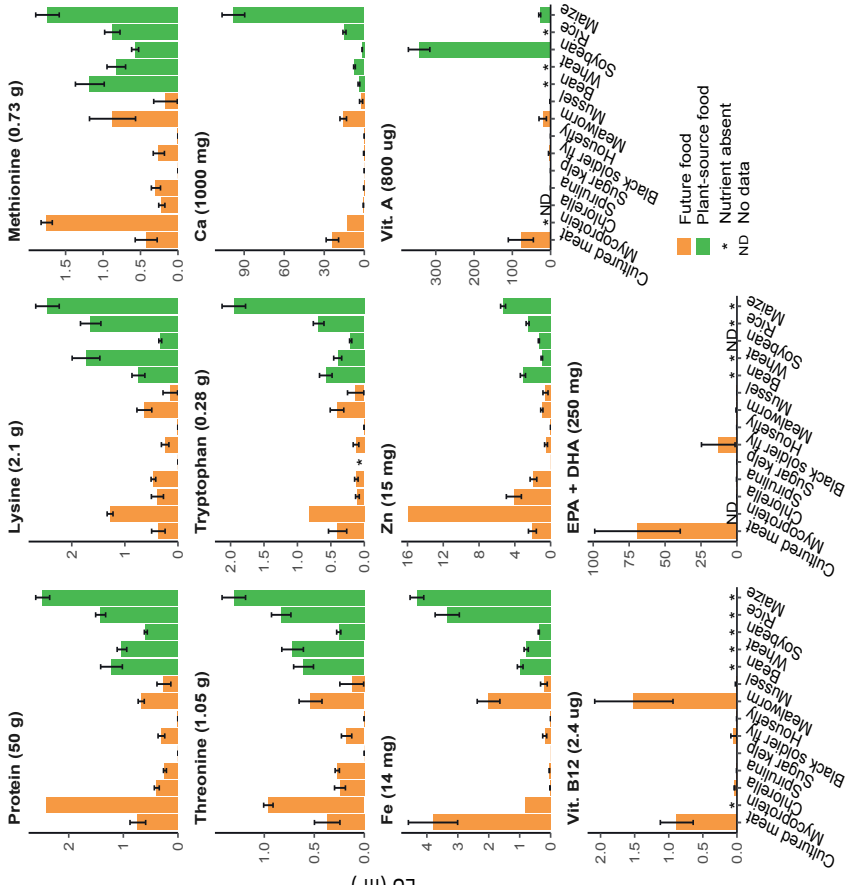

Figure 2.6: Land use needed to satisfy the daily recommended intake of each essential nutrient with future foods and plant-source foods. The mean and standard error of the mean (s.e.m.) are shown for each case.

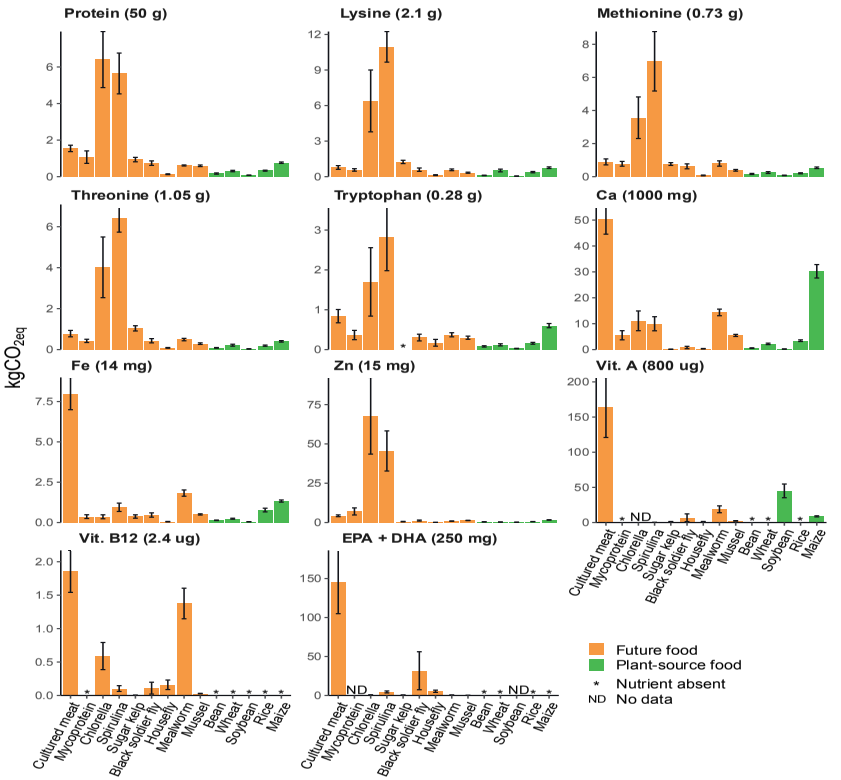

Figure 2.7: Greenhouse gas emissions to produce the daily recommended amount of each nutrient with each protein source. The mean and standard error of the mean (s.e.m.) are shown for each case.

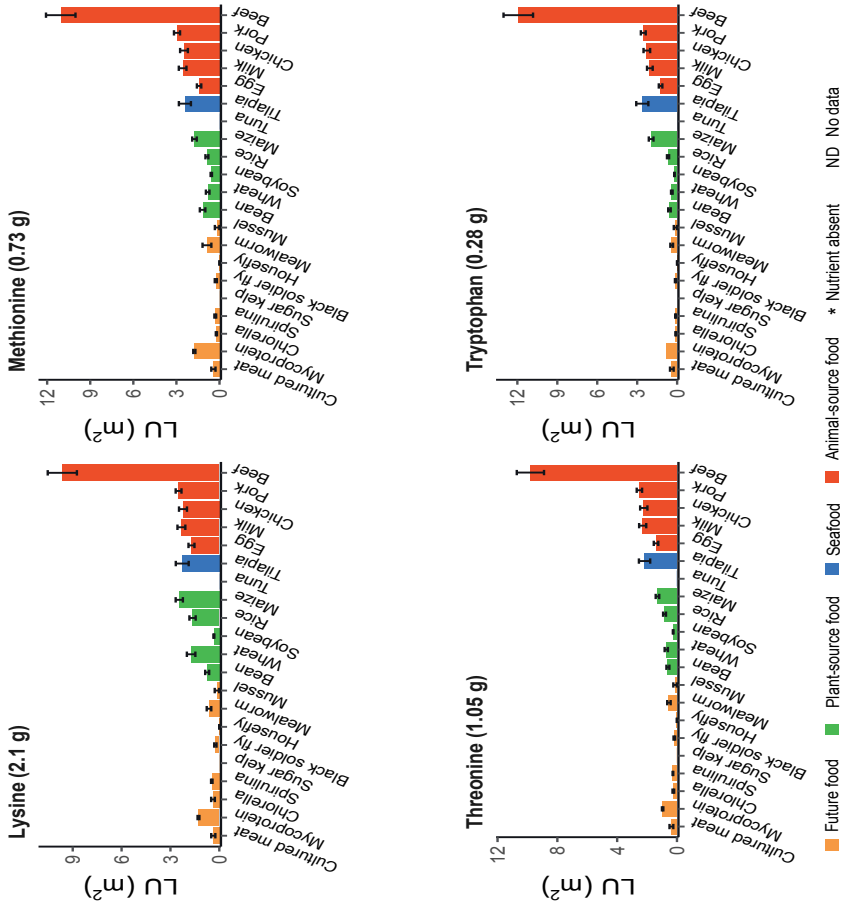

Figure 2.8: Land use of various foods expressed per daily recommended intake of each essential amino acid. Expressing land use per unit of daily recommended intake does not imply that the daily nutrient requirements should be fulfilled by one type of food. The mean and standard error of the mean (s.e.m.) are shown for each case.

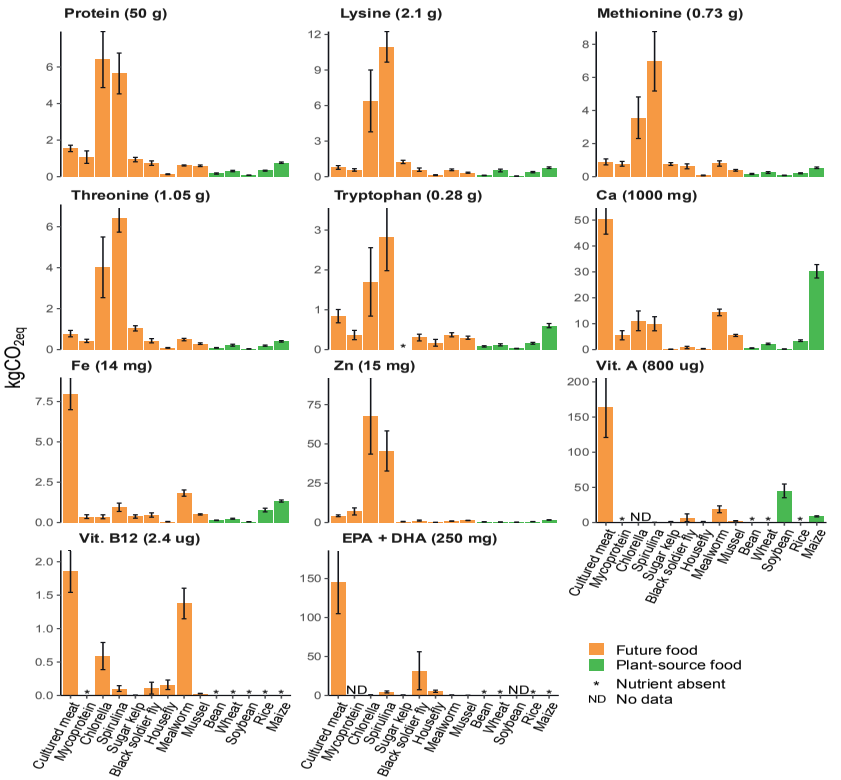

Figure 2.9: Greenhouse gas emissions resulting from producing the daily recommended amount of each amino acid with each protein source. The mean and standard error of the mean (s.e.m.) are shown for each case. We selected these four amino acids for their limited availability in certain poor-quality proteins.

Appendix 2C - Supplementary Discussion

Carbon sequestration in grazing systems

It is worth to highlight that well-managed grazing livestock systems could potentially aid the process of carbon sequestration in the soil (Garnett et al., 2017), and thus help to reduce atmospheric CO_2 concentrations. However, so far, evidences indicate that the sequestration potential from grazing management is time-limited (Smith, 2014)), has a negligible effect on overall livestock emissions, and that practices optimal for achieving carbon sequestration might have trade-offs with other environmental goals (Garnett et al., 2017). Future research on carbon sequestration rates at deeper soil levels (deeper than 1 m) are needed to reveal the true potential of managed grasslands to mitigate GHG emissions. However, given that grasslands are an important stock of carbon and that it is easier and faster for soils to lose carbon that it is for them to gain it (Johnston et al., 2009), it is crucial to maintain the existing soil carbon stocks by reducing land-use change. For that, future foods might play an important role.

Food safety issues

The micro-algae Chlorella and Spirulina have been cultivated and consumed for a long time (Draaisma et al., 2013; Tang and Suter, 2011; Vigani et al., 2015; Wells et al., 2016). Due to the long history of use, these species are, in general, considered to be safe for consumption, provided that cultivation is controlled and takes place in clean water (European Commission, 2015; Tang and Suter, 2011; Wells et al., 2016). The micro-algae species Tetraselmis chuii was the first micro-algae to be approved by the EU as novel food (European Commission, 2015).

Seaweeds are found with different types and concentration of metals (e.g. arsenic, copper, etc.), depending on the species, collection time, and collection site. Some studies found excess metals, while other studies found no harmful quantities (Van der Spiegel et al., 2013). Some seaweeds also contain iodine in concentrations that limit consumption (Maehre et al., 2014; Yeh et al., 2014)).

For blue mussels, it is important to be aware of that they may not be fit for consumption in certain periods due to the accumulation of algal toxins during blooms of toxic algae, which occur regularly. All commercially farmed blue mussels are controlled for content of algal toxins (DSP, ASP, PSP) before being sold. After the algal bloom is over, the mussels can be harvested, as the toxins disappear. Another safety risks associated with mussels is the presence of *Vibrio* bacteria in the water bodies where mussels are grown. In Norway this pathogen was found in low concentrations and did not pose any health risk to consumers (Bauer et al., 2006), while in Germany, *Vibrio* pathogens were common in blue-mussel growing areas, posing a relevant risk to public health (Lhafi and Kühneb, 2007). In addition, microplastics have been found in commercial mussels in levels that

present a route for human exposure (Li et al., 2018). All these aspects should be taken into account when selecting adequate places for the cultivation of mussels.

As for conventional animals, the safety of edible insects is ensured with the choice of appropriate and safe feeds (EFSA, 2015). As a plus for insects, aflatoxins which spoil crops and therefore increase food waste, are well tolerated by BSF and mealworms, and do not accumulate in the edible portion (Bosch et al., 2017).

Specific compounds limiting digestibility

The specific compounds present in human or animal foods that reduce nutrient utilization are also known as "anti-nutritional factors" (Thangaraj, 2016). We have avoided the use of the term "anti-nutritional factors" because many of these compounds also have positive effects on human and animal health. We found no publications documenting the presence of these compounds in mycoprotein, mussels, cultured meat, spirulina and chlorella. The information found for seaweeds, microalgae and cyanobacteria and insects is presented below.

Seaweeds

Compounds that reduce the digestibility of nutrients such as polyphenols, trypsin and alpha- amylase inhibitors, tannins and phytic acids have been found in seaweeds (Cofrades et al., 2016; Murugan et al., 2015; Oliveira et al., 2009; Vijayabaskar P et al., 2012). However, no study has directly explored the effects in the human digestibility of seaweed caused by these compounds. Instead, most of the literature related to seaweed focus on the bioactive properties and their associated human health effects of the same components reported as "anti-nutritional factors" (see Brown et al. (2014)). For instance, it has been found that polyphenols (commonly referred as an "anti-nutritional factor") create insoluble high molecular complexes that limit the bioavailability of proteins and polysaccharides, but also show positive health benefits with their anti-oxidant (Khairy and El-Sheikh, 2015; Sathya et al., 2017; Vijayabaskar P et al., 2012), anti-inflammatory (Shibata et al., 2008), anti-proliferative and potential anti-diabetic properties (Nwosu et al., 2011).

Some of the compounds present in seaweeds that reduce their digestibility can be found in lower concentrations than in common plant-source foods. For instance, phytic acid, a compound that cannot be absorbed by monogastric (including humans) and that binds to minerals (e.g. iron, zinc, calcium) making them unavailable (Gupta et al., 2015), was found in lower concentration in some seaweeds (0.45%) than in beans (1.45%), refined wheat (2% to 9.6 %), corn (0.77%) and soybean (1.5%) (Oliveira et al., 2009). Trypsin inhibitors, common in legumes (Avilés-Gaxiola et al., 2018) and also found in some seaweeds (Oliveira et al., 2009), inhibits the activity of the key pancreatic enzymes trypsin and chymotrypsin and therefore limit the digestion and absorption of dietary proteins (Gemede and Ratta, 2014). Many treatments have been used in plant-source foods to reduce trypsin inhibition below threshold limits (Avilés-Gaxiola et al., 2018; Gupta et al., 2015), including physical

processes (e.g., heat, extrusion, ultrasound, soaking), chemical processes (reducing agents, chemical bases) and biological processes (e.g., fermentation). Even though, Oliveira et al. (2009) found that trypsin inhibitors from a mixture of seaweeds were not inactivated by heat processing, other treatments currently used for plant-source foods should be further explored.

In addition, it is important to consider that the presence and concentration of compounds that reduce the digestibility as well as the effect of the treatments to reduce them vary among the species. For instance, García-Casal et al. (2007) did not find phytic acid in four edible algae and reported them as a good sources of bioavailable iron, and Maehre et al. (2016) found in an in-vitro model that a heat treatment resulted in an 64%–96% increase in liberated amino acids in the red seaweed dulse (*Palmaria palmata*), but not in the brown seaweed winged kelp (*Alaria esculenta*).

Microalgae and cyanobacteria

So far, there is no publication documenting negative effects of compounds that reduce the utilization of nutrients from chlorella and spirulina. Again, the literature is mainly related to health applications of compounds present in these sources such as protease inhibitors (Ishihara et al., 2006), phycocyanobilin (McCarty, 2007), carotenoids (Singh et al., 2005) and others (de Jesus Raposo et al., 2013; Fu et al., 2017).

Insects

Compounds that reduce digestibility such as phytic acid, tannin and oxalates have been reported for edible ants, termites, and moths, but in very small amounts, mostly lower than in many cereals and legumes (Chakravorty et al., 2016; Omotoso, 2006; Schlemmer et al., 2009). Instead, insects have developed ways of dealing with the presence of these compounds in plants in order to increase the bioavailability of the nutrients contained in the plants (Klasing et al., 2000).

Moreover it has been found that chitin, a compound present in fungi, crustaceans and insects, is the main factor affecting the in vitro digestibility of insects (mealworms and black soldier fly) used as feed (Marono et al., 2015). However, substantial increases in protein digestibility (20%) have been achieved when chitin is removed (DeFoliart, 1992). In addition, human gastric juice contains enzymes (chitinases) which can degrade chitin (Paoletti et al., 2007). These enzymes, however, are not present in all humans, as it was found to be absent in some Caucasian individuals and present with high activity in populations exposed to chitin-rich foods (Paoletti et al., 2007). Feeding behaviour has also been found to affect chitinase enzymes in farm and wild animals (Strobel et al., 2013; Tabata et al., 2018). It should be also highlighted that chitin and its derivatives also have antitumor, antioxidant and antimicrobial activity (Younes et al., 2014).

Overall, we conclude that literature about the compounds that reduce digestibility (also called "anti-nutritional factors") in future foods still needs further development and that

these compounds can play a negative (reduced digestibility of some nutrients) as well as having a positive impact (improved health) on humans.

Chapter 3

Nutrient flows during black soldier fly larvae rearing on agri-food residues

This chapter is based on:

A. Parodi, I. J. M. De Boer, W. J. J. Gerrits, J. J. A. Van Loon, M. J. W. Heetkamp, J. Van Schelt, J. E. Bolhuis, and H. H. E. Van Zanten (2020a). "Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing – A mass balance approach". *Journal of Cleaner Production* 271, 122488. DOI: 10.1016/j.jclepro.2020.122488

Abstract

Black soldier fly larvae (BSFL) are acknowledged for their potential to upcycle waste biomass into animal feed, human food or biofuels. To ensure sustainable BSFL rearing, insight into nutrient bioconversion efficiencies and nutrient losses via gaseous emissions is key. This study used a mass balance approach to quantify nutrient bioconversion efficiencies (i.e., carbon, energy, nitrogen, phosphorus and potassium) and gaseous emissions (i.e., greenhouse gasses and ammonia) of BSFL reared on a substrate used in industrial production. On this substrate, bioconversion efficiencies ranged from 14% (potassium) to 38% (nitrogen). The proportion of dietary inputs found in the residues ranged from 55% (energy) to 86% (potassium), while the proportion of dietary inputs lost via gaseous emissions ranged from 1% (nitrogen) to 24% (carbon). Direct emissions of methane and nitrous oxide during rearing were 16.8 ± 8.6 g CO_2equivalents per kg of dry BSFL biomass. Even though ammonia emissions were minimal, these could have been avoided if larvae would have been harvested before the CO_2 peak was reached. Our results provide the first complete mass balance and comprehensive quantification of BSF larval metabolism and GHG emissions, required to assess and improve the environmental sustainability of BSFL production systems.

3.1 Introduction

The interest in farmed insects as a future source of food, feed and energy is increasing. The Food and Agriculture Organization of the United Nations has acknowledged the potential of edible insects to contribute to healthy and sustainable diets, and has encouraged their adoption in the diets of people all around the world (Van Huis et al., 2013). Animal feed regulation agencies are authorizing the use of insect proteins as feed for poultry (McDougal, 2018) and farmed fish (Regulation 2017/893/EC, 2017) with the ambition to replace protein-rich feed ingredients with high environmental footprints, such as soybean and fish meal. The energy sector has envisioned high-fat farmed insects as a potential feedstock for future biodiesel production (Nguyen et al., 2019). Among all farmed insects, the black soldier fly (BSF) is one of the focal species due to the capacity of its larvae (BSFL) to quickly grow on different organic waste streams (Lalander et al., 2019; Tomberlin and Van Huis, 2020). This capacity not only makes BSFL a promising source of food, feed or feedstock for bioenergy, but also an attractive alternative for organic waste management (Čičková et al., 2015).

To ensure sustainable BSFL production, understanding and improving bioconversion efficiencies are key. The bioconversion efficiency is defined as the proportion of nutrients provided in the substrate which are incorporated into the larval biomass (Bosch et al., 2020). The higher these conversion efficiencies, the better the sustainability performance of a system. In the last decades, research mainly focused on reporting dry matter, carbon and nitrogen bioconversion efficiencies of BSFL grown on a wide variety of organic substrates, such as animal manures (Beskin et al., 2018; Li et al., 2018; Myers et al., 2008; Xiao et al., 2018), vegetable waste (Diener et al., 2011; Lalander et al., 2015; Parra Paz et al., 2015; Spranghers et al., 2016), and sludge (Lalander et al., 2019). These studies showed that the substrate for rearing of BSFL strongly influences the bioconversion efficiency and life-history traits (e.g., BSFL nitrogen efficiency of 2% if fed with undigested sludge and 80% if fed with chicken feed (Lalander et al., 2019)). Bioconversion efficiencies reported thus far, however, have not been calculated based on a complete mass balance (Bosch et al., 2020), a basic methodological requirement for bioconversion studies. Moreover, no bioconversion efficiencies have been reported for customized substrates currently used for industrial BSFL production in which different organic streams are mixed together to get a homogeneous substrate. Lastly, energy bioconversion efficiency has remained largely underexplored.

Besides improving bioconversion efficiencies, lowering gaseous emissions during larvae rearing is also an important aspect for sustainable BSFL production. Gases such as of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and ammonia (NH_3), are of particular interest due to the negative impact that these have on the global climate, air quality and eutrophication (Gruber and Galloway, 2008; IPCC, 2013). Only recently, the

first reports on gaseous emissions produced during the rearing of BSFL appeared (Chen et al., 2019; Ermolaev et al., 2019; Mertenat et al., 2019; Pang et al., 2020). All of these studies were framed in using BSFL for bio-waste management. They reared BSFL on non-homogeneous substrates, such as food waste and pig manure, and under different levels of moisture (Chen et al., 2019), pH (Pang et al., 2020) and substrate microbial inoculation (Ermolaev et al., 2019). Although these studies have produced valuable knowledge on gas emissions patterns, gas sampling was performed with a frequency of once every day or every five days, leading to measurements that did not quantify all gaseous emissions and therefore did not allow the construction of complete mass balances. Moreover, such time gaps between measurements increased the chance of missing details which occur at shorter time scales.

Given the need for complete mass-balances, and comprehensive gas measurements, the aim of this study was to quantify bioconversion efficiencies and gaseous emissions during BSFL rearing on a substrate currently used for its industrial production. To this end, we quantified the flows of energy and nutrients (i.e. nitrogen, carbon, potassium and phosphorus) and the emission of CO_2, CH_4, N_2O, NH_3, total N, as well as the heat production, to achieve a match of inputs and outputs.

3.2 Materials and methods

3.2.1 Insect rearing and sample collection

Just-hatched larvae of the Texas strain of BSF (*Hermetia illucens* L.; Diptera: Stratiomyidae; 100 generations; 38 days egg to egg cycle) were fed with a substrate made of 30% wheat bran and flour, and 70% water for 7 days at the facilities of Bestico B.V., the Netherlands. Once larvae were 7 days old (hereafter called starter larvae), they were sieved, packaged at 10–15 °C and shipped to the facilities of Wageningen University & Research. The same shipping also included sealed buckets with a substrate composed of a mixture of three feed ingredients, i.e. yeast concentrate from wheat (ProtiWanze®), a starch-rich by-product from wheat and potato industry (DB-blend) and a binding agent. On a fresh matter basis, the substrate contained 47% ProtiWanze®, 47% DB-Blend and 6% binding agent, and had an acid pH (near to pH 4) as the two main ingredients were acidified prior to commercialization. The nutrient composition of the substrate is given in Table 1. This substrate, is used in the mass-rearing operations of Bestico B.V. Upon arrival, three plastic crates (each 50 × 30 × 10 cm) were filled with 4 kg of fresh substrate and 10,000 starter larvae each. The three crates (in total 30,000 larvae and 12 kg of substrate) were stacked (space between crates was approx. 5 cm) and placed inside an open-circuit climate respiration chamber of 265 L (80 × 50 × 45 cm) (Figure 3.1). Larvae were fed only once, given that the chamber remained closed for the 7-day experimental period. Inside the

chamber, air temperature was set to 27 ± 0.5 °C, relative humidity to 70 ± 5% and L:D (light:day) ratio to 1:23. All these settings were specifically selected to mimic those used by Bestico B.V. Ventilation air flow through the respiration chamber was set to 27 L/min and two fans were used to ensure proper mixing of air (Figure 3.1). Air speed varied from 0.2 to 0.8 m/s due to the turbulence generated by the presence of the crates. The experiment was repeated 12 times. Since two identical respiration chambers were available in parallel, these 12 repetitions were obtained using six different batches of starters and substrates (two repetitions for every batch, one in each chamber). All batches of substrates contained the same ingredients and both chambers contained the same treatment. One repetition was discarded due technical problems with one of the respiration chambers, implying we finished with 11 repetitions.

Table 3.1: Nutrient composition of the substrate, starter larvae, 14-day old larvae and residual substrate (mean ± sd) . Except for DM, all values are expressed per 100 g of dry matter product.

Sample	Dry matter (%)	Carbon (g)	Energy (KJ)	Nitrogen (g)	Phosphorus (g)	Potassium (g)	Crude fat (g)	Starch (g)
Substrate	28.3±0.7	49.8±0.6	1976±18	2.74±0.08	0.45±0.03	1.34±0.11	6.6±0.2	22.6±0.9
Starter larvae	26.1±1.1	53.8±1.7	2366±96	9.71±0.78	1.83±0.15	1.78±0.12	13.4±4.4	-
14-day old larvae	35.8±1.5	59.5±0.4	2710±18	6.94±0.2	0.89±0.04	1.29±0.04	26.4±1.53	-
Residues	79.6±4.3	46.5±0.5	1812±18	2.94±0.14	0.57±0.06	2.02±0.22	3.1±0.3	17.2±0.6

3.2.2 Material sampling and analysis

Homogeneous samples of substrate and starter larvae were collected in 1 L plastic containers. After the 7-day experimental period, chambers were opened and samples of residues (i.e., mixture of larval excreta, their exuviae and uneaten feed) and 14-day old larvae were collected. Given that the three crates stacked in one respiration chamber were part of the same experimental unit (Figure 3.1), equal amounts of larvae and residues from each crate were sampled in the same container. All samples were stored at -20 °C for subsequent nutrient analysis. Samples of condensed water (referring to the water that got condensed from the cooling unit of the chamber in the 7 days period) and 25% sulfuric acid solution containing the NH_3 trapped from the outgoing air stream, were collected and stored at 5 °C for subsequent nitrogen analysis (for calculations see section 3.2.4).

3.2.3 Nutrient analysis

Prior to nutrient analysis, samples of substrate, starter larvae and 14-day old larvae were freeze-dried, whereas samples of residues were oven-dried at 70 °C for 48 h. Samples of substrate and residues were grounded to pass a 1 mm screen (Retsch ZM200). Due to their high fat content, samples of starter and 14-day old larvae were grounded three times with the same mill, but without a screen. Nutrient analyses were performed in duplicates at the

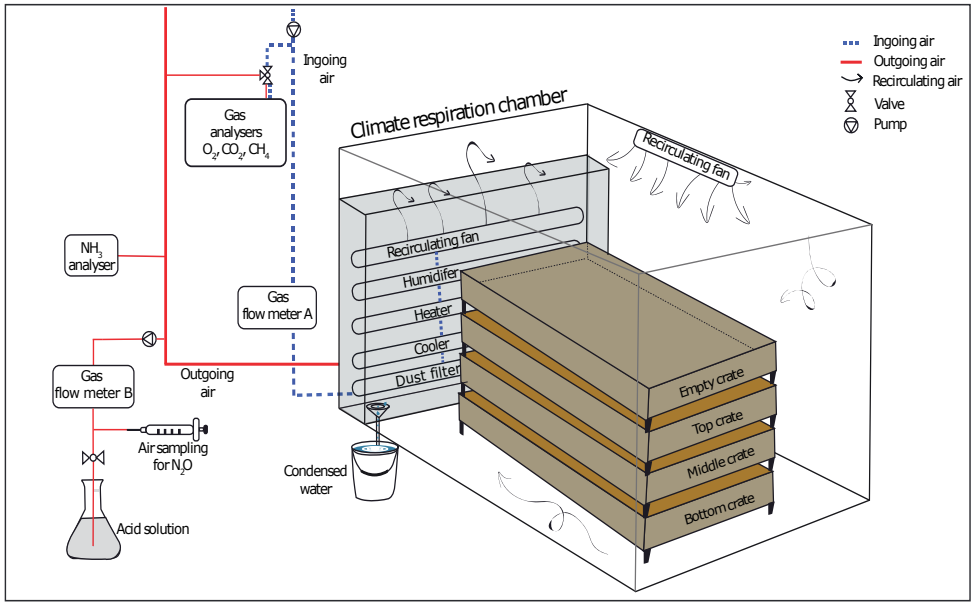

Figure 3.1: Schematic representation of the respiration chamber: air flows, climate control unit (shaded box), gas analysers and rearing crates. The shaded box was separated from the "animal space" in which the crates were located.

Animal Nutrition Laboratory of Wageningen University & Research, except for potassium which was analysed at Nutricontrol Laboratories, Veghel, the Netherlands. Samples of substrate, residues, starter and 14-day old larvae were analysed for contents of dry matter (ISO6496, 1999), nitrogen and carbon (Dumas method, ISO1634-1 (2008)), gross energy (oxygen bomb method, ISO9831 (1998)), phosphorus (spectrophotometry method, ISO6941 (1998)), potassium (CP-OES method, ISO21033 (2016)), and crude fat (hydrolysis method, ISO6492 (1999)). Samples of substrate and residues were also analysed for contents of starch (Amyloglucosidase method, ISO14914, 2004). Samples of condensed water and acid were analysed for nitrogen (Kjeldahl method, ISO5983-2 (2005)).

3.2.4 Gas measurements and calculations

CO_2, CH_4 and metabolic heat losses

Concentrations of O_2, CO_2 and CH_4 were measured in a cycle time of 9 min in the ingoing and outgoing air stream of each climate respiration chamber. Consumption of O_2 and production of CO_2 and CH_4 by the larvae, substrate and residues were therefore calculated based on the difference in gas concentrations measured in the ingoing (L/h)

and the outgoing (L/h) air streams multiplied by the amount of ingoing and outgoing ventilation air respectively, plus the change in each gas volume in the chamber between successive measurements. For a detailed explanation of the calculations used to determine total flows of CO_2, CH_4 and O_2 see Alferink et al. (2015). Each chamber operated under hyperbaric conditions (75 Pa as an ongoing check of airtightness). Ingoing air volumes were measured with a calibrated gas flow meter (Schulemberger/Itron G1.6), and were corrected for air temperature, pressure and humidity. Outgoing air volumes were calculated assuming that N_2 gas volumes in the outgoing and ingoing air were equal. O_2, CO_2 and CH_4 concentrations were measured in gas dried in a +2 °C dew-point cooler, using a paramagnetic analyser for O_2 and non-dispersive infrared analysers for CO_2 and CH_4 (ABB A02020). Two successful recovery tests were performed at the start of the measurements to ensure the correct calibration of all individual parts of the system (see Supplementary Methods for details). In addition, calibrating gases were daily flushed through all analysers to check and account for zero and span drift.

For the carbon balance, the overall carbon losses via gaseous emissions were quantified by the sum of the carbon contained in the CO_2 and CH_4 produced.

To quantify the amount of energy lost as heat from the complete oxidation of substrates, we calculated the heat production (Q) using Brouwer's equation (Brouwer, 1965):

$$Q = 16.175VO_2 + 5.021VO_2 + 2.167VCH_4 \tag{3.1}$$

where VO_2 is the consumption of O_2 (in L/h), VCO_2 (in L/h) is the production of CO_2 and VCH_4 (in L/h) is the production of CH_4. The respiratory quotient (RQ), used as an indicator of the type of substrate oxidized, was calculated using the following equation (Brouwer, 1965):

$$RQ = \frac{VCO_2}{VO_2} \tag{3.2}$$

Nitrogen lost as ammonia

Nitrogen air losses were measured with two methods. The first method quantified the overall amount of nitrogen lost (mainly NH_3) in the whole experimental period. With this method, here called "washing-bottle method", we quantified the nitrogen leaving the chamber in air and in condensed water (see Figure 3.1). Total nitrogen emissions were determined using the following equation:

$$TN = \left[\frac{N_{acid}}{1000} \times \frac{V_t A_s}{V_f}\right] + \left[\frac{N_{cond}}{1000} \times D\right] \tag{3.3}$$

where TN is the total nitrogen emissions (in g) during the whole time that the larvae remained in the climate respiration chamber, N_{acid} is the concentration of nitrogen in the acid sample (in g/kg), V_t (measured by gas flowmeter A, see Figure 3.1) is the total air ventilated volume (in m^3), is the grams of acid (in g), V_f (measured by gas flowmeter B) is the total air ventilated volume (in m^3) that went through the acid bottle, A_s is the concentration of nitrogen in the condensed water sample (in g/kg) and D is the total amount of condensed water (in g).

With the second method, NH_3 concentrations were continuously measured (every 9 min) in the outgoing air stream (see Figure 3.1) using a calibrated NH_3 sensor (Dräger Polytron® 8100 EC with sensor type NH_3-FL, range 0–100 ppm NH_3). This method allowed us to see the development of NH_3 losses over time. NH_3 emissions (L/h) were calculated with the same procedures as applied for CO_2 and O_2. Nitrogen losses in condensed water were not accounted for in this method. The NH_3 sensors were damaged during the last two repetitions, and therefore emissions could only be presented for nine repetitions.

Nitrogen lost as nitrous oxide

Open air N_2O concentrations (outside the chamber) were measured on the first day of each repetition and were assumed to remain constant until the end of each replicate. N_2O concentrations in each chamber were measured every 24 h (at 12:00 h), by taking an air sample of 60 mL of outgoing air with a syringe (BD Plastipak). Syringes were stored for 1–48 h in polyethylene zip bags at room temperature (20–25 °C) and analysed in a gas chromatograph (Interscience GC 8000 top), using a Haysep Q 80–100 mesh 3m × 1/8" SS column at 60 °C and with an injection volume of 2 mL. Total N_2O emissions during the 7 days were estimated using the following equation (adapted from Alferink et al. (2015)):

$$TN_2O = \sum g_i \times 10^{-4} \times W_i \times \frac{44}{0.0224} \tag{3.4}$$

where TN_2O is the amount (in grams) of N_2O during the whole time that the larvae remained in the climate respiration chamber, g_i is the averaged N_2O concentration (in ppm) of two subsequent measurements in time period i, 10^{-4} is used to convert gas concentrations from ppm to %, W_i is the air ventilation volume in the time period i, 44 (in g/mol) is the molar mass of N_2O and 22.4 (in L/mol) is the molar volume of an ideal gas.

Nutrient bioconversion efficiency

To determine the nutrient bioconversion efficiency, we adapted the bioconversion efficiency equation of Bosch et al. (2020) as follows:

$$NUE_n = \frac{(B_m Y_n - B_s Y_s)_n}{B_d Y_d} \tag{3.5}$$

where NUE_n is the nutrient bioconversion efficiency of nutrient n, B_m is the harvested DM biomass of 14-day old larvae (in g), Y_n is the content of n in dry 14-day old larvae (in g/kg), B_s is the DM biomass of starter larvae introduced at the beginning of the experiment (in g), Y_s is content of n in dry starter larvae (in g/kg), B_d is the DM substrate added at the beginning of the experiment (in g) and Y_d is the content of n in dry substrate (in g/kg).

3.3 Results and discussion

In all balances, the sum of outputs (larval biomass, residues and gaseous emissions) nearly equalled the inputs, provided through the substrate, indicating that our methods successfully quantified the nutrient flows through the system. Recovery rates were 95 ± 0.5% for carbon, 97 ± 0.4% for energy, 101 ± 0.7% for nitrogen, 100 ± 0.8% for phosphorus and 102 ± 1% for potassium.

3.3.1 Dry matter, carbon and energy

The dry matter, carbon and energy balances showed similar partitioning. Between 16 and 22% of the outputs were found in the 14-day old larval biomass, 55–58% in the residues and 23–27% was lost to the air via gas emissions and metabolic heat (Figure 3.2A–C). Although comparison of bioconversion efficiencies with other studies should be made with caution due to the different nutrient content of diets, rearing time, densities, and other experimental conditions, our dry matter bioconversion efficiency (16%) was within the ranges (4–28%) reported in studies that used non-manure/non-sludge substrates (Diener et al., 2011; Ermolaev et al., 2019; Lalander et al., 2019; Oonincx et al., 2015). We found a higher carbon bioconversion efficiency (20%) than those (2–14%) reported for BSFL fed with a substrate composed of food waste and rice straw at different pH values (1.95–13.71%; Pang et al. (2020)). While many factors apart from pH might have affected the bioconversion efficiencies in the study by Pang et al. (2020) (e.g., particle size and moisture), the high levels of total fibre present in rice straw could be the cause of the high retention of carbon in the residues and therefore low presence in the larvae.

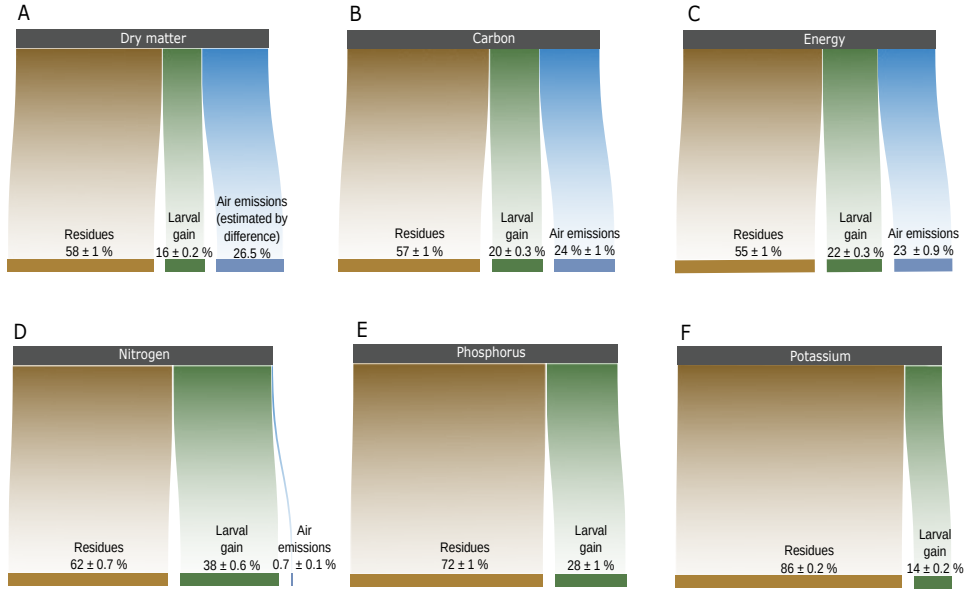

Figure 3.2: Dry matter, carbon, energy, nitrogen, phosphorus and potassium balances. All balances are expressed in percentage of each output ± standard error of the mean. Substrate was considered as the only input and larval gain shows how much of each input was incorporated as larval biomass (after subtraction of the inputs contained in the starter larvae). See Table S3.1 for the basic mass values.

Between 37 and 53% of the starch provided with the substrate was recovered in residues (Figure S1), indicating that not all the carbon and energy contained in the substrate as starch was used by the larvae. Fly larvae and other insects defecate into their feeding substrate which can led to more than one round of digestion (Weiss, 2006; Wotton, 1980). In addition, it is known that BSFL produce amylases to digest starch (Kim et al., 2011). It is therefore likely that even though most of the starch found in the residues was consumed, part of it might have been resistant to enzymatic degradation and therefore not digested by the larvae. Research has shown starch to be resistant to enzymatic degradation because of its granular structure (e.g. in native potato) or retrogradation, caused by heat processing (Champ et al., 2003). In addition to resistant starch, it cannot be excluded that a portion of the starch found in the residues was not digested by the larvae due to water limitation (see section 3.3.5). When carbon and energy inputs were corrected for the resistant starch to explore potential bioconversion efficiencies, the bioconversion efficiencies for carbon increased from 19.5% to 21.4% and for energy from 22% to 24% (see Supplementary Material for calculations). Although the efficiency gains in these cases are minor, any unconsumed or unused inputs would result in lower efficiencies than those that were

attained by the animal. This points to the importance of ingredient digestibility for optimal BSFL conversion efficiencies and the relevant role that microbial inoculation strategies could have to increase both digestibility and bioconversion efficiencies (Rehman et al., 2017; Yu et al., 2011).

Carbon losses via gas emissions occurred mainly as CO_2 (Figure 3.3A), and nearly no CH_4 (Figure 3.3D) was detected. This is in line with the few studies that have measured CO_2 and CH_4 emissions (Mertenat et al., 2019; Perednia et al., 2017). Despite CO_2 emissions occurring throughout the experiment, a clear peak in CO_2 production was observed between day 5 and day 6 (Figure 3.3A). A trial parallel to our main experiment, in which fresh substrate was supplemented after the peaks of CO_2 showed that following the addition of new fresh feed, both parameters peaked again (data presented in Figure S2). This finding indicates that the drop in CO_2 emission observed on day 5 was caused by the physiological response of BSFL to either limited availability or accessibility of fresh feed. In the same pilot study, we measured CO_2 emissions after the addition of fresh feed, but without larvae, and concluded that microbial metabolism in the substrate contributed to 34% of the overall CO_2 emissions. This demonstrates that the contribution of microbial respiration to the overall CO_2 production during BSFL rearing is substantial. While it is known that inoculation of beneficial bacteria can help to increase bioconversion efficiencies and improve larval growth (Xiao et al., 2018; Yu et al., 2011), excessive microbial fermentation could also lead to inefficiencies, such as excessive production of CO_2 and the modification of substrate conditions (e.g., elevated substrate temperatures) which can negatively affect larval growth. Thus, even though both larval and microbes coexist in the same system (Jeon et al., 2011), future research efforts should focus on disentangling the contribution of each component to the overall GHG emissions, and exploring maximum tolerable levels of microbial emissions to avoid unnecessary substrate fermentation without benefits for larval growth and bioconversion efficiencies.

Respiratory Quotient (RQ) values peaked in the first two days, dropped to values slightly above 1 until the fifth day, and decreased below 1 in the last three days (Figure 3.3C). The RQ values above 1 observed in the first five days might be associated with anaerobic fermentation and/or *de novo lipogenesis* from carbohydrates. During anaerobic fermentation, CO_2 is produced without the need for O_2. During *de novo lipogenesis* only a portion of the C in carbohydrates (e.g., glucose) is sequestered in fatty acids, and the rest is excreted as CO_2 without the need for O_2 (Gerrits et al., 2015). In a pilot study, we observed RQ values higher than 1 when only substrate and residues were present in the respiration chambers (Figure S2), confirming that anaerobic fermentation can take place in the absence of larvae. Thus, it is likely that anaerobic fermentation occurred during the first days, when larval biomass was small and larval movement had a limited influence on substrate aeration. Following the RQ peak, it is likely that *de novo lipogenesis* could have still occurred but at lower rates, and together with increasing oxidation rates of starch, lactic acid, fats and proteins during the last days.

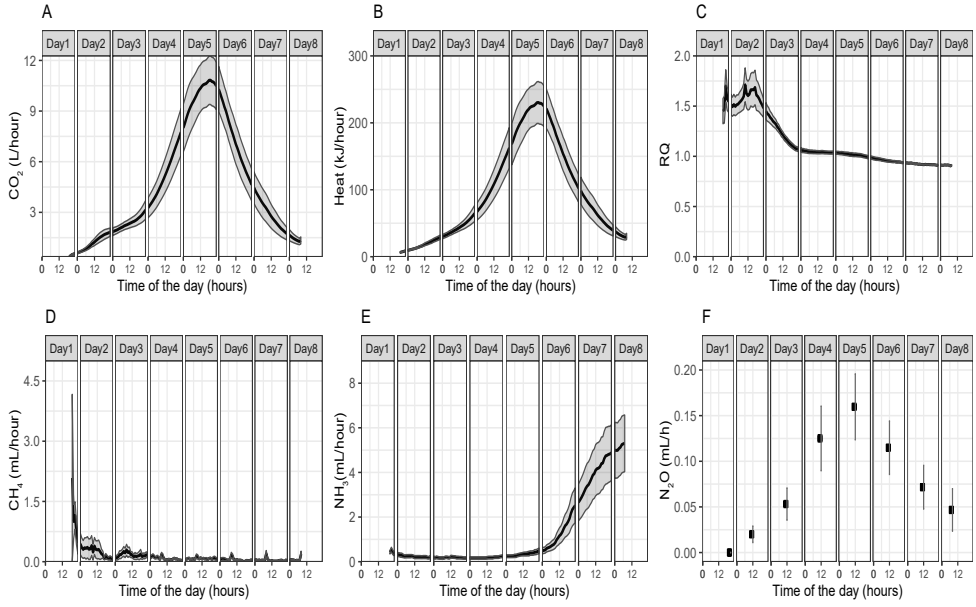

Figure 3.3: Parameters registered over time in the climate respiration chambers for A) CO_2, B) heat, C) RQ, D) CH_4, E) NH_3 and F) N_2O. The black lines show the mean value obtained from 11 replicates (except for NH_3 which were 9), and the grey shaded area and error bars (panel F) the standard error of the mean. N_2O was measured once per day. See Figure S3 for figures per replicate.

3.3.2 Nitrogen

The nitrogen bioconversion efficiency was 38%, meaning that to get 1 unit of nitrogen gain from BSFL 2.6 units of input-nitrogen were needed (Figure 3.2D). This bioconversion efficiency was close to those found for BSFL fed with dog feed (46%), fruits and vegetables (34%) and abattoir waste (31%), but lower than those found for BSFL fed with food waste (59%) and chicken feed (80.4%) (Lalander et al., 2019). Although it should be noticed that other studies have reported lower nitrogen bioconversion efficiencies for food waste (12.5% in Lalander et al. (2015); 5–19% in Pang et al. (2020)) and chicken feed (52% in Oonincx et al. (2015)). Bosch et al. (2019) summarised the nitrogen bioconversion efficiencies from five studies presenting data on 13 substrate types and found these to vary greatly. This variation shows the dominant effect that substrate composition has on nitrogen bioconversion and points to the necessity to identify the key factors that affect it.

The residues, containing nearly 62% of the total nitrogen input, were found to be the main nitrogen output (Figure 3.2D). Although we did not measure the different forms of nitrogen

in the residues (i.e., organic-nitrogen, ammonium-nitrogen or nitrates), Lalander et al. (2015) found that nitrogen in BSFL residues consisted of 78% of organic-nitrogen and 19% of ammonium-nitrogen. Given that under certain temperatures, moisture, pH and other physicochemical conditions, the organic and ammonium-nitrogen are prone to air losses via ammonia volatilization (Koerkamp, 1994), it is crucial to implement good post-harvest management practices to avoid gaseous nitrogen losses. Such practices, which are already described for the prevention of NH_3 emissions from poultry litter (Koerkamp, 1994), should tackle two processes. First, the reduction of microbial activity in the residues to prevent additional microbial breakdown of uric acid and undigested proteins into ammonium (NH_4^+). This could be achieved by keeping the dry matter content of the residues above 60%. Second, the maintenance of the equilibrium between NH_4^+ and NH_3 to avoid NH_3 volatilization. This could be achieved by keeping an acidic pH, temperatures below 20 °C, and by reducing as much as possible the exposure surface of the residues to air (Koerkamp, 1994).

With 1% of the total nitrogen, the proportion of nitrogen leaving the system via gaseous emissions was minor. While some studies have found similar results (Ermolaev et al., 2019; Pang et al., 2020), others have reported losses up to 40% (Lalander et al., 2015). Large-scale BSFL producers have also reported high levels of NH_3 emissions during the rearing process (Yang, 2019). The low levels of NH_3 quantified in our study might have had two causes. First, the low dry matter content of the substrate which might have limited the microbial degradation of organic nitrogen into NH_4^+. Second, the relatively low acidity of the initial substrate (pH = 4) which might have prevented a rapid shift of the equilibrium between NH_4^+ and NH_3 equilibrium towards NH_3 (Pang et al., 2020).

Although nitrogen losses via NH_3 emissions in our system were very low, the production of this gas had a defined temporal pattern. NH_3 was produced from day 5 onwards, right after the peak of CO_2 and metabolic heat production was reached (Figure 3.3E). This pattern is more evident when CO_2 and NH_3 emissions are observed per replicate (Figure S3). The timing of NH_3 emissions might be explained by the high excretion rates of uric acid during the larval metabolic peak, followed by the microbial breakdown of uric acid into NH_4^+ (favoured by substrate temperatures above 40 °C at this timing, unpublished data), and the subsequent volatilization of NH_3 due to pH substrate turning alkaline (Ma et al., 2018; Meneguz et al., 2018; Pang et al., 2020). When CO_2 peaked late, NH_3 was barely produced or absent (Figure S3). The fact that only some batches of BSFL produced NH_3 has also been observed at industrial scale (Bestico B.V., personal communication). We did not find any effect of the energy and nitrogen content of the substrate, size of starter larvae, nor proportion of resistant starch found in the residues that could explain the emission patterns of NH_3 (see Table S2). However, considering that the occurrence and intensity of NH_3 emissions are associated with the timing and total production of CO_2 (Figure S3 ; Figure S4), it is likely that NH_3 emissions are the result of changes taking place in the substrate when larval metabolism is high (e.g., changes in temperature, moisture, pH

and microbial activity). Further research is needed for a deeper understanding of this process, as its elucidation could help to minimize nitrogen gaseous losses by management practices such as early larval harvesting or the application of low-pH feeds in multiple feeding systems to avoid NH_3 volatilization.

3.3.3 Phosphorus and potassium

The outcome of the phosphorus and potassium balances showed that 27% of the phosphorus and 14% of the potassium were retained in the larvae, and the remaining 73% and 86% in the residues, respectively (Figure 3.2E-F). Both minerals were not quantified in the air given that these are almost exclusively found in the solid phase. As the variation in the bioconversion efficiency of phosphorus and potassium was low and air losses were nearly zero, the low bioconversion efficiencies reported here typically reflect high dietary concentrations. Hence, these efficiencies are highly diet-dependent and care should be taken in using them as benchmark values for other systems. The nutrient analysis showed that the concentrations of both minerals were higher in the residues than in the initial substrate, as it was reported in other studies (Lalander et al., 2015; Sarpong et al., 2019).

The residues had a C:N:P:K ratio of 81:5:1:4. Given that the C:N ratio of the residues was lower than 20:1, and the C:P lower than 200:1 (both values usually used as benchmarks), it is expected that if residues are intended to be used as fertilizers and applied directly to the soil, nitrogen and phosphorus mineralization will be favoured (Stevenson and Cole, 1999). Compared to pig slurry manure, the residues of the system studied could supply the same amounts of nitrogen but with 22% less phosphorus and 10% more potassium (Table S3). This could be a potential advantage for soils with already high amounts of phosphorus, but a disadvantage for soils with low levels of this mineral. Compared to cattle slurry manure, the application of larval residues would not offer considerable benefits as phosphorus inputs would be almost the same while potassium inputs will be reduced by 38% (Table S3). Compared to composted food waste, larval residues had very similar C:N and C:P ratios and could supply the same levels of nutrients (Table S3). So far, some studies report similar crop yields and growth rates in crops fertilized with BSFL residues, compared to those reached with artificial fertilizers and compost (Choi et al., 2009; Zahn, 2017), while others report reduced plant growth (Alattar et al., 2016). The quality of larval residues as a crop fertilizer would heavily depend on the choice of ingredients to feed BSFL. Hence, generalizations about its fertilizer value and its effects on crop yields should be made with caution.

3.3.4 Emissions

The total direct emissions of CO_2, CH_4, N_2O and N produced during the rearing process of BSFL are shown in Table 3.2. Overall, the direct emissions from CH_4 and N_2O per kg

Table 3.2: Gas emissions per kg of dry matter larvae biomass (mean ± standard deviation). Global Warming Potential (GWP) was expressed as g CO_2 equivalents based on the GWP_{100} of CH_4 (34) and N_2O (298) with carbon feedback (IPCC, 2013).

Study[a]	CO_2 (g)	CH_4 (mg)	N_2O (mg)	N (g)	GWP - CO_2eq (g)
Ermolaev et al. (2019)	1750 ± 170	49 ± 29	21 ± 13	-	8 ± 4.8
Mertenat et al. (2019)	-	5.5	118	-	35
Pang et al. (2020)	1394 ± 343	14 ± 6	7 ± 1	-	2.5 ± 0.5
This study	2750 ± 314	28 ± 29 [b]	53 ± 27	1.2 ± 0.7	17 ± 8.6

[a] For Ermolaev et al. (2019) we used the values of treatment "L". For Pang et al. (2020) we used the values of treatments pH 5, pH 7 and pH 9. For calculations and assumptions see Supplementary Methods.

[b] Standard deviation was large because in one of the repetitions (R11) much more CH_4 was produced than in all other repetitions. Without this outlier, CH_4 mg per kg of dry matter larvae biomass would be 19 ± 10 (mean ± standard deviation).

of fresh larval gain were 6 ± 3.23 g CO_2eq per kg of fresh larvae and 16.8 ± 8.6 g CO_2eq per kg of dry larvae (see Figure 3.3A-F for emissions over time). CO_2 emissions resulting from larvae and substrate respiration were not accounted in GHG emission calculations as respiration carbon is part of the short carbon cycle (Clais et al., 2013) and is assumed to be rapidly assimilated in plant biomass.

GHG emissions vary largely between BSFL studies. Our estimates of Global Warming Potential (GWP) double the emissions quantified by Ermolaev et al. (2019), halve those estimated by Mertenat et al. (2019) and exceed by six times the values obtained by Pang et al. (2020) (Table 3.2). All studies listed in Table 3.2 were performed with the main motivation of using BSFL for waste management rather than to maximize larvae production per unit of time. Thus, parameters such as treatment duration, feed substrate ration, experimental scale (i.e., number of larvae, kg of substrate) feeding strategy (i.e., single and multiple feeding), and ambient temperature, differed between studies and likely played a substantial role in the reported variation of the available estimations. For instance, the higher final larval weight (287 mg per larva) and nitrogen conversion efficiency (56%) reported by Ermolaev et al. (2019) could have caused the lower gas emissions compared to our values. Furthermore, a very important factor that distinguished our measurements from others was the frequency of gas sampling. While we measured the concentration of most gases (except N_2O, which was done daily) every 9 min, others sampled only once every 24 h (Mertenat et al., 2019; Pang et al., 2020) or 48 h and 96 h (Ermolaev et al., 2019). Thus, with longer periods without data, it is more likely to miss emission peaks or gas fumes and therefore underestimate the total gas production.

The quantification of gaseous emissions is relevant for sustainability assessments at a larger scale. Previous life cycle assessments (LCA) on BSFL relied on emission data

quantified for insect species other than BSF (Salomone et al., 2017; Smetana et al., 2015; Smetana et al., 2016) to account for the direct GHG emissions produced during BSFL rearing. Due to the lack of basic data presented in these studies, we were unable to estimate the contribution of direct GHG emissions presented here to the overall GWP found in these systems. However, recent evidence indicates that direct BSFL emissions do have a small but still important role in the overall GWP when looked at an LCA level. If direct GHG emissions resulting from waste pre-processing, larvae rearing, colony rearing, product harvesting and larvae processing are included, direct BSFL and substrate emissions resulting from these processes contributed to approx. 10–15% of the overall GWP (Mertenat et al., 2019). This value is larger than that reported by Oonincx and Boer (2012) for mealworms, in which direct GHG emissions from larvae and substrate contributed to less than 1% of the overall GWP in a cradle-to-farm gate LCA. It should be noticed, however, that the direct GHG emissions from mealworm rearing were reported to be 7.58 ± 2.29 g CO_2eq, per kg of dry larvae which is nearly half of those reported here (Oonincx et al., 2010).

Given the variations that can exist between studies reporting direct GHG emissions from BSFL rearing (Table 3.2), and the contribution that these might have on the overall GWP of a system, we advise future researchers relying on direct GHG emissions from the literature for the elaboration of life cycle assessments of BSFL production systems, to be cautious and perform sensitivity analysis using the available values of direct GHG emissions reported for BSFL in their estimations.

3.3.5 Limitations

Even though we successfully quantified the inputs and outputs of the system, our results were likely affected by the experimental conditions. Larval yields were found to be 30% lower than those aimed under industrial conditions. We believe that the lower yields might be linked to water limitation. Water losses from the substrate might have been larger than in industrial conditions given the higher exposure to circulating air inside the chambers that were needed to ensure homogenous mixing of air for gas analysis. Thus, it is likely that under optimal growing conditions, the nutrient and energy efficiencies could be higher and the gaseous emissions of GHG and nitrogen per kg of larvae gain lower.

3.4 Conclusions

Bioconversion efficiencies of BSFL reared on a substrate currently used for its industrial production ranged from 14% (potassium) to 38% (nitrogen). The proportion of inputs found in the residues ranged from 55% (energy) to 86% (potassium), while the proportion of inputs lost via gas emissions ranged from 1% (nitrogen) to 24% (carbon). Substantial

amounts of starch were found back in the residues, indicating that there is room to improve carbon and energy efficiencies. Direct GHG emissions associated to BSFL rearing were 16.8 ± 8.6 g CO_2eq per kg of dry larvae gain. Even though nitrogen losses via NH_3 emissions were very low, we observed that NH_3 was produced only after the peak of CO_2 production was reached. This trend should be further explored as its understanding could be relevant to minimize nitrogen losses in BSFL production systems.

3.5 Supplementary Information

The supporting information of this chapter includes:

Appendix A - Supplementary Methods

Appendix B - Supplementary Figures (S1-S4)

The original publication also contained a section of Supplementary Tables. Along the chapter there are references to items that belong to this section, however these were not included in this thesis due to space constraints. The Supplementary Table can be accessed through the online version of the publication.

Appendix A - Supplementary Methods

Recovery tests – climate chambers

Before the start of the experiment, two CO_2 recovery tests per respiration chamber were performed to ensure the correct calibration of all individual parts of the system. We measured recovery rates of 98.2% and 97.9% in chamber 1, and 99.3% and 98.2% in chamber 2. In these tests, the CO_2 injection diluted the O_2 content in the chamber. The calculated O_2 consumption, expressed as a percentage of the injected amount of CO_2 (which should be near zero), was 0.2% and 0.2% in chamber 1, and 0 and 0.1% in chamber 2. A more detailed explanation of the system used to analyse CO_2, CH_4 and O_2 in the climate respiration chambers is given by Heetkamp et al. (2015).

Carbon and energy efficiencies corrected for resistant starch

In order to recalculate the carbon and energy efficiencies, we first calculated the amount (in grams) of starch found in the residues and then we estimated the amounts of carbon and energy contained in that starch. For carbon we assumed 423 g of C per kg of dry matter starch (Gerrits et al., 2015) and for energy we assumed 17.5 kJ per g dry matter starch (Van Erp et al., 2018).

The corrected larval conversion efficiency was estimated with the following equation:

$$LCE_n = \left(\frac{N_{larvae}}{N_{diet} - N_{resistantstarch}} \right) \times 100 \tag{3.6}$$

where LCE_n is the corrected larval conversion efficiency for carbon or energy, N_{larvae} is the carbon (in grams) or energy (in kJ) content in the larval biomass, N_{diet} is the carbon (in grams) or energy (in kJ) content provided in the diet and $N_{resistantstarch}$ is the carbon (in grams) or energy (in kJ) content present in the starch found in the residues.

Microbial contribution to CO_2 emissions

We performed a pilot study to understand if the drop in CO_2 emissions observed between days 5 and 6 was a natural physiological response of larvae development or if it was a physiological response to feed availability or accessibility. We had two climate respiration chambers available (i.e., chamber 1 and chamber 2). In each chamber we placed 30,000 starter larvae and 12 kg of fresh substrate distributed in three crates (10,000 starters and 4 kg of substrate per crate). Chambers were closed and six days later, when CO_2 emissions had already peaked and were dropping, both chambers were opened. We added 6 kg of fresh substrate to the crates of chamber 1 (2 kg per crate) (treatment 1), while for the crates in chamber 2 , we removed all the larvae (by sieving), put the sieved residues back into the crates and added 6 kg of fresh substrate (2 kg per crate) (treatment 2). Crates were placed back in the chambers for four additional days. The treatment applied to chamber 2 (i.e., larvae removed, residues left and fresh feed added) was made to disentangle microbial from larval emissions in case an increase in CO_2 was observed. A graphical design of the experiment is shown in Figure S2-A.

Estimation of gaseous emissions in other studies

Different studies reported the production of CO_2, CH_4 and N_2O during biowaste treatments with BSFL. Most of the studies reported the emissions expressed per kg of waste treated. We calculated the emissions expressed per kg of dry matter larvae. Calculations were made in the following way:

Ermolaev et al. (2019)

We used the mean of CH_4 and the mean of N_2O for treatment "L" listed in Table 3 and the GWP_{100} conversion factors for CH_4 (34) and N_2O (298) to estimate the relative contribution of each gas to the total GWP. We estimated that CH_4 contributed 21% and N_2O 79% of the overall GWP. Table 4 showed that 7.90 ± 4.73 kg CO2eq (considering CH_4 and N_2O) were produced per ton of DM larvae produced. Thus, using the contribution of each gas estimated in the previous step, we were able to calculate how much mg of each gas were produced per kg of dry larvae. To report mean values ± standard deviation, we repeated the calculations 3 times. The first with the mean (7.90 kg CO2eq), the second (minimum value) with the mean – sd (7.90 – 4.73) and third with (maximum value) with the mean + sd (7.90 + 4.73).

Mertenat et al. (2019)

We used the values listed in Table 3 (grams CH_4 and N_2O per ton of wet waste) and a larvae yield of 1087 grams per 15 kg of wet substrate (A. Mertenat, personal communication, March 27 2020) to estimate the emissions per kg of dry larvae.

Pang et al. (2020)

We used the CO_2, CH_4 and N_2O values (g/kg dry substrate) of the treatments pH=5, pH=7 and pH=9 from Table 3. The other two treatments (pH=3 and pH=11) were excluded as these are extreme pH values not likely to be applied in practice. To estimate the emissions per kg of larvae, the larvae yield per kg of dry matter waste product was needed but this value is not shown in the study. Therefore we estimated it in the following way.

The authors mention that the individual larval weight for each of the treatments was 60 mg (pH=3), 68 mg (pH=5) and 80 mg (pH=7). The authors also mentioned that each treatment consisted of 1800 larvae per 1.2 kg of substrate. By assuming that the survival rate was 100% in all treatments we estimated the fresh larval yield per kg of wet substrate. We converted the fresh larval yield per kg of wet substrate to dry larval yield per kg of dry substrate by using a substrate dry matter content of 35% (mentioned in section 2.2) and assuming a larval dry matter content of (35.8%) which was obtained from our own values We used the larval yield per kg of dry mater to estimate the production of each gas using the values provided in Table 3. We estimated the mean value from the 3 treatments and calculated the standard deviation.

Appendix B - Supplementary Figures

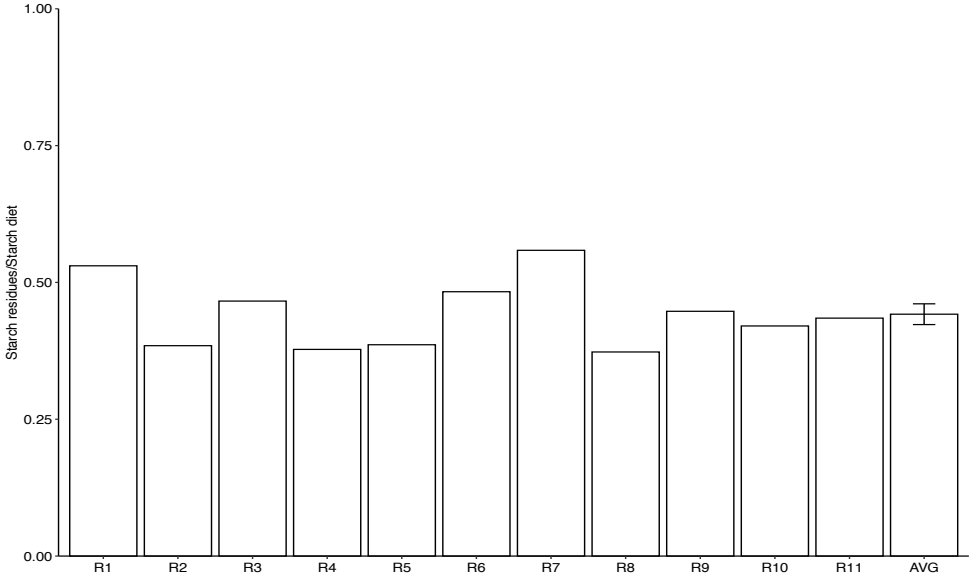

Figure S1. Proportion of starch found in the residues relative to the amounts given in the diet. R1-R11 refer to each replicate. AVG is the average value of all replicates with the standard error.

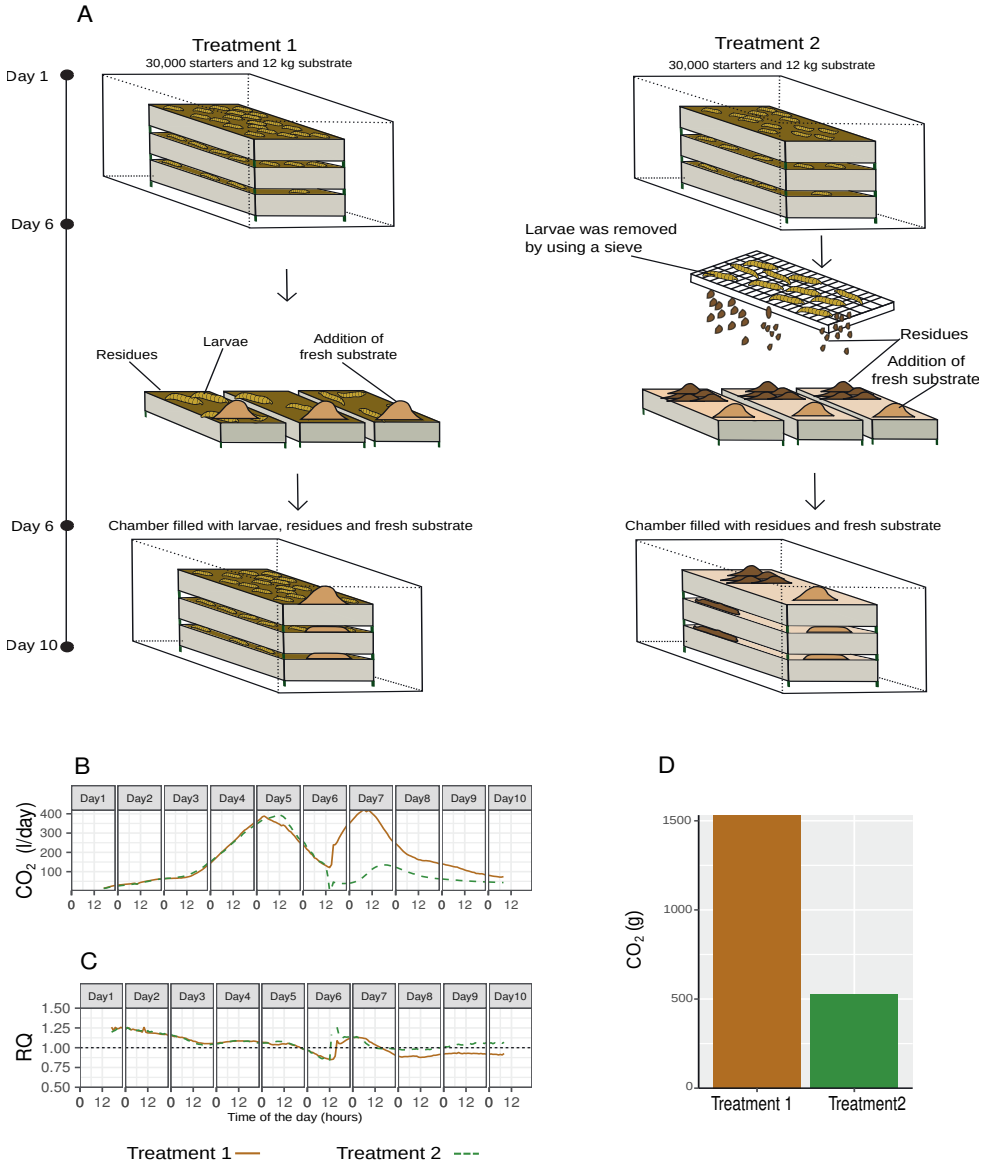

Figure S2. **A**. Experimental setup. **B**. CO_2 emissions over time. **C**. Respiratory Quotient (RQ) over time. The black dashed line shows RQ = 1. **D**. Total production of CO_2 (grams) from day 6 to day 10 per treatment.

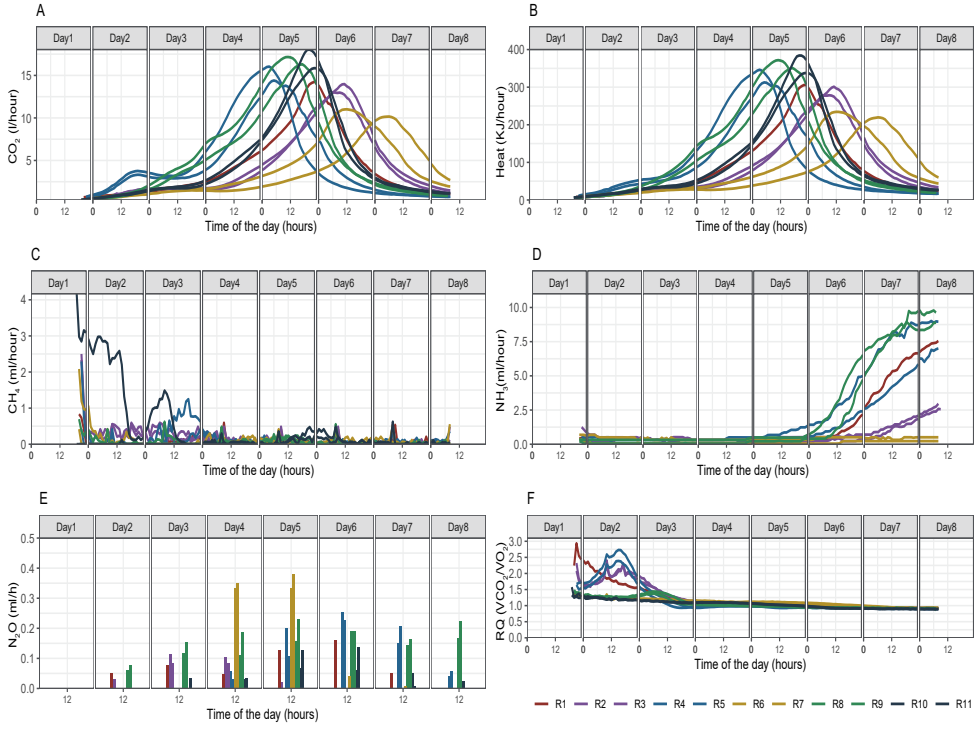

Figure S3. Parameters registered over time in the climate respiration chambers for carbon dioxide (panel A), heat (panel B), methane (panel C), ammonia (panel D), nitrous oxide (panel E) and RQ (panel F). Each line represents one replicate. Lines with the same colour correspond to the same batch of starters and diet, but conducted in a different chamber. Nitrous oxide (panel E) was measured once per day (at 12:00 h) per replicate and therefore each bar represents the production of nitrous oxide for a specific day and replicate.

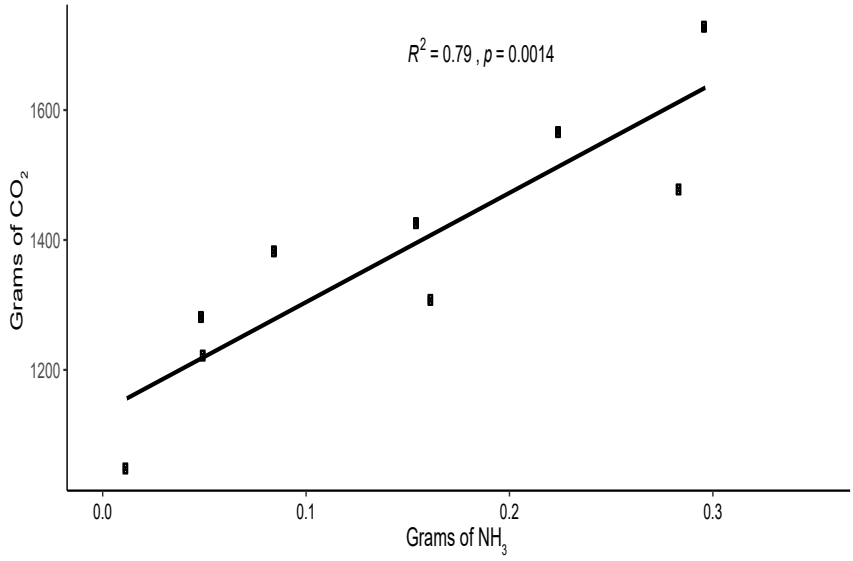

Figure S4. Correlation between overall aerial N emissions and CO_2.

Chapter 4

Nutrient flows during black soldier fly larvae rearing on pig manure

This chapter is based on:

A. Parodi, W. J. J. Gerrits, J. J. A. Van Loon, I. J. M. De Boer, A. J. A. Aarnink, and H. H. E. Van Zanten (2021). "Black soldier fly reared on pig manure: Bioconversion efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions". *Waste Management* 126, 674–683. DOI: 10.1016/J.WASMAN.2021.04.001

Abstract

There is an increased interest for using insects, such as the black soldier fly, to treat surplus manure and upcycle nutrients into the food system. Understanding the influence that BSFL have on nutrient flows and nutrient losses during manure bioconversion is key for sustainability assessments. Here we quantified and compared nutrient balances, nutrient levels in residual materials and emissions of greenhouse gases and ammonia between manure incubated with black soldier fly larvae (BSFL) and manure without BSFL, during a 9-day experimental period. We obtained high analytical recoveries, ranging between 95 and 103%. We found that of the pig manure supplied, 12.5% of dry matter (DM), 13% of carbon, 25% of nitrogen, 14% of energy, 8.5% of phosphorus and 9% of potassium was stored in BSFL body mass. When BSFL were present, more carbon dioxide (247 vs 148 g/kg of DM manure) and ammonia-nitrogen (7 vs 4.5 g/kg of DM manure) emitted than when larvae were absent. Methane, which was the main contributor to greenhouse gas emissions, was produced at the same levels (1.3 vs 1.1 g/kg of DM manure) in both treatments, indicating the main role that manure microbial methane emissions play. Nitrous oxide was negligible in both treatments. The uptake of nutrients by the larvae and the higher carbon dioxide and ammonia emissions modified the nutrient composition of the residual material substantially relative to the fresh manure. Our study provides a reliable basis to quantify the environmental impact of using BSFL in future life cycle assessments.

4.1 Introduction

Insect farming for feed and food purposes is considered a new emerging agricultural sector (Van Huis, 2020). Factors such as the development of accessible technologies to upscale insect farming and legislation changes allowing the use of mass-produced insects as human food and animal feed have contributed to the sector's growth over the last years (Van Huis, 2020). The increased interest in farmed insects as an alternative food and feed source, however, is rooted in their potential to improve the sustainability of food systems. By feeding farmed insects with organic residual streams, insects can recover part of the nutrients contained in these streams, and the insect biomass obtained can be used in the food system either as human food or animal feed. This innovation is expected to reduce the environmental impact of the food system as wasted resources are reconverted and reused. To efficiently use insects as recyclers in our food systems, these should be produced using the same principles proposed for livestock in circular food systems (Van Zanten et al., 2019). Thus, insects envisioned for food should be only fed with organic streams inedible to humans such as by-products from the food industry, food waste, crop residues (Van Hal et al., 2019a; Van Hal et al., 2019b),, while insects envisioned for feed should be fed with streams that cannot be directly consumed by humans nor by livestock or fish. An abundant organic stream that is not directly consumed by livestock and fish, and that in addition is considered as an environmental burden when not properly managed (Strokal et al., 2016), is animal manure.

Surplus manure produced in livestock-dense regions where animals are intensively produced and where adjacent croplands are saturated with nutrients, is a source of environmental pollution (Gerber et al., 2013; Leip et al., 2015; Strokal et al., 2016; Wang et al., 2013; Yang et al., 2017) and a public health threat (Venglovsky et al., 2009; Xie et al., 2018; Zhu et al., 2013). Regional surpluses of manure are often transported to other regions. In addition, diverse manure processing technologies have been developed to modify the physical, chemical and/or biological properties of manure and hence reduce its environmental impact or facilitate its transport. However, despite the wide range of manure processing techniques available (Flotats et al., 2011) and the potential that some of these have to reduce some of the environmental impacts caused by manure (Hou et al., 2017; Zhang et al., 2019), these technologies have not been widely adopted in global livestock chains (Cai et al., 2019; China Ministry of Agriculture, 2016; Foged et al., 2011). High investment and operational costs with low returns, logistical complexity for implementation at the farm level, low social acceptance and environmental concerns due to the emission of malodorous volatile organic compounds and greenhouse gas (GHG) emissions, have constrained the large scale adoption of these technologies (Cai et al., 2019; Chadwick et al., 2015; Martinez et al., 2009). Although policies to reduce animal numbers in livestock-dense regions would directly reduce the environmental and health problems caused by surplus manure,

simultaneously new short-term approaches for surplus manure management are needed to guarantee the transition towards more sustainable food systems.

Insects, specifically larvae of fly species (Diptera), are being considered promising candidates to treat surplus manure and upcycle it into the food system (Van Huis, 2019). Manure treatment with black soldier fly larvae (BSFL) is an innovation that could bring multiple benefits at once. BSFL can modify the physical, chemical and biological properties of manure in one to two weeks and thus modify the initial moisture and nutrient levels (Sanchez Matos et al., 2020). When safe (Charlton et al., 2015; Van Raamsdonk et al., 2017), the larvae could be used as feed for aquaculture or livestock (Moula et al., 2018) and hence decrease the dependency on imported high-protein feeds with high environmental impact such as soybean and fish meal (Heuel et al., 2021; Van Zanten et al., 2015b). Alternatively, the fat contained in the larvae could be used for biofuel production (Li et al., 2011). The residual material obtained from the process (i.e., insect excreta, uneaten manure and larval exuviae) can be used as a soil amendment (Bortolini et al., 2020). In addition, manure bioconversion with BSFL can strongly reduce the emission of malodorous compounds present in fresh manure (Beskin et al., 2018), reduce the loads of pathogens (Erickson et al., 2004; Liu et al., 2008) and mitigate the risk of antibiotic resistance genes in manure by degrading antibiotics (Cai et al., 2018a; Cai et al., 2018b). Despite the increasing evidence supporting the benefits of using BSFL as a manure management strategy, primary data on complete nutrient balances and emission of GHG during manure bioconversion with BSFL are scarce. This information is crucial to assess the transformational potential of manure-fed BSFL systems towards sustainable food systems.

Complete nutrient balances and quantification of gaseous emissions during the rearing of BSFL have been mainly reported for systems in which BSFL were reared on food waste (Mertenat et al., 2019; Pang et al., 2020) and by-products from the food industry (Parodi et al., 2020a). Only recently, Chen et al. (2019) quantified the GHG and ammonia (NH_3) emissions of BSFL reared on pig manure at different moisture levels. Although Chen et al. (2019) provided valuable estimations of the emissions occurring when BSFL was reared on pig manure, the nitrogen balance reported was not complete, indicating that not all outputs were accurately quantified (i.e., larvae, residual material and gaseous emissions). Moreover, a missing link in the literature is the quantification of nutrient losses from manure itself (i.e., without larvae) during the same time and under climatic conditions equal to those for manure bioconversion by larvae. Manure, being a material with high microbial activity, is expected to have nutrient losses via gaseous emissions caused by microbial fermentation. Quantifying such fermentation-related losses and comparing these with those occurring when manure is incubated with BSFL, is key to accurately understand the effect that BSFL have on the final nutrient levels in the residual material and the emission of GHG and NH_3. Therefore, the aims of this study were to 1) construct a complete balance of nutrient inputs and outputs used to quantify the nutrient and energy bioconversion efficiency of pig manure by BSFL, 2) quantify the levels of DM content, carbon, nitrogen,

ammonia-nitrogen, energy, phosphorus and potassium in the residual material of manure incubated with BSFL and without BSFL, and 3) determine the time course and cumulative gaseous emissions of CO_2, CH_4, N_2O and NH_3 in both treatments.

4.2 Material and methods

4.2.1 Manure collection

Pig manure was collected from a pig farm located in the surroundings of Rhenen, the Netherlands. The manure was produced by fattening pigs (Topigs 50 × PIC 408) fed with a commercial feed produced by Kamphuis Mengvoeders (see SI.1 for the nutrient composition of the feed). The pigs were 35 weeks old when the first batch of manure was collected and 39 weeks old when the last batch of manure was collected (i.e., 4th batch). Two days before the manure collection day, the pig farmer stopped cleaning the slopping concrete floors on which the pigs were kept. The manure samples consisted mainly of fresh faeces (i.e., maximum 2-day old) that were in contact with the urine present on the floor. In every batch, approximately 50 kg of manure was collected with a shovel, stored in three sealed plastic buckets of 20 L and transported to the facilities of Wageningen University & Research. The same day, a small (i.e., less than 80 g) but representative sample of manure was dried with a moisture analyser Ohaus MB-90 (Parsipany, United States) to determine its DM content (see SI.2 for details). The buckets with manure were kept at 21 °C for the subsequent 5 h until the start of the experiment. In total, four batches of manure were collected over a period of 31 days following the procedure described above.

4.2.2 Insect sourcing and experimental set-up

Just-hatched larvae of the Texas strain (Zhou et al., 2013) of BSF (Hermetia illucens L.; Diptera: Stratiomyidae; 100 generations; 38 days egg to egg cycle) were fed with a substrate containing 30% wheat bran and wheat flour, and 70% water for 7 days at the facilities of Bestico B.V., the Netherlands. Once larvae were 7 days old (hereafter called starter larvae), they were sieved, packaged at 10–15 °C and shipped to the facilities of Wageningen University & Research. Upon arrival, one plastic crate (100 × 50 × 15 cm) was filled with fresh manure in an amount equivalent to 4500 g of DM and 20,000–25,000 starter larvae. The height of the feed layer in the crate was around 5 cm high, the density of larvae was 4.7 ± 0.5 per cm^2, 0.9 ± 0.1 per cm^3 and the amount dry manure provided per larvae was 22 ± 2 mg DM manure $larvae^{-1}$ day^{-1} (mean ± std. deviation). The ratio between DM manure and larvae was selected based on pilot studies (see SI.3 for details). Another identical crate was filled with the same amount of manure without starter larvae added. Each crate was placed inside an open-circuit climate respiration chamber of 1800

L (1.00 × 0.8 × 1.1 m) and kept inside for a 208-hour experimental period (i.e., start time day 1–17:00 and end time day 10–09:00). Both climate respiration chambers were designed and built at Wageningen University & Research (Heetkamp et al., 2015). No manure was added during the experimental period. Inside the chambers, air temperature was maintained at 27 ± 0.5 °C, relative humidity at 70 ± 5% and L:D (light:dark) periods were set to 12:12. Ventilation air flow through the respiration chambers was set to 27 L/min and internal fans were used to ensure proper mixing of air. The experiment was repeated four times, here referred as four trials. Since two identical respiration chambers were available in parallel, every trial was made with a different batch of manure and starter larvae. Overall, we had four trials, four batches of manure and hence four replicates for both manure incubated with BSFL and for manure incubated without BSFL (Figure S1).

4.2.3 Material sampling and nutrient analyses

Homogeneous samples of fresh manure and starter larvae were collected in 1 L plastic containers prior to the start of every trial. At the end of every trial, chambers were opened and samples of mature larvae and residual material from both treatments were collected in 1 L plastic containers. For manure incubated with BSFL, the residual material consisted on a mixture of larval excreta, larval exuviae and dry uneaten manure (i.e., crust layer on top). For manure incubated without BSFL, the residual material consisted of pig manure, partly decomposed by microbial activity. All samples were stored at -20 °C for subsequent nutrient analyses. Condensate from the heat exchanger of the climate respiration chamber and 25% sulphuric acid solution containing the NH_3 trapped from the outgoing air stream, were collected and stored at 5 °C for subsequent nitrogen analyses (see Parodi et al. (2020a) for details).

Prior to freeze drying all solid samples, subsamples of manure and residual material were collected for the colorimetric determination of ammonium nitrogen. Freeze dried samples of manure and residual material were ground to pass a 1 mm screen (Retsch ZM200), while samples of starter and mature larvae, due to their high fat content, were ground three times with the same mill, but without a screen. Nutrient analyses were performed in duplicate at the Animal Nutrition Laboratory of Wageningen University & Research, except for potassium which was analysed at Nutricontrol Laboratories, Veghel, the Netherlands. Samples of fresh manure, residual material, starter and mature larvae were analysed for contents of DM (ISO6496, 1999), nitrogen and carbon (Dumas method, ISO1634-1 (2008)), gross energy (oxygen bomb method, ISO9831 (1998)), phosphorus (spectrophotometry method, ISO6941 (1998)), potassium (ICP-OES method, ISO21033 (2016)), and crude fat (hydrolysis method, ISO6492 (1999)). Samples of condensed water and acid were analysed for nitrogen (Kjeldahl method, ISO5983-2 (2005)). The results of these analyses are presented in Table 4.1.

Table 4.1: Nutrient composition of the fresh pig manure, starter larvae, 16-day old larvae and residual materials (mean ± standard deviation, n=4). Except for dry DM, all values are expressed per 100 g of DM. Nitrogen values include both ammonium and non-ammonium nitrogen.

Component	Dry matter (%)	Carbon (%)	Nitrogen (%)	Energy (kJ/100 g)	Ammonium-nitrogen (%)	Potassium (%)	Phosphorus (%)
Fresh manure	23.9 ± 1.0	44.8 ± 0.4	3.4 ± 0.0	1860 ± 17	2.1 ± 0.2	2.2 ± 0.1	1.8 ± 0.1
Starter larvae	26.3 ± 0.3	52.3 ± 1.1	9.5 ± 0.4	2373 ± 59	-	1.8 ± 0.1	1.9 ± 0.1
16-day old larvae	27.6 ± 0.4	46.1 ± 0.4	6.9 ± 0.2	2042 ± 33	-	1.6 ± 0.1	1.2 ± 0.1
Residues manure with BSFL	32.5 ± 1.2	41.9 ± 0.4	2.4 ± 0.0	1682 ± 15	1.6 ± 0.1	2.6 ± 0.2	2.1 ± 0.2
Residues manure without BSFL	29.4 ± 1.2	43.5 ± 0.4	3.2 ± 0.0	1787 ± 23	2.0 ± 0.2	2.4 ± 0.1	2.0 ± 0.1

4.2.4 Gas measurements

The consumption of O_2, the emissions of CO_2, CH_4, NH_3, N_2O, the production of heat, and the elaboration of the nutrient balances were calculated following the same procedures described in Parodi et al. (2020a). As a complete system validation, two CO_2 recovery tests per chamber were performed prior to the start of the experiment (see Heetkamp et al. (2015) for details). Measured CO_2 recoveries were 99.7% and 100.1% in chamber 1, and 99.4% and 99.6% in chamber 2. For NH_3 we used two methods as described in Parodi et al. (2020a). In short, with the acid washing bottle method, we measured the NH_3 lost by exhaust air, and added the ammonium in the condensed water that was produced from the cooling of the recirculating air. To obtain the time course emissions of NH_3, we measured every 18 min the NH_3 concentrations in the outgoing air stream using a calibrated NH_3 sensor (Dräger Polytron® 8100 EC with sensor type NH_3-FL range 0–300 ppm NH_3, Lübeck, Germany). N_2O loss by air was quantified and calculated as described in Parodi et al. (2020a). Air samples were collected once per day with a syringe (BD Plastipak, Drogheda, Ireland) from the ingoing and outgoing air streams of the climate respiration chambers and subsequently analysed in a gas chromatograph (Trace1300 GC, Waltham, United States), using a Haysep Q80-100 mesh 3 × 1/8 " SS column at temperature 60 °C and with an injection volume of 1 ml.

4.2.5 Nutrient balances and recoveries

To quantify how much of the initial nutrients and energy provided in the pig manure were recovered as larval biomass (i.e., also known as BSF bioconversion efficiency as described by Bosch et al. (2020) , residual material and gaseous emissions, we used equations (4.1), (4.2), (4.3), respectively.

$$BE_n = \frac{ML_n - SL_n}{I_n} \tag{4.1}$$

Where BE_n is the BSFL bioconversion efficiency (%) of nutrient n, ML_n is amount of nutrient n contained in mature larvae (in g), SL_n is the amount of nutrient n contained in the starter larvae (in g) and I_n is the amount of nutrient n contained and provided in the manure (in g).

$$RM_n = \frac{R_n}{I_n} \times 100 \tag{4.2}$$

where RM_n is the percentage of nutrient n recovered in the residual material and R_n is the amount of nutrient n found in the residual material (in g).

$$GE_n = \frac{E_n}{I_n} \times 100 \tag{4.3}$$

where GE_n is the percentage of nutrient n recovered as gaseous emissions and E_n is the amount of nutrient n lost as emissions or heat (in g or kJ). E was only quantified analytically for carbon, energy and nitrogen. Gaseous emissions of phosphorus and potassium were not quantified given that these two elements and compounds in which they occur are not volatile. DM lost via emissions (E_{DM}) was calculated by difference (equation 4).

$$E_{DM} = 100 - (BE_{drymatter} + RM_{drymatter}) \tag{4.4}$$

To verify if we had a matching balance in which all inputs provided were recovered in the outputs, we calculated the total recovery (RE) (in %) for each nutrient n using equation (5). For manure incubated without BSFL, BE was zero because larvae were absent. As E_{DM} was calculated by difference (equation 4), the total recovery of DM was 100%.

$$RE_n = \sum (GE_n + RM_n + BE_n) \tag{4.5}$$

4.2.6 Data analysis

Experimental data were analysed using R (R Core Team, 2019). All analyses and visualizations are reproducible and accessible at https://doi.org/10.4121/14318780.v1. Results were expressed as mean ± standard error ($n = 4$). To test whether the production of N_2O was not different from zero, we performed a two-sided t-test. To test for differences between treatments in the recovery of nutrients in residual materials and gaseous emissions, nutrient ratios and production of GHG, we used ANOVA with treatment and trial as fixed effects. We were not able to robustly test for normality and homogeneity of variance due to the small sample size ($n = 4$), and therefore assumed that data was normal and variances homogeneous.

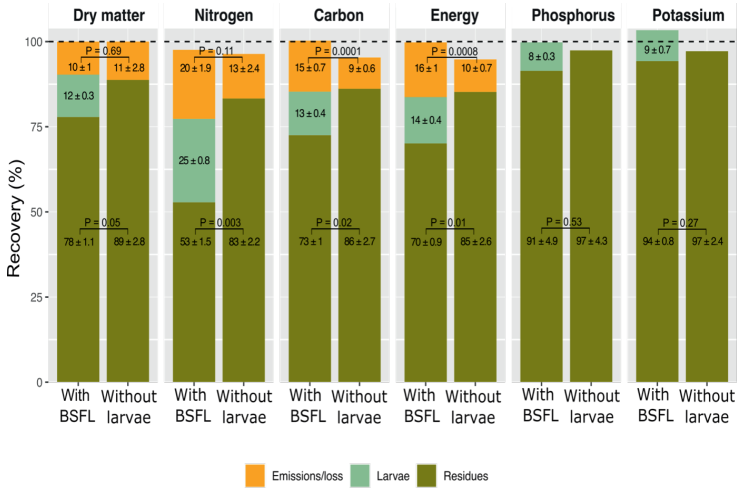

Figure 4.1: Recovery of DM, nitrogen, carbon, energy, phosphorus and potassium of pig manure incubated with or without BSFL in residual material, larvae, and gaseous emissions and heat loss. DM recovery is by definition 100% because emissions were calculated by difference. See SI.4 for the detailed statistical parameters. See Fig. S2 for the raw nutrient balances per trial.

4.3 Results

In both treatments, the recovery of the initial quantity of nutrients present in the manure at the start of the incubation were close to 100%. For manure incubated with BSFL, the recovery was 100 ± 2% for carbon, 98 ± 6% for nitrogen, 100 ± 2% for energy, 103 ± 1% for potassium and 100 ± 1% for phosphorus. For manure incubated without BSFL, the recovery was 95 ± 4% for carbon, 96 ± 8% for nitrogen, 95 ± 4% for energy, 97 ± 5% for potassium and 97 ± 9% for phosphorus. Considering that a recovery of 100% indicates that all inputs were recovered in the outputs, we were able to successfully quantify the outputs of the system in both treatments.

4.3.1 Bioconversion efficiency

The bioconversion efficiency of manure by BSFL was 13 ± 0.3% for DM (mean ± std. error), 25 ± 0.6% for nitrogen, 13 ± 0.4% for carbon, 14 ± 0.5% for energy, 9 ± 0.5% for phosphorus and 9 ± 0.7% for potassium (Figure 4.1). The fresh larvae yield per kg of fresh manure was 110 ± 3 g, while the dry larvae yield per kg of dry manure was 127 ± 3 g. The final larval individual fresh weight was 84 ± 9 mg. Additional parameters of the bioconversion process are presented in Table S1.

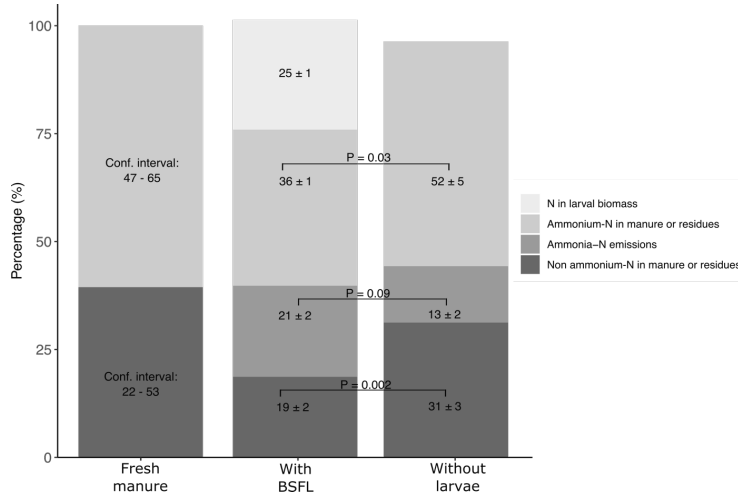

Figure 4.2: Nitrogen balances partitioned among ammonium-nitrogen and non-ammonium nitrogen in fresh manure and residual material, ammonia-nitrogen in gaseous emissions and nitrogen accumulated in larval biomass. All bars show mean values (n = 4). For fresh pig manure, text labels within each bar show the 95% confidence interval, while for the two treatments, text labels show mean ± std. error. See SI.4. for detailed statistical parameters

4.3.2 Nutrients in the residual material

Incubating pig manure with BSFL substantially decreased the levels of total nitrogen, carbon, energy, phosphorus and potassium in the residual material (Figure 4.1). The residual material of manure incubated with BSFL had 12% less DM, 37% less nitrogen, 20% less carbon, and 9% less phosphorus and potassium than the residual material of manure incubated without BSFL. Moreover, the presence of BSFL decreased substantially the fractions of ammonia and non-ammonia nitrogen found in fresh manure (Figure 4.2). In contrast, in the absence of BSFL, both nitrogen fractions were within the confidence interval found in fresh manure (Figure 4.2).

The presence of BSFL also modified markedly the C:N, N:P and N:K ratios in the residual material. The C:N ratio of the residual material of manure incubated with BSFL was higher than the ratio found in fresh pig manure, while the N:P and N:K ratios were lower (Figure 4.3). The nutrient ratios of the residual material of manure without BSFL did not differ from those initially present in the fresh pig manure, and the P:K ratio did not change from the initial levels as a result of the treatment (Figure 4.3). Overall, these results confirm that when manure is incubated with BSFL, there are substantial changes in the nutrient composition of the residual material relative to the original manure. Compared to the original manure, the residual material contains a C:N ratio which is closer to the 24:1 ratio that soil microbes need, while still ensuring nitrogen mineralization. In addition, the

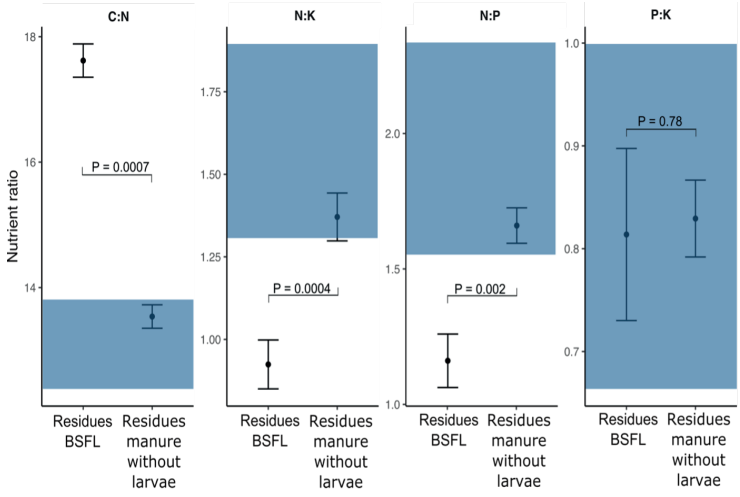

Figure 4.3: Nutrient ratios (mean ± std. error) in the residual material of manure incubated with BSFL, and the residual material of manure incubated without BSFL. Shaded areas show the lower and upper confidence interval (95%) of each nutrient ratio found in fresh pig manure. See SI.4 for the detailed statistical parameters.

reduced N:P and N:K ratios are beneficial for the fertilization of soils with high levels of nitrogen and potassium.

4.3.3 Nutrient losses in the form of gaseous emissions

Incubating manure with BSFL increased carbon emissions and heat loss compared with incubating manure without BSFL (Figure 4.1). Although nitrogen emissions between treatments were not significantly different (Figure 4.1), nitrogen emissions varied largely between trials. In two trials nitrogen emissions were only 5% higher when BSFL was present, but in the other two, nitrogen emissions were 50% higher (Fig. S1). Volatilization of DM was similar for both treatments (Figure 4.1).

In both treatments, carbon losses occurred mainly as CO_2, although losses of CH_4 were also detected (Table 4.2). The CO_2 emissions produced when manure was incubated with BSFL nearly doubled those emitted in the absence of BSFL (Table 4.2). The development of CO_2 emissions over time started on the same time and were similar in both treatments until the second day. After the second day, CO_2 emissions were always higher in the treatment with BSFL (Figure 4.4A) and reached its peak on day 7. The CO_2 emissions of treatment without BSFL did not show any peak and remained constant until the end of the experiment. CO_2 emissions were 1956 ± 105 g per kg of DM larvae (mean ± standard error, see Table 4.3).

Table 4.2: Gaseous emissions, heat production and oxygen consumption per kg of DM initial manure. Means ± std. error followed by the same superscript in each column do not differ significantly (P >0.05). N_2O emissions were not significantly different from zero for both treatments, but original mean and standard error are shown. See SI.4 for the detailed statistical parameters. See Table S2 for detailed emissions expressed in other metrics and per trial.

Treatment	CO_2 (g)	CH_4 (g)	N_2O (mg)	CO_2 eq (g)*	O_2 (g)	NH_3-N (g)	Energy (kJ)
Manure incubated with BSFL	247 ± 12a	1.27 ± 0.17a	0.73 ± 0.95a	43 ± 5.4a	213 ± 15a	7.2 ± 0.70a	3048 ± 201a
Manure incubated without BSFL	148 ± 10b	1.12 ± 0.02a	0.56 ± 1.10a	38 ± 1.1a	124 ± 10b	4.5 ± 0.83 a	1780 ± 132b

* Global Warming Potential (GWP) was expressed as g CO_2 equivalents based on the GWP_{100} of CH_4 (34) and N_2O (298) with carbon feedback (IPCC, 2013)

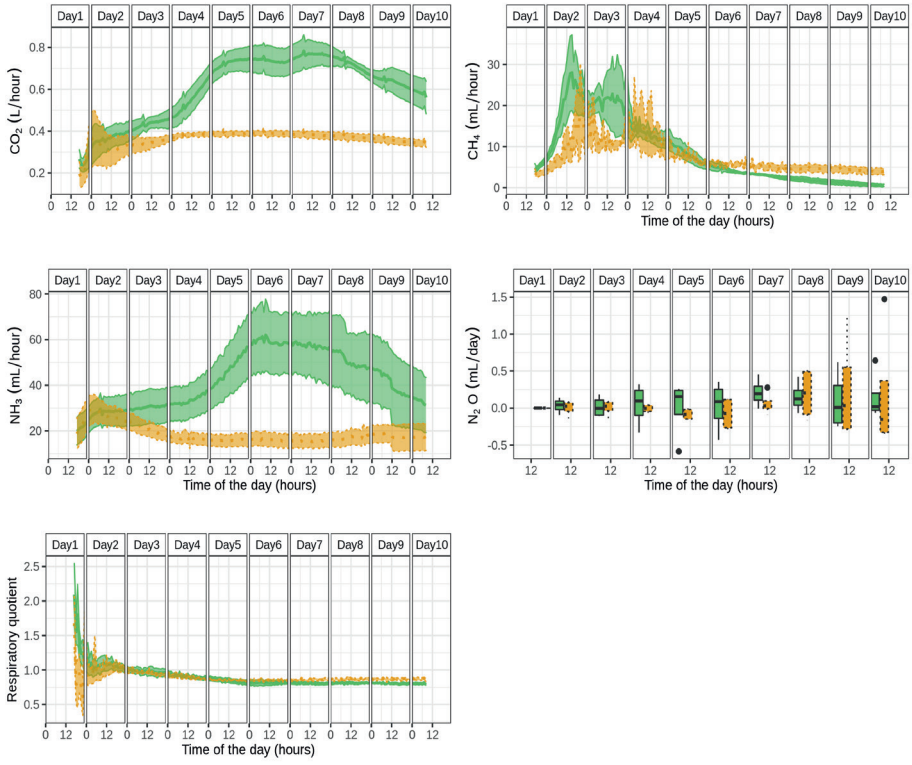

Figure 4.4: Gaseous emissions (panels A-E) over time during the incubation of manure with black soldier fly larvae and without larvae. Lines in panels A, B, C and E show the mean of each treatment (n = 4) and the shaded areas the standard error of the mean. See Fig. S3 for emissions figures per trial.

Table 4.3: Direct gaseous emissions during BSFL rearing expressed per kg of dry matter larvae (mean ± standard deviation). These values correspond to the emissions during the growth of BSFL and do not account for feed, energy-use and processing-related emissions.

*Study	Diet	CO_2 (g)	CH_4 (mg)	N_2O (mg)	Nitrogen (g)	GWP - CO_2 eq (g)
Ermolaev	Restaurant food waste	1750 ± 170	49 ± 29	21 ± 13	-	8 ± 4.8
Mertenat	Kitchen food waste	-	5.5	118	-	35
Pang	Restaurant food waste with rice straw	1394 ± 343	14 ± 6	7 ± 1	-	2.5 ± 0.5
Parodi	Food by-products	2750 ± 314	28 ± 29	53 ± 27	1.2 ± 0.7	17 ± 8.6
This study	Pig manure	1956 ± 105	10066 ± 2652	6 ± 14	58 ± 7	344 ± 43

* For Ermolaev et al. (2019) we used the values of treatment "L". For Pang et al. (2020) we used the values of treatments pH 5, pH 7 and pH 9.

Emissions of CH_4 did not differ between the two treatments (Table 4.2). During the first four days both treatments had similar time dynamics with peaks of CH_4 production (Figure 4.4B) and Fig. S3). However, after day 4, CH_4 emissions in the treatment with BSFL declined. Such decline was not observed in the treatment without BSFL. CH_4 emissions were 10.1 ± 1.33 g per kg of dry larvae.

While statistical tests did not show significant differences, on average, nitrogen emissions were higher when manure was incubated with BSFL (Fig. 1, Table 4.2). The intensity of the nitrogen emissions, however, varied between trials (Fig. S2). In line with this observation, the recorded time course in NH_3 emissions (Figure 4.4C) showed higher emission levels for manure inoculated with BSFL and a high variability between trials. The variability was more pronounced in the treatment with BSFL (see larger standard error in Figure 4.4C, and Fig. S3). The NH_3 emission time patterns differed between the treatments. While NH_3 emissions of manure incubated with BSFL peaked on day 6 and dropped in the following days, the NH_3 emissions of manure without BSFL remained constant over time. Overall nitrogen emissions were 57.5 ± 6.84 g per kg of DM larvae (mean ± standard error, Table 4.3).

Unlike CO_2, CH_4 and NH_3 which were measured continuously, N_2O was measured once a day. With this setup, the recorded N_2O emissions for both treatments were very low and not significantly different from zero (Table 4.2 and SI.4). The negative values near zero were caused by normal analytical errors (Figure 4.4D and Fig. S3).

The GHG emissions in grams of CO_2 eq per kg of DM input manure did not differ significantly between treatments. As the calculation of CO_2 eq combines both CH_4 and N_2O, and N_2O emissions were negligible (Table 4.2), CO_2 eq were determined mainly by the CH_4 emissions. Overall GHG emissions were 344 ± 43 g CO_2 eq per kg of DM larvae (mean ± standard error, Table 4.3).

4.4 Discussion

To evaluate the environmental sustainability of using BSFL as a manure management technology, broad-scale assessments, such as life cycle assessments and food system modelling, are needed. Our study, and specifically the results for manure incubated with BSFL, provides a reliable quantitative basis based on high analytical recoveries and complete nutrient balances for the elaboration of such broader scale studies. For instance, future life cycle assessments on manure processing with BSFL could use not only the emissions reported here, but also the chemical composition and bioconversion efficiencies at the larval density studied to estimate the avoided impacts associated with the use of BSFL ingredients for feed formulations and residual material as a fertilizer. In addition, our values could be used to quantify the potential of manure bioconversion with BSFL to increase the nitrogen use efficiency in livestock supply chains. These studies, combined with assessments of economic feasibility and technological adoption, should be used for decision making to evaluate whether manure bioconversion with BSFL can bring environmental, health and social benefits compared to other alternatives for treating surplus manure.

To accurately quantify the influence of the larvae on the nutrient flows and emissions when reared on manure we included the treatment consisting of manure without larvae. It is important to highlight that the treatment without larvae and its associated measurements (i.e., nutrients in residual material, gaseous emissions) should not be considered representative for current manure management practices (i.e., composting).

4.4.1 Bioconversion efficiencies

Attaining high nutrient bioconversion efficiencies in a manure bioconversion system with BSFL is desirable from an environmental perspective. As surplus manure leads to environmental pollution due to the excessive concentration of reactive nutrients susceptible to microbial decomposition, storing as much nutrient mass as possible in the larval biomass (i.e., high bioconversion efficiencies) reduces the amount of nutrients vulnerable to microbial decomposition in the residual material. In addition, high nutrient bioconversion efficiencies lead to higher total larval biomass fed with recycled nutrients that could decrease in a larger degree the need of producing raw materials (e.g., feed ingredients, biofuels) and therefore avoid the impacts associated with their production.

Bioconversion efficiencies of pig manure with BSFL have been reported mainly for DM and only a few studies covered carbon, nitrogen and potassium. While we found a DM bioconversion efficiency of 12.5%, previous studies with pig manure and BSFL reported DM bioconversion efficiencies of 1.8–2.1% (Miranda et al., 2019), 2% (Liu et al., 2018) and 5% (Oonincx et al., 2015). Similarly, our carbon (i.e., 12.8%) and phosphorus (i.e., 8.5%)

bioconversion efficiencies, were higher than the 0.3–4.7% carbon bioconversion efficiency reported by Chen et al. (2019), and the 5% phosphorus bioconversion efficiency reported by Oonincx et al. (2015). Among the many factors that could explain such variation (Bosch et al., 2020), the different metrics used to calculate bioconversion efficiencies (e.g., fresh vs dry weight), the different experimental conditions (e.g., all cited studies were performed with 100 to 450 larvae per container and different larval densities), the different nutrient composition of manure samples, and the different feeding regimes (i.e., single vs. multiple feeding) are likely the four most important.

4.4.2 Residual material

We determined the effect of incubating manure with BSFL on the reduction of the quantity of nutrients in the residual material (Figure 4.1, Figure 4.2). The lower nutrient levels of the residual material after BSFL treatment compared to manure incubated without larvae, are in line with Liu et al. (2019) who found 20% less organic matter, 13% less dissolved organic carbon and 25% less nitrogen in the residual material after manure bioconversion with BSFL compared to manure composted without BSFL. The lower nutrient levels and subsequent change in nutrient ratios in the residual material of manure incubated with BSFL can be explained by the sequestration of nutrients in the larval biomass and the slightly higher CO_2 and NH_3 emissions occurring during the bioconversion process (Figure 4.1, Figure 4.2).

Although the presence of BSFL increased NH_3 emissions during the rearing, if the residual material of manure bioconversion with BSFL is used as fertilizer, the lower levels of ammonium-nitrogen in the residual compared to fresh or stored manure, could lead to lower rates of ammonia volatilization when applied to the soil (Jiang et al., 2017; Sommer and Hutchings, 2001). Lower ammonia-nitrogen levels might not affect the fertilizer quality as NO_3 -nitrogen levels in the residual material are 15% higher than composted pig manure without BSFL (Liu et al., 2019).

4.4.3 Gaseous emissions

While Parodi et al. (2020a) detected NH_3 emissions only after day 5 of the bioconversion process of food residues with BSFL, in this study NH_3 was emitted from day 1 (Figure 4.4). Considering that microbial enzymes are abundant in manure, the NH_3 emissions occurring early in the process are likely explained by the activity of microbial ureases breaking down manure urea into NH_3 and CO_2 (Aarnink and Elzing, 1998; Dai and Karring, 2014). Nitrogen emissions were not only earlier when BSFL was reared on manure but were also 48 times higher than those recorded when larvae grew on food residues (Table 4.3). This large difference between the two diets might be due to the fact that two thirds of the nitrogen contained in pig manure was present as ammonia-nitrogen, which can volatilize

faster compared to the predominantly organic nitrogen likely contained in the food residues used by Parodi et al. (2020a).

Even though most of the ammonia-nitrogen losses observed in both treatments were likely driven by the activity of microbial ureases, our results showed that after day 3, nitrogen losses were higher when BSFL were present (Figure 4.4. It is known that NH_3 volatilization in manure is a function of pH, temperature, moisture and air velocity over the surface of manure (Koerkamp, 1994). Although we did not measure these parameters, we hypothesize that moisture and temperature were not important drivers for the higher NH_3 emissions observed for the treatment with larvae, and instead pH played a main role. Chen et al. (2019) showed that when BSFL is present in manure with 25% DM content, there were no substantial changes in substrate temperature and moisture levels in the first five days of bioconversion. As we started to observe differences in NH_3 production between treatments after the third day, it is likely that neither temperature nor moisture played a main role in the higher NH_3 emissions observed when BSFL were present. Instead, Liu et al. (2019) reported that the substrate pH is higher when black soldier fly is present in manure (pH 9) compared to when it is absent (8.5). Considering that slight changes in pH between pH 8 and 11 can lead to large changes in the NH_3-NH_4^+ equilibrium, and that nitrogen is only volatilized as NH_3 (Koerkamp, 1994), a higher pH caused by the larval activity might have caused the larger NH_3 losses. Additional factors that might have influenced the larger NH_3 emissions are the higher exposure of the residual substrate to air currents due to the mechanical perturbation of the substrate caused by larval movement, and the larval excretion of uric acid which could be converted to NH_3 by microbes (Gold et al., 2018; Green and Popa, 2012).

A key topic that requires more attention is the quantification of the nitrogen contained in the larval body mass that originated from ammonium-nitrogen. The ammonium and non-ammonium nitrogen balances presented in Figure 4.2 revealed the existence of a potential mechanism allowing BSFL or associated microbes to use ammonium-nitrogen. Quantifying this potential flow of nitrogen will allow the estimation of how much ammonium-nitrogen is involved in BSFL anabolism and will hopefully lead to the exploration of ways on how to utilize this mechanism to reduce NH_3 emissions.

Different processes might explain the temporal dynamics and occurrence of CO_2, CH_4 and N_2O emissions observed in this study. While microbial respiration was the main source of CO_2 emissions in the treatment without BSFL, the presence of BSFL increased the CO_2 emissions as both microbial and larval respiration occurred. Compared to the emissions reported for BSFL farmed in non-manure diets (Table 4.3), CO_2 emissions per kg of DM larvae were 40% and 11% higher than Pang et al. (2020) and Ermolaev et al. (2019) respectively, but 40% lower than Parodi et al. (2020a). Besides the influence that factors such as type of diet, larval density, age of starter larvae at the start of the experiment could have on CO_2 emissions, a key factor that might explain the lower CO_2 emissions

reported by Pang et al. (2020) and Ermolaev et al. (2019), is the lower gas sampling frequency (i.e., daily basis or once every 2 to 5 days). Such low sampling frequencies might have led to an underestimation of true emissions. On the other hand, the higher CO_2 emissions reported by Parodi et al. (2020a) might be linked to differences in the quality of the diet. The respiratory quotient (RQ) (i.e., ratio between CO_2 produced and O_2 consumed that indirectly shows if protein, carbohydrates or fats are used as oxidation substrate) reported by Parodi et al. (2020a) were above or equal to 1 in most of the growing periods indicating that larvae had access and were mainly using carbohydrates as oxidation substrate. Instead, the RQ for BSFL fed on manure was relatively constant around 0.8 (Figure 4.4), suggesting that protein and fats were the main macromolecules used to sustain larval metabolism.

Manure CH_4 emissions are mainly linked to the presence and abundance of Archaea (Petersen et al., 2014). CH_4 emissions, however, can decrease with aeration (Martinez et al., 2003). Even though CH_4 emissions were only slightly higher with the presence of BSFL (Table 4.2), the slight decline in CH_4 after day 4 in the BSFL treatment (Figure 4.4B), might have been linked to the aeration caused by the larval movement through the crate when the larvae had grown to a larger size, and to the reduction in the quantity of substrate present. Compared to other studies, CH_4 emissions were much higher when larvae were reared on manure than when reared on food waste or food residues (Table 4.3). This highlights the importance of the substrate on the emissions during BSFL bioconversion, as in this case it is the manure Archaea and not the larvae that cause the high emissions. Therefore, judging whether manure-fed BSFL production systems can bring environmental benefits should be done comparing emissions with other manure uses at a life cycle level, and not comparing food waste fed BSFL with manure fed BSFL.

The N_2O emissions registered with our experimental setup were almost null in both treatments and did not have a defined time pattern. In addition, our N_2O measurements are in line with Chen et al. (2019) who found that N_2O emissions were close to zero after 9 days of manure bioconversion with BSFL. Compared to other diets N_2O emissions were equal or lower than those for food waste and food residues (Table 4.3).

4.5 Conclusions

In this study we provide a quantitative nutrient balance for pig manure bioconversion with BSFL. With recoveries ranging between 95 and 103%, our nutrient balances were virtually complete. BSFL incorporated 12, 25, 14, 8.5 and 9% of the carbon, nitrogen, energy, phosphorus and potassium initially contained in fresh manure. By storing nutrients in the larval body mass and releasing additional carbon dioxide, ammonia and heat, BSFL substantially reduced the carbon, nitrogen and energy levels originally found in fresh pig manure. CH_4 was the main GHG produced during manure bioconversion with larvae and

was likely produced by manure's Archaea. Our study provides a reliable quantification of GHG and NH_3 emissions to quantify the environmental consequences of using BLFL in future life cycle assessments.

4.6 Supplementary Information

The supporting information of this chapter includes:

Appendix A - Supplementary Figures (S1-S3)

Appendix B - Supplementary Tables (S1)

The original publication also contained a section of Supplementary Methods. Along the chapter there are references to items that belong to this section, however these were not included in this thesis due to space constraints. The Supplementary Methods can be accessed through the online version of the publication.

4.6.1 Supplementary Figures

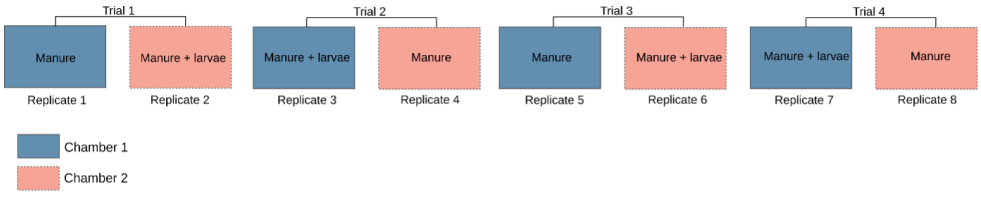

Figure S1. Experimental design showing the trials, treatments and replicates. Every trial was made with a different batch of manure and starter larvae.

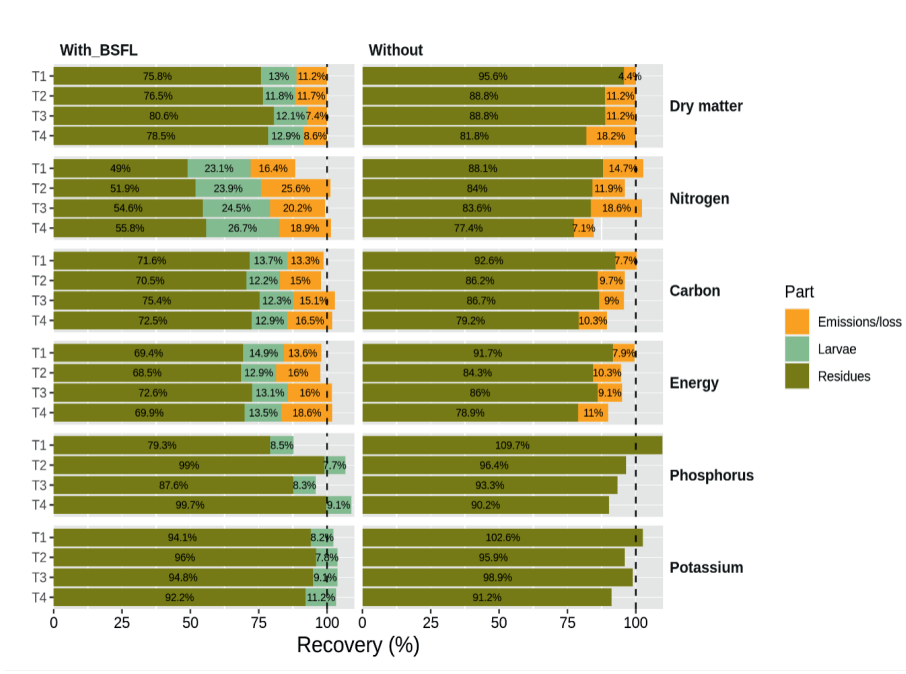

Figure S2. Raw balance (without correction to reach 100%). T1-T4 correspond to each trial. The dotted vertical line indicates a recovery of 100%.

Figure S3. Production of gaseous over time per repetition. Panel A shows the emissions of CO_2, CH_4, heat and NH_3, the consumption of O_2 and the respiratory quotient per kg of DM manure. Panel B shows the emissions of N_2O.

4.6.2 Supplementary Tables

Table S1. Parameters of the bioconversion process

Parameter	Value
Fresh larvae yield per kg of fresh manure (g)	109 ± 3
Dry manure provided (g)	4416 ± 112
Fresh larvae gain weight (g)	1784 ± 106
Dry larvae yield (g)	559 ± 26
Larvae dry matter content (%)	28 ± 0.4
Manure dry matter (%)	24 ± 1
Final larvae individual weight (mg)	84 ± 1
Dry larvae gain weight (g)	496 ± 32
Fresh larvae yield (g)	2024 ± 82
Dry larvae yield (g) per kg of dry manure	126 ± 3
Fresh manure provided (g)	18569 ± 855

Chapter 5

Incorporation of manure ammonia-nitrogen into black soldier fly larvae

This chapter is based on:

A. Parodi, Q. Yao, W. J. J. Gerrits, M. Mishyna, C. M. M. Lakemond, D. G. A. B. Oonincx, and J. J. A. Van Loon (2022b). "Upgrading ammonia-nitrogen from manure into body proteins in black soldier fly larvae". *Resources, Conservation and Recycling* 182, 106343. DOI: 10.1016/J.RESCONREC.2022.106343

Abstract

Nitrogen (N) losses via ammonia (NH_3) emissions from manure is one of the main environmental burdens resulting from livestock production. Feeding manure to black soldier fly larvae (BSFL) is envisioned as a new circular strategy to recover manure-N, reduce its environmental impact, and upgrade it into insect proteins to be used in animal feed. However, so far, it remained unknown if BSFL could incorporate N from NH_3 in manure into the larval body mass. Here, using the stable isotope ^{15}N in NH_3, we demonstrate that at least 13% of pig manure NH_3-N can be incorporated into BSFL body mass. Within the larval body, the tracer was found in both insoluble and soluble nitrogen fractions, including proteins. We discuss interventions that could increase the incorporation of NH_3-N into larval proteins and with that reduce NH_3 emissions from manure. Our results provide the first reliable quantification of NH_3-N assimilation in manure-fed larvae and contributes to quantifying the potential of BSFL for manure management, and as a circular protein source.

5.1 Introduction

Surplus manure in livestock-dense regions is a source of environmental pollution, with ammonia (NH_3) emissions being one of the main environmental burdens (Gerber et al., 2013; Leip et al., 2015). Ammonia emissions contribute to acidification and eutrophication, and are an indirect source of the potent greenhouse gas (GHG) nitrous oxide (Cameron et al., 2013; Chadwick et al., 2015; Strokal et al., 2016; Wang et al., 2017). Ammonia can be released from manure through ammonification via two pathways. In the first pathway, the organic nitrogen (N) present in the solid feces (e.g., as undigested dietary or fecal-endogenous proteins) is degraded to NH_3 or its ionic form ammonium (NH_4^++) by microbial fermentation (Chen et al., 2020; Maeda et al., 2011). In the second and most important pathway, NH_3 is released from manure due to microbially produced ureases present in faces, which hydrolyzes urinary urea into NH_3 and CO_2 (Aarnink and Elzing, 1998; Dai and Karring, 2014). Some groups of microbes assimilate this NH_3 as a source of N for *de novo* amino acid synthesis and other metabolic processes (Kenealy et al., 1982; Kuypers et al., 2018). However, by hydrolyzing urea with microbial ureases, NH_3 molecules are volatilized, thus causing environmental pollution.

A key requirement to improve the environmental sustainability of the livestock sector is to reduce manure-related NH_3 and GHG emissions (Gerber et al., 2013). Even though the biological and physicochemical mechanisms of manure-related emissions are well known (Dai and Karring, 2014; Sigurdarson et al., 2018), and different manure management methods and mitigation strategies for emissions are available (Flotats et al., 2011), manure-related emissions are a persistent environmental problem in livestock-dense regions. While manure NH_3 emissions due to urea hydrolysis could be largely avoided by collecting livestock´s solid feces separately from urine (**Vries2013**), in most large-scale livestock housing systems solid feces and urine are mixed and stored together. In such systems, the mitigation of NH_3 and greenhouse gas (GHG) emissions mainly depend on costly strategies such as the use of urease inhibitors and acidifiers, the export of manure outside manure-surplus areas, and the use of manure valorization practices such as composting and anaerobic digestion (Kuhn et al., 2018; Sigurdarson et al., 2018). Many of these practices are known to reduce manure-related emissions (Hou et al., 2017; Wang et al., 2017), but most are costly and not widely adopted by farmers unless incentives for their adoption are available. In this context, novel manure management methods which are sustainable, circular and accessible are being explored.

Manure bioconversion with black soldier fly larvae (BSFL) is envisioned as a new potentially sustainable, and circular management method to treat surplus manure (Van Huis, 2019). If safety standards for metals, antibiotics, pathogens and parasites are met (Sanchez Matos et al., 2020) the larval biomass obtained after bioconversion could be used as protein-rich ingredient. Thereby, it can replace feed ingredients having a high environmental impact,

such as soybean and fish meal, and contribute to the circular economy (Heuel et al., 2021; Van Zanten et al., 2015b).

In addition, the residual material of the bioconversion process (i.e., mix of uneaten manure, and insect frass and exuviae) is considered an attractive fertilizer because of improved humification properties relative to fresh manures (Wang et al., 2021). In the last years, various studies reported the bioconversion efficiency of BSFL on different manure types (see Sanchez Matos et al. (2020) for an overview), and recent studies quantified NH_3 and GHG emissions during this process (Chen et al., 2019; Parodi et al., 2021). Although more quantitative environmental data is becoming available for broader sustainability assessments, the role that BSFL bioconversion can have in reducing NH_3 emissions through its direct use has so far received limited attention.

After pig manure bioconversion with BSFL, one quarter of the N originally contained in the manure can be accumulated in the larval body mass (Parodi et al., 2021). However, it remains unknown how much of this N is derived from NH_3. Pathways for glutamine synthesis from NH_3-N has been found in moths (Drews et al., 2000; Hirayama et al., 1997), and in cockroaches by the endosymbiont Blattabacterium (Sabree et al., 2009). In addition, the microbiota present in the rumen of ruminants, and to some extent also in the digestive tract of monogastric animals, provides their hosts with microbial amino acids derived from NH_3-N (Columbus et al., 2014; Pengpeng and Tan, 2013; Torrallardona et al., 2003; Van Erp et al., 2020). Given the high content of intestinal microbes in manure and the influence that the diet has on BSFL microbiota (Bruno et al., 2019), it is hypothesized that some NH_3-N is incorporated in the larval body mass via exogenous (i.e., in the substrate) or endogenous (i.e., in the larval digestive system) microbial assimilation, followed by the absorption of microbial amino acids by the larvae. Thus, the objectives of this research are to quantify the incorporation of NH_3-N into the larval body mass and larval proteins after pig manure bioconversion with BSFL using the stable isotope ^{15}N ammonium in $^{15}NH_4{}^+Cl$, and to construct a NH_3-N mass balance of the bioconversion process among the larvae and residues. The outcomes of this study are relevant to elucidate the potential of manure bioconversion with BSFL for reducing manure NH_3 emissions and to upgrading NH_3 as a circular protein source for animal feed.

5.2 Material and methods

5.2.1 Experimental design

A flow diagram of the experimental design is presented in Figure 5.1. BSFL were reared under three different treatments named Control, Enriched, and Enriched_Digesta (Figure 5.1). The larvae reared under the treatment Control were placed in a container that

contained fresh pig manure. After 208 h (i.e., 8.6 days), the average time on which the first prepupae were observed in previous experiments, larvae were removed from the containers, counted, washed with water, dried with paper towel, and weighed. The residual material was collected. Subsequently, the washed larvae were placed overnight in a substrate made of a nitrogen-free diet. This was done to replace the content of the larval intestines with a nitrogen-free material to maintain the same $^{15}N/^{14}N$ ratio, and thus ensure that during the isotopic quantification only absorbed ^{15}N in the larval body mass was measured. Then, larvae were removed, counted, washed, weighed, and stored at -20 °C. The larvae reared under the treatment Enriched passed through the same steps described for the Control treatment, except that the larvae were initially reared in fresh pig manure mixed with $^{15}NH_4^+Cl$ (Figure 5.1). The Enriched_Digesta larvae were reared as in the Enriched treatment, but without the subsequent nitrogen free diet; thus, once harvested from the ^{15}N-enriched residual material, they were directly stored at -20 °C. The Enriched_Digesta treatment was included to compare larval total nitrogen and ^{15}N values when guts were filled with enriched digesta, and hence have a benchmark value for larval samples which also contained unabsorbed ^{15}N. No residual material was collected for this treatment as it was expected to have the same composition as the residual material from the Enriched treatment. Each treatment had five replicates.

5.2.2 Larvae rearing

Pig feces excreted within the last 24 h, that were in contact with urine, were collected in a commercial pig fattening farm in Rhenen, the Netherlands, and transported to the experimental facilities of Wageningen University & Research. A preliminary dry matter (DM) analysis was performed to rapidly determine the amount of manure that needed to be added to each container. This was done by drying a homogeneous 2 g sample of fresh manure in a moisture analyzer (Ohaus MB-90, Parsipany, United States). Once the DM was known (i.e., 24.5 %), 15 cylindric plastic containers (diameter 8 cm, height 11 cm) were filled with 75 g of pig manure, equivalent to 18.4 g of dry manure. The height of the manure layer inside each container at the start was 1.3 cm. Subsequently, 9.8 mg of ^{15}N ammonium-chloride ($^{15}NH_4^+Cl$, Sigma-Aldrich, see Supplementary Methods for details), diluted in 400 µL of distilled water were pipetted in the Enriched and Enriched_Digesta treatments and mixed with the manure using a small spoon. The containers of the Control treatment received 400 µL of distilled water. Subsequently, 100 seven-day old BSFL, also referred to as starter larvae, were added to each container. The larval density in manure was 1 larvae/cm^3, the individual weight of each starter larva was 3.4 ± 0.2 mg (mean ± standard deviation) and the feed rate was equivalent to 23 mg DM manure/larva/day, as suggested by Parodi et al. (2021), although all manure was provided at once. The starter larvae were reared on a substrate containing 30% wheat bran and wheat flour, and 70% water by Bestico B.V. (Berkel and Rodenrijs, the Netherlands). All containers were placed

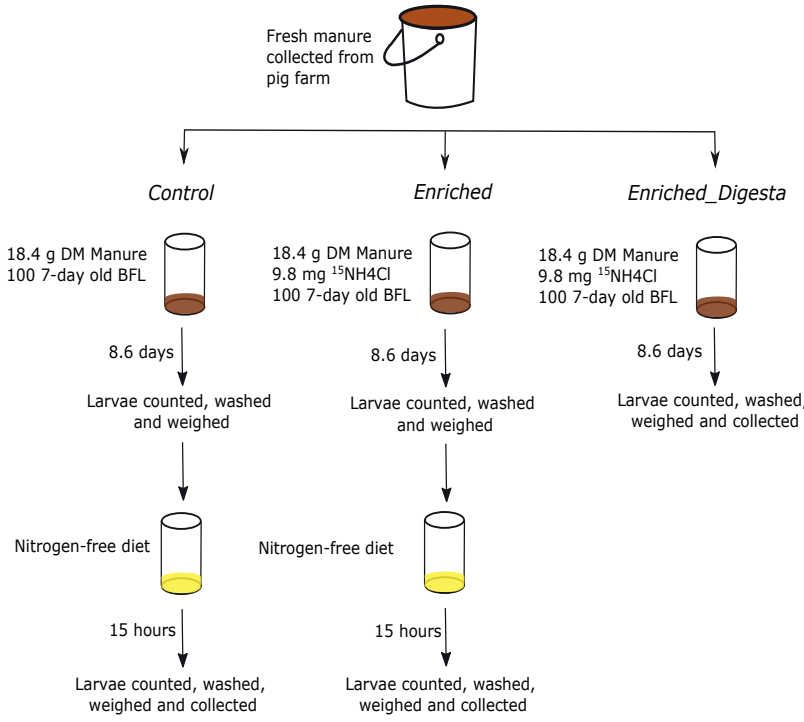

Figure 5.1: Flow diagram of the experimental design. Each treatment had five replicates.

in a custom-made climate chamber at 28 °C and 70 % relative humidity under a 12:12 light:dark regime for 208 hours (8.6 days). The residual material from the Control and Enriched treatments and the larvae from the Enriched_Digesta treatment were collected, weighed, and stored at -20 °C. The larvae from the Enriched and Control treatments were moved for 15 h to new plastic containers containing 50 g of a nitrogen-free diet mixed with 40 ml of deionized water (see Supplementary Methods for details on the ingredient composition of the diet) prior to sample collection and storage (see Figure 5.1). Larval survival ranged between 99 and 100%, and the average fresh larval weight was determined by weighting all larvae obtained per container and dividing the weight by the number of surviving larvae.

5.2.3 Sample processing and nutrient analyses

Samples of the Enriched and Control residual material were freeze-dried for 96 h starting at -80 °C until stable weight was reached, grounded by mortar and pestle, and stored at -20°C for DM, total N and ^{15}N analysis. Samples of larvae from all three treatments were

subjected to the same processing steps as for the residual material, but after grinding, passed through a defatting process and subsequently a protein extraction process. This defatting was a precautionary measure to protect the equipment used to measure ^{15}N and was not suitable to quantify true fat content. Protein extraction was done to quantify ^{15}N in larval insoluble and soluble N fractions.

Defatting

The ground, freeze-dried larvae were defatted by mixing and centrifuging 2-3 g twice with n-hexane at a ratio of 1:4 g:ml. Centrifugation was done for 5 min at 1000 g and 23 °C. To remove n-hexane, the solid centrifuged material was placed in an incubator for 4 h at 30 °C, and subsequently placed overnight in a fume hood for drying. Homogenous samples of defatted larvae were freeze-dried and stored at -20°C for analysis of DM, total nitrogen and ^{15}N content, and used for protein extraction.

Protein extraction

Protein was extracted by immersing 1.5 to 2.5 g of defatted larvae in 0.2 M Na_2HPO_4 / 0.2 M $NaH_2PO_4 \bullet 2H_2O$ buffer in a ratio of 1:4 (g:ml), at pH 8. The solution was vortexed, shaken (Multi Reax, Germany) for 60 min, and centrifuged for 5 min, at 4500 g and 23 °C. After centrifugation, the supernatant was collected, and the remaining pellet passed again through the same protein extraction process. The second supernatant was then mixed with the first one, and the pellet was freeze-dried and stored at -20 °C for DM, total N and ^{15}N analysis. The supernatant, which was the soluble larval fraction, was expected to be mainly composed of soluble protein. The pellet, the insoluble larval fraction, was mainly assumed to contain insoluble protein and other N non-protein compounds (Janssen et al., 2017). To corroborate if ^{15}N was found in soluble larval proteins and not in other N-containing compounds, the supernatant was centrifuged (5 min, 1000 g, 23 °C) and dialysed. Dialysis was performed at 4 °C with a cut-off of 3 kDa (SnakeSkin™ Dialysis Tubing, 3.5K MWCO, 22 mm) using 2000 ml ultrapure water in two rounds of 3 h each. The dried and dialysed supernatant (i.e., here referred as dialysed soluble larval fraction) were freeze-dried and stored at -20 °C for DM, total N and ^{15}N analysis. Overall, the protein extraction process yielded an insoluble larval fraction and a dialysed soluble larval fraction.

5.2.4 DM, total N and ^{15}N analysis

The DM content of freeze-dried samples was estimated by oven drying samples at 70 °C overnight. Sample quantity varied; the quantities of freeze-dried samples were 0.5 g for manure, 0.2 g for ground larvae, 0.2 g for defatted larvae, 0.5 g for pellet of insoluble larval

fraction, 0.1 g for dialysed soluble larval fraction and 0.5 g for residual material, on average. As the experimental unit was one container, we had five biological replicates per sample. Dry matter and total N were determined *in simplo*, except for fresh manure for which DM was done in duplicate. For N-NH_3 and ^{15}N determination, analyses were performed in duplicate. Total N was quantified with the Dumas method using Flash EA 1112 N/Protein analyzer (Thermo Fisher Scientific, USA). Sample quantities used for Dumas averaged 15 mg for manure, 9 mg for defatted larvae, 14 mg for insoluble larval fraction, 10 mg for dialysed soluble larval fraction and 14 mg for residual material. Ammonia-nitrogen was determined via colorimetric determination in fresh manure samples of 5 g. ^{15}N enrichment was measured after combustion in an elemental analyzer (Flash 2000 organic elemental analyzer HT O/H- N/C, Thermo Scientific) with the use of a continuous flow isotope ratio mass spectrometer (Conflo IV, Thermo Scientific) in samples of 1 to 1.5 mg. Given that ^{15}N was not measured in the starter larvae, it was assumed starter larvae had the same ^{15}N content as the larvae at the end of the Control treatment.

5.2.5 ^{15}N and nutrient balance calculations

The total amount of ^{15}N stored in the larvae ($15NL_{Total}$, in mg) was calculated using equation (5.1):

$$15NL_{Total} = FW \times DM \times TN \times 15N_{at} \tag{5.1}$$

Where FW is the fresh weight (mg) of the larvae harvested from each replicate at the end of the experiment, DM is the dry matter (%) of fresh larvae, TN is the total nitrogen content (%) in DM larvae, $15N_{at}$ is the percentage of ^{15}N atoms (at %) relative to the total N atoms (^{14}N +^{15}N) in larvae.

While the $15NL_{Total}$ of the control treatment shows the background content of ^{15}N in the larvae, for the enriched treatments the total $15NL_{Total}$ shows the sum of the background^{15}N in the larvae and the tracer ^{15}N that was incorporated in the larvae. Equation (5.2) was used to calculate the total amount of ^{15}N (mg) from tracer origin in the larvae of the Enriched and Enriched_Digesta treatments ($15NL_{tracer}$):

$$15NL_{tracer} = 15NL_{Total} - 15NL_{Background} \tag{5.2}$$

Where, $15NL_{Background}$ (mg) is the estimated background ^{15}N content in the enriched larvae. $15NL_{Background}$ was calculated using equation (5.1), using the average $15N_{at}$ for the larvae of the control group for all replicates.

Equation 5.3 was used to calculate the percentage (%) of tracer ^{15}N found in the larvae (tL):

$$tL = \frac{15NL_{tracer}}{15N_{tracer}} \times 100 \tag{5.3}$$

Where, $15N_{tracer}$ is the amount (mg) of ^{15}N contained in the tracer added at the start of the experiment. Considering that 9.8 mg of $^{15}NH_4{}^+Cl$ were added to each replicate of the enriched treatments, and that N constitutes 27% of the molecular weight of $NH_4{}^+Cl$ and the purity of ^{15}N atoms in the tracer was 98%, $^{15}Ntracer$ was 2.61 mg.

The total amount of ^{15}N in the residual material, the amount of ^{15}N from tracer origin in the residual material and the percentage of tracer ^{15}N found in the residual material (tR) were calculated similarly to equations (5.1), (5.2), and (5.3) used for the larvae. Because gaseous ^{15}N and other aerial losses were not measured, for the ^{15}N balance the percentage of ^{15}N losses (%) (tLo) was estimated using equation (5.4):

$$tLo = 100 - (tL + tR) \tag{5.4}$$

The total amount of ^{15}N in the larval insoluble and dialysed soluble fractions was calculated using equation (5.5).

$$15N_f = FW \times DM \times Y_f \times TN_f \times 15Nat_f \tag{5.5}$$

Where, $15N_f$ is the amount of ^{15}N (mg) in fraction f (i.e., insoluble larval fraction and dialysed soluble larval fraction), FW is the fresh weight (mg) of the larvae harvested from each replicate at the end of the experiment, DM is the dry matter (%) of fresh larvae, Y is the percentage of the dry mass recovered (%) after extraction of each nitrogen fraction f, TN_f is the total nitrogen content of fraction f and is the ^{15}N content (at %) of fraction f. The amount of ^{15}N from tracer origin and the percentage of tracer ^{15}N in fraction f were calculated similarly to equations (5.2) and (5.3).

5.2.6 Statistical analyses

Data was analyzed in R version 4.0.3 (R Core Team, 2019). All analyses and visualizations are reproducible and accessible online. Results were expressed as mean ± standard error (n = 5). Student's t-test and two-way ANOVA with post-hoc Tukey tests were used to test whether there were differences among treatments, after verifying normality (Shapiro test) and homogeneity of variances.

Table 5.1: Fresh weight (mg) of individual larvae (mean ± std error, n = 5) before and after exposure to the nitrogen free diet for each treatment. The larvae from treatment Enriched_Digesta were not exposed to the nitrogen free diet. Values within columns and rows without letters in common are significantly different ($P < 0.05$). See Table S1 for statistical parameters.

Treatment	After manure	After N free diet
Enriched	82 ± 1.76 ^{ab}	81 ± 1.07 ^{ab}
Control	82 ± 1.06 ^{ab}	85 ± 1.26 ^{a}
Enriched_Digesta	79 ± 1.16 ^{b}	-

5.3 Results and Discussion

5.3.1 Larval performance

The larval weight once harvested from manure were similar between the Enriched, Control and Enriched_Digesta treatments (Table 5.1). Considering this, and that larval weights ranging from 79 to 82 mg were almost the same to the 84 ± 9 mg as reported by Parodi et al. (2021), who used the same diet (i.e., pig manure), feeding rate and incubation time, it can be presumed that the tracer ^{15}N nor experimental conditions limited larval growth. Furthermore, the larvae from the Enriched and Control treatments that passed through the nitrogen free diet had equal final weights, which were similar to those prior to the exposure to the nitrogen free diet (Table 5.1). These results show that the larvae of the Enriched and Control treatments did not lose weight after being exposed to the nitrogen free diet and suggest that the nitrogen free diet did not influence larval final weights.

5.3.2 Total nitrogen and ^{15}N content in larval samples

Larval total N concentration differed between treatments. The larvae and the dialysed soluble larval fraction from the Enriched_Digesta treatment had higher nitrogen levels than those from the Enriched and Control treatments (Figure 5.2). On average, the insoluble larval fraction from the Enriched_Digesta treatment contained more nitrogen than the other two treatments, however, statistically the difference was marginally non-significant ($P = 0.069 – 0.093$, see Table S2 for details). There were no differences in the larval N content between the Enriched and Control treatments (Figure 5.2, $P = 0.806$), confirming that the tracer did not alter the N deposition in the larvae.

The higher nitrogen content in larvae from the Enriched_Digesta treatment was likely due to nitrogen contained in their gut, whereas this was replaced by nitrogen free diet in the larvae from the Enriched and Control treatments. This is supported by the fact

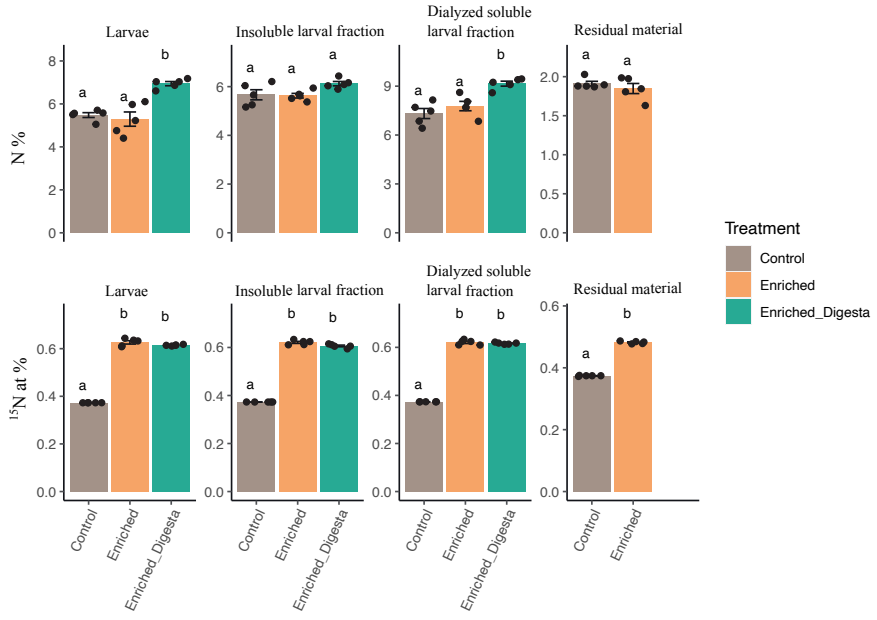

Figure 5.2: Total nitrogen (N) and ^{15}N enrichment (^{15}N) of larvae, insoluble larval fraction, dialysed soluble larval fraction and residual material (mean ± std error, n = 5). N values expressed as % are reported on a DM basis. ^{15}N expressed as at% (atom percentage of ^{15}N atoms relative to total N (^{14}N +^{15}N)). Different letters indicate that values are significantly different (P<0.05). See Table S2 for exact N and ^{15}N values and statistical parameters, and Table S3 for background data.

that larval total N in the Enriched_Digesta (Figure 5.2) was equal to 6.9 ± 0.2 g/100 g DM as reported by Parodi et al. (2021) for manure-fed larvae, while the larval total N of the Enriched and Control treatments were slightly lower (Figure 5.2). The midgut is the most important digestive organ in insects (Caccia et al., 2019), with feed residence times ranging from 154 to 195 minutes in BSFL (Gold et al., 2020). It is therefore expected that during the 15 h that the larvae were exposed to the nitrogen free diet their gut content was replaced several times effectively removing N contained in their digestive tract.

The higher ^{15}N enrichment of almost a factor 2 in the larval samples of both Enriched and Enriched_Digesta compared to the Control (Figure 5.2), confirmed that the tracer-derived ^{15}N was incorporated in the larval body mass. Unlike total N, ^{15}N enrichment was equal for most larval samples (i.e., larvae, insoluble N and soluble N fractions) of both Enriched and Enriched_Digesta treatments (Figure 5.2), suggesting that the ^{15}N concentration in the frass-digesta was too low to change the relative atom abundance of ^{15}N in the larval samples. This is supported by the fact that the ^{15}N abundance in the residual material

was lower (Figure 5.2) than in the larval samples, and that most of the tracer ^{15}N was lost (Figure 5.3B). The background ^{15}N enrichment found in the larvae of the Control treatment was within the 0.355 – 0.377 at % range present in most naturally-occurring nitrogen materials (Robinson, 2001).

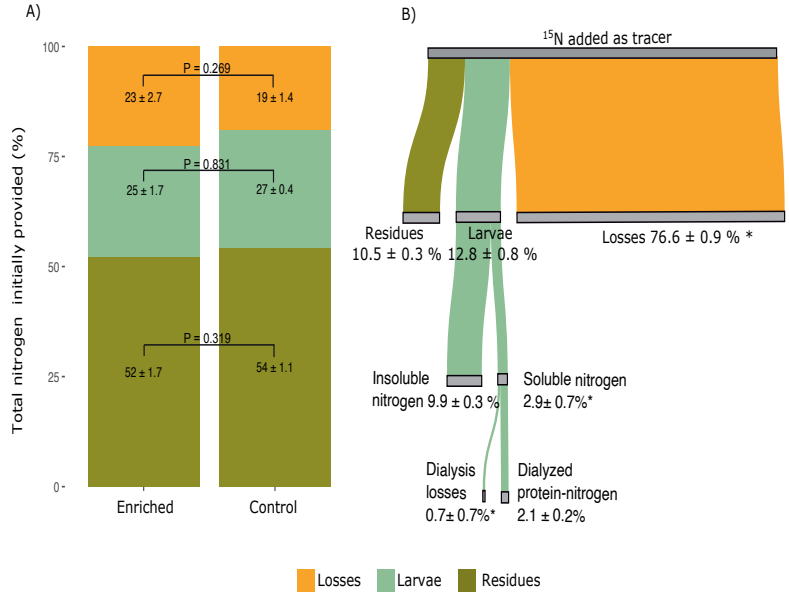

Figure 5.3: A) Total nitrogen balance of the Enriched and Control treatment. B) ^{15}N tracer balance for the Enriched treatment. Percentages refer to the initial amount of total N or ^{15}N tracer. Values with an asterisk * were calculated by difference. See Table S3-S4 for background data for both nutrient balances.

5.3.3 Total N, ^{15}N tracer balance and ^{15}N allocation in larvae

The nitrogen balance shows that between 25 to 27 % of the nitrogen contained in the manure was deposited in the larvae, 52 to 54 % was found in the residues and 19 to 23 % was likely lost to the air as NH_3 emissions (Figure 5.3A). In addition, the nitrogen balance reported here was similar to Parodi et al. (2021), who also quantified emissions in a complete mass balance approach and found that 25% of the nitrogen provided in pig manure was found in the larvae, 53% in the residues and 20% was lost as NH_3 emissions.

The balance of the tracer ^{15}N showed that 13% of the tracer ^{15}N was found in the larvae, 11% in the residues, and 76% was lost (Figure 5.3B). ^{15}N proportional losses were much higher than proportional total nitrogen losses. Considering that 80 to 90 % of the nitrogen contained in the manure was NH_3-N (see Table S5 for total nitrogen and NH_3-N in fresh

manure, and Table S6 for N content in supernatant from manure), it was expected that total nitrogen losses were going to be similar to the ^{15}N losses, but this was not the case. It is therefore likely that while some ^{15}N was lost via NH_3 emissions, a substantial amount of ^{15}N was lost from the residues pool during the cleaning of larvae once extracted from the enriched residual material, or during gut digesta cleaning with the nitrogen free diet.

Within the larvae, 17% of the ^{15}N was found in dialysed soluble fraction and 78% in the insoluble fraction. The remaining 10% was likely lost during dialysis either as soluble protein or other N-containing compounds. The main pool of ^{15}N was in the insoluble larval fraction. Likely, at pH 8 this primarily consisted of proteins, and chitin (Caligiani et al., 2018). A smaller proportion could have been present in other non-protein nitrogen compounds such as melanin (Caligiani et al., 2018), nucleic acids and phospholipids (Janssen et al., 2017). A previous study that quantified the composition of nitrogen-containing compounds in whole BSFL, found 84% of the nitrogen in proteins, 10% in chitin, and 6% in other N-compounds such as melanin (Caligiani et al., 2018). If all the chitin and other N-compounds were in the insoluble larval fraction (i.e., assuming 13% of insoluble larval fraction was chitin and 8% in melanin), at least 79% of the ^{15}N in the insoluble larval fraction could have been present in insoluble proteins. Thus, when considering soluble and insoluble proteins, at least 10% of the NH_3-N contained in manure could be incorporated into larval proteins and 3% into other N-compounds such as chitin and melanin. Future studies could quantify ^{15}N enrichment in amino acids, chitin and melanin to confirm this claim.

5.3.4 Opportunities and outlook

In this study we show that 13% of manure NH_3-N can be stored in the larval body mass and incorporated into proteins. Elucidating the mechanisms by which NH_3-N is assimilated into BSFL is key to explore interventions aimed to maximize the 13% reported here. Maximizing manure NH_3-N assimilation in BSFL reduces manure NH_3 emissions and increases the amount of circular protein that BSFL supply. Two simultaneous pathways could be involved in the assimilation of NH_3-N in BSFL larval proteins. One of the pathways is via transamination inside the larvae, where NH_3 is used as a N-donor during amino acid synthesis (Chen and Bachmann-Diem, 1964; Seshachalam et al., 1992). If the tracer ^{15}N was the N donor, amino acids and proteins could be enriched with tracer ^{15}N. As with higher larval densities more larvae compete for the same substrate, the chances of larvae incorporating NH_3-N as an N donor for the synthesis of non-essential amino acids via transamination might increase. The second pathway is via microbial biomass acting equivalent to an "external rumen", where NH_3-N is used for the synthesis of microbial amino acids, subsequently used by larvae feeding on these microbes. The assimilation of NH_3 by microbes is enzymatically driven by glutamate deshydrogenase (GDH) and glutamine synthetase (Reitzer, 2014). The gene that encodes for GDH is

present in animals, plants and microorganisms (Hudson and Daniel, 1993). Unlike the larval transamination pathway, the microbial biomass pathway could reduce manure NH_3 emissions if NH_3-N assimilation in larvae is maximized via rearing conditions. For instance, substrates inoculated with microbes can increase both bioconversion and larval weights (Mazza et al., 2020; Wong et al., 2021), although this is not always the case (Gold et al., 2021; Hasnol et al., 2020). Future assessments should elucidate if increased bioconversion due to specific microbial inocula is correlated with larger NH_3-N assimilation in manure-based diets. Inocula from *Enterococcus faecium* strain C2 and *Bacillus coagulans* strain B1 can reduce NH_3 emissions from manure by 53% and 31%, respectively (Xiao et al., 2021), and could be good candidates to improve NH_3-N assimilation in BSFL. Another potential intervention to increase NH_3-N microbial and larval assimilation is to increase the C:N ratio of manure to foster microbial growth and promote higher microbial nitrogen utilization. Such carbon sources could be supplied from low value fiber-rich leftovers or high-carbohydrate leftovers which are non-edible to livestock. However, negative effects on larval performance parameters such as final weight, survival and development time, and GHG emissions should be evaluated to determine the net benefits of this intervention (Barragan-Fonseca et al., 2019).

If the limit of microbial NH_3-N assimilation is reached, as can occur in the rumen (Pengpeng and Tan, 2013), interventions should prevent NH_3 volatilization from the residual material. Keeping the substrate slightly acidic to favor the NH_4^+: NH_3 equilibrium towards NH_4^+, without hampering the growth of beneficial bacteria that assimilate NH_3-N, would contribute to this. Instead of the expensive acidifiers used for manure management, the substrate pH could be kept slightly acidic by mixing manure with an acidic feed ingredient that might improve the nutrient quality of manure and increase larval yields. Such acidic ingredient could come from food waste pre-treated with lactic acid bacteria (Sabater et al., 2020), or acidic fruit wastes such as citrus and tomatoes. After BSFL harvest, however, it is key to implement management practices of the residual material to avoid NH_3 volatilization during storage and application. The residual material is the main pool of nitrogen after BSFL bioconversion (Parodi et al., 2021) and its emissions are one of the main sources of GHG in BSFL bioconversion systems (Mertenat et al., 2019). Thus, without proper post-harvest management of the residual material, the NH_3 reduction gains obtained via larval NH_3-N assimilation could be offset.

BSFL have the potential to become an important element in the circular economy. Future holistic assessments covering not only the net environmental benefits and disadvantages of BSFL at a supply-chain and food systems level, but also the economic, social and safety dimensions associated to its use, are needed to elucidate the role of manure-fed BSFL in future food systems.

5.4 Conclusions

In this study we show that during manure bioconversion with BSFL 13% of the NH_3-N contained in manure was incorporated into the larval biomass. Within the larval body, 77% of the NH_3-N was found in the insoluble larval fraction, and 17% in the dialysed soluble fraction. Our results suggest that manure-fed black soldier fly larvae can reduce ammonia pollution from manure and can thereby contribute to a circular and sustainable protein supply.

5.5 Supplementary Information

The supporting information of this chapter includes: Appendix A - Supplementary methods Appendix B - Supplementary tables (S1-S6)

5.5.1 Supplementary methods

Composition of the nitrogen-free diet

The nitrogen free diet used in this study was specially elaborated for the study 'Determination of True Ileal Amino Acid Digestibility in the Growing Pig for Calculation of Digestible Indispensable Amino Acid Score (DIAAS)' (Hodgkinson et al., 2020). The ingredient composition is presented in Table S1. We analyzed the nitrogen-free diet for ^{15}N in the Isotope Ratio Mass Spectrometer, and ^{15}N was not detected.

Ingredient composition of the nitrogen-free diet.

Ingredient composition (g/kg DM)	N-free diet
Purified maize starch	764
Purified cellulose	30
Rapeseed oil	50
Sucrose	100
Premix (vit+min)	1.5
Dicalcium phosphate	25
Magnesium oxide	1
$CaCO_3$	3
K_2CO_3	7
$NaHCO_3$	3
Salt extra	4
Titanium dioxide	4
Celite	7.5
Sum	1000

Isotopic determination

The 9.8 mg of $^{15}NH_4^+Cl$ added to the containers of the treatments Enriched and Enriched_Digesta was estimated based on a pilot experiment. In the pilot experiment, 25 starter larvae were reared on 10 g DM pig manure enriched with 5.4 mg of $^{15}NH_4^+Cl$. A control group was reared using the same amount of manure and larvae but without

$^{15}NH_4{}^+Cl$. The amount of $^{15}NH_4{}^+Cl$ added was theoretically estimated to get a ^{15}N delta ($^{15}\delta N$) value at least higher than 100 ‰ compared to the control group reared without $^{15}NH_4{}^+Cl$. The $^{15}\delta N$ scale (in per mille ‰) is used to express the ^{15}N:14N ratio of samples containing only slight ^{15}N enrichments using the ^{15}N abundance in atmospheric N2 as a standard (i.e., $^{15}\delta N = 0$ ‰) (Robinson, 2001). Given that the $^{15}\delta N$ of the defatted control larvae was 10 ‰ and the $^{15}\delta N$ of the enriched defatted larvae was above 240 ‰, it was concluded that the proportion of $^{15}NH_4{}^+Cl$ and dry matter manure used in the pilot was satisfactory to test if tracer ^{15}N incorporation into BSFL was occurring. Thus, for this study we used the same proportion, which resulted on 9.8 mg of $^{15}NH_4{}^+Cl$ for 18.4 g of DM manure. The following section contains general information on the background abundance of ^{15}N in manure and the calculations done to theoretically estimate the amount of $^{15}NH_4{}^+Cl$ used in the pilot study.

Background abundance of ^{15}N in manure

The abundance of ^{15}N is expressed on the sigma scale (in per mill ‰):

$$^{15}\delta N = 1000 \times \frac{(s-a)}{a} \tag{5.6}$$

Whereas, s is the relative abundance (%) of ^{15}N atoms in a sample, and a is the relative abundance (%) of ^{15}N atoms in the standard sample (the atmosphere N is 99.6337% ^{14}N and 0.3663% ^{15}N) (Bedard-Haughn et al., 2003). The higher the $^{15}\delta N$, the higher the abundance of ^{15}N. Most naturally occurring N containing materials, are within a $^{15}\delta N$ of -30‰ and + 30‰ (this would be between 0.355 and 0.377 % of ^{15}N atoms) (Robinson, 2001). ^{15}N is present in animal manure naturally. This is because plants (and therefore animal feed) contain ^{15}N. The abundance of ^{15}N in plants varies depending on the type of soil where they grew, fertilization type, N deposition, etc (Robinson, 2001). Published studies report a $^{15}\delta N$ of 13.9 ‰ for composted pig manure (Choi et al., 2002). Another study reports a $^{15}\delta N$ of 14.9 ‰ for poultry litter (Caio de Teves et al., 2013). For the upcoming calculations we assume a $^{15}\delta N$ of 13.9 ‰, which means that the atom ^{15}N relative abundance is 0.371392 %. This atomic relative abundance is also expected to be present in the larvae.

Estimating how much tracer is needed

The pilot experiment will be performed with 10 g of fresh manure and 25 black soldier fly larvae. The following parameters have been used for the calculations (Table A1).

To calculate what is the background content of ^{15}N of manure-fed BSFL (this is the amount of ^{15}N in milligrams that is expected to be naturally found in 1.035 grams of dry matter manure fed-larvae) we do the following calculation:

Parameters used in the calculations to determine how much tracer is needed per replicate

Parameter	Value	Unit	Source
15N atom abundance in larvae	0.371392	%	(Choi et al., 2002)
Larvae per replicate	25	Number of larvae	-
Expected final weight per larva	150	mg	Previous pilot
Dry matter content of manure-fed larvae	25.6	%	(Parodi et al., 2021)
Expected larvae DM yield per replicate	1.035	g	Calculated
Expected N content as protein in DM larvae	5.5	%	Calculated based on (Janssen et al., 2017)
Manure nitrogen bioconversion with BSFL	25	%	(Parodi et al., 2021)

$$
\begin{aligned}
{}^{15}N_{BackgroundLarvae} &= Larvae_{yield} \times Larvae_{DMnitrogen} \times {}^{15}N_{\%} \times 1000, \\
{}^{15}N_{BackgroundLarvae} &= 1.035 \times \frac{5.5}{100} \times \frac{0.371392}{100} \times 1000, \\
{}^{15}N_{BackgroundLarvae} &= 0.21350958
\end{aligned}
$$

The isotope-mass spectrometer is a very precise instrument. It can detect differences of $^{15}\delta$N 20 ‰. For this case, we aim to enrich the sample to get a $^{15}\delta$N of 100 ‰. To calculate what should be the ^{15}N atom relative abundance to get a $^{15}\delta$N of 100 ‰ we do the following calculation:

$$
\begin{aligned}
{}^{15}N_{Desired\%} &= \left((\frac{{}^{15}\delta N_{desired}}{1000}) \times {}^{15}N_{atm} \right) + {}^{15}N_{atm}, \\
{}^{15}N_{Desired\%} &= \left((\frac{100}{1000}) \times 0.3663 \right) + 0.3663, \\
{}^{15}N_{Desired\%} &= 0.402932\%
\end{aligned}
$$

Given that we know the desired ^{15}N atom relative abundance that we need to achieve to get a $^{15}\delta$N of 100 ‰, we can calculate the amount of ^{15}N (in milligrams) that will be found (includes both background and tracer):

$$
\begin{aligned}
{}^{15}N_{Background+tracer} &= Larvae_{yield} \times Larva_{DMnitrogen} \times {}^{15}N_{Desired\%} \times 1000, \\
{}^{15}N_{Background+tracer} &= 1.035 + \frac{5.5}{100} \times \frac{0.402930}{100} \times 100, \\
{}^{15}N_{Background+tracer} &= 0.23164073mg
\end{aligned}
$$

By subtracting the amount of ^{15}N found in background from the amount of ^{15}N of the enriched larvae (background + tracer), we can know how much tracer (in milligrams) should end up in larvae to get a $^{15}\delta$N of 100 ‰.

$$^{15}N_{Needed_larvae} = {}^{15}N_{Background+tracer} - {}^{15}N_{BackgroundLarvae}$$
$$^{15}N_{Needed_larvae} = 0.23164073 + 0.21350958$$
$$^{15}N_{Needed_larvae} = 0.018131152mg$$

To end up with 0.018131152 milligrams of tracer in the larvae, we need to add larger amounts of tracer to the manure. This is because not all the tracer is expected to end up in the larvae. A portion will be lost as ammonia emissions and another portion will remain in the residues. The nitrogen bioconversion efficiency with manure BSFL larvae is 25%, and in this experiment, we want to test if at least 5% of the ammonia nitrogen can be found as larval proteins. Therefore, to finally estimate how much ^{15}N tracer is needed, we do the following calculation:

$$^{15}N_{Neededmanure} = \frac{\frac{^{15}Nneeded_{larvae}}{N_{bioconversion\%}}}{^{15}N_{minimumuptake}}$$
$$^{15}N_{Neededmanure} = \frac{\frac{0.018131152}{\frac{25}{100}}}{\frac{5}{100}}$$
$$^{15}N_{Needed_manure} = 1.450492134mg$$

So far, calculations have been done for ^{15}N. However, the commercial tracer used (ammonium chloride, $^{15}NH_4Cl$ also contains other atoms. To estimate how much ammonium chloride ($^{15}NH_4Cl$) is needed to have 1.450492134 mg of ^{15}N, we do the following calculation using the contribution of nitrogen to the total molecular weight of $^{15}NH_4Cl$ (i.e., 27%) and the purity of ^{15}N atoms in $^{15}NH_4Cl$ according to the manufacturer (i.e., 98%):

$$^{15}NH_4Cl_{Neededmanure} = \frac{\frac{^{15}Nneeded_{manure}}{N_{proportioninNH4Cl}}}{^{15}N_{tracerpurity}},$$
$$^{15}NH_4Cl_{Neededmanure} = \frac{\frac{1.450492134}{0.27}}{0.98}$$
$$^{15}NH_4Cl_{Neededmanure} = 5.48mg$$

5.5.2 Supplementary Tables

Table S1. Statistical results for tukey post-hoc test presented in Table 1.

Comparison	null.value	estimate	conf.low	conf.high	adj.p.value
Control_beforeNfree - Control_afterNfree	0	-2.424	-7.871	3.023	0.675
Enriched_Digesta_beforeNfree - Control_afterNfree	0	-5.8	-11.247	-0.353	0.033
Enriched_afterNfree - Control_afterNfree	0	-3.258	-8.705	2.189	0.406
Enriched_beforeNfree - Control_afterNfree	0	-2.822	-8.269	2.625	0.544
Enriched_Digesta_beforeNfree - Control_beforeNfree	0	-3.375	-8.822	2.072	0.372
Enriched_afterNfree - Control_beforeNfree	0	-0.834	-6.281	4.613	0.99
Enriched_beforeNfree - Control_beforeNfree	0	-0.398	-5.845	5.049	0.999
Enriched_afterNfree - Enriched_Digesta_beforeNfree	0	2.542	-2.905	7.989	0.637
Enriched_beforeNfree - Enriched_Digesta_beforeNfree	0	2.978	-2.469	8.425	0.493
Enriched_beforeNfree - Enriched_afterNfree	0	0.436	-5.011	5.883	0.999

Table S2. A) Exact N and ^{15}N enrichment values presented in Figure 5.2 and statistical results for Tukey post-hoc test and t-test. N (%) is given for DM samples.

Treatment	Larvae		Insoluble larval fraction		Dialysed soluble larval fraction		Residual material	
	N %	^{15}N enrichment (at %)	N %	^{15}N enrichment (a)	N %	^{15}N enrichment (at %)	N %	^{15}N enrichment (at %)
Enriched	5.29 ± 0.332a	0.6263 ± 0.007a	5.63 ± 0.0947a	0.6211 ± 0.0042a	7.78 ± 0.2878a	0.6206 ± 0.0047a	1.85 ± 0.0647a	0.4821 ± 0.0018a
Control	5.48 ± 0.1122a	0.3729 ± 0.0001b	5.67 ± 0.2071a	0.3729 ± 0b	7.32 ± 0.3076a	0.3734 ± 0.0001b	1.91 ± 0.0298a	0.3741 ± 0.0005b
Enriched_Digesta	6.94 ± 0.0981b	0.6145 ± 0.0012a	6.12 ± 0.0895a	0.6063 ± 0.0035c	9.15 ± 0.1522b	0.6168 ± 0.0017a	-	-

Table S2 B) Post-hoc for larval fractions.

Sample	nutrient	term	contrast	null.value	estimate	conf.low	conf.high	adj.p.value
Insoluble_N	N	Treatment	Enriched-Control	0	-0.037589537	-0.570577742	0.495398668	0.980703903
Insoluble_N	N	Treatment	Enriched_Nfree-Control	0	0.457115673	-0.075872532	0.990103878	0.09627731
Insoluble_N	N	Treatment	Enriched_Nfree-Enriched	0	0.49470521	-0.038282995	1.027693415	0.069830707
Insoluble_N	N15	Treatment	Enriched-Control	0	0.2482403	0.236210578	0.260270022	3.10E-14
Insoluble_N	N15	Treatment	Enriched_Nfree-Control	0	0.233421	0.221391278	0.245450722	3.74E-14
Insoluble_N	N15	Treatment	Enriched_Nfree-Enriched	0	-0.0148193	-0.026849022	-0.002789578	0.016577812
Larvae	N	Treatment	Enriched-Control	0	-0.187326116	-0.97996014	0.605307908	0.806487242
Larvae	N	Treatment	Enriched_Nfree-Control	0	1.462858845	0.670224821	2.255492869	0.000946902
Larvae	N	Treatment	Enriched_Nfree-Enriched	0	1.65018496	0.857550936	2.442818984	0.000339883
Larvae	N15	Treatment	Enriched-Control	0	0.253464	0.238064775	0.268863225	1.12E-13
Larvae	N15	Treatment	Enriched_Nfree-Control	0	0.2416893	0.226290075	0.257088525	1.66E-13
Larvae	N15	Treatment	Enriched_Nfree-Enriched	0	-0.0117747	-0.027173925	0.003624525	0.144991233
Residual material	N	Treatment	Enriched-Control	0	-0.062395627	-0.226705367	0.101914113	0.406721297
Residual material	N15	Treatment	Enriched-Control	0	0.1079995	0.103617447	0.112381553	3.02E-12
Soluble_dialysed_N	N	Treatment	Enriched-Control	0	0.45901413	-0.516530781	1.43455904	0.445383651
Soluble_dialysed_N	N	Treatment	Enriched_Nfree-Control	0	1.834531105	0.858986195	2.810076015	0.000811111
Soluble_dialysed_N	N	Treatment	Enriched_Nfree-Enriched	0	1.375516975	0.399972065	2.351061886	0.00707348
Soluble_dialysed_N	N15	Treatment	Enriched-Control	0	0.2472385	0.236451542	0.258025458	2.78E-14
Soluble_dialysed_N	N15	Treatment	Enriched_Nfree-Control	0	0.2433704	0.232583442	0.254157358	2.79E-14
Soluble_dialysed_N	N15	Treatment	Enriched_Nfree-Enriched	0	-0.0038681	-0.014655058	0.006918858	0.616601243

Table S2 C) T-test for residual material

Nutrient	estimate	estimate1	estimate2	statistic	p.value	parameter	conf.low	conf.high	method	alternative
N	0.062395627	1.910768885	1.848373258	0.875691086	0.417010077	5.626435416	-0.114799055	0.239590309	Welch Two Sample t-test	two.sided
N15	-0.1079995	0.3740816	0.4820811	-56.8334677	7.66E-08	4.692086333	-0.112982336	-0.103016664	Welch Two Sample t-test	two.sided

Table S3. Input (i.e., Manure, Starter) and output (i.e., Larvae, Residues) data for total N and 15N balances. For DM% n=2 for manure, and n=1 for the rest of samples. For N% n = 1 for all samples. For 15N (at%) n = 2 for all samples.

Type	Sample	Treatment	Replicate	Total_fresh_g	DM%	N%	^{15}N(at %)
Input	Manure	Enriched	R1	75	24.79	2.64	0.3676065
Input	Manure	Enriched	R2	75	24.79	2.64	0.3676065
Input	Manure	Enriched	R3	75	24.79	2.64	0.3676065
Input	Manure	Enriched	R4	75	24.79	2.64	0.3676065
Input	Manure	Enriched	R5	75	24.79	2.64	0.3676065
Input	Manure	Enriched_Digesta	R1	75	24.79	2.64	0.3676065
Input	Manure	Enriched_Digesta	R2	75	24.79	2.64	0.3676065
Input	Manure	Enriched_Digesta	R3	75	24.79	2.64	0.3676065
Input	Manure	Enriched_Digesta	R4	75	24.79	2.64	0.3676065
Input	Manure	Enriched_Digesta	R5	75	24.79	2.64	0.3676065
Input	Manure	Control	R1	75	24.79	2.64	0.3676065
Input	Manure	Control	R2	75	24.79	2.64	0.3676065
Input	Manure	Control	R3	75	24.79	2.64	0.3676065
Input	Manure	Control	R4	75	24.79	2.64	0.3676065
Input	Manure	Control	R5	75	24.79	2.64	0.3676065
Input	Starter	Enriched	R1	0.321	26.10	9.50	0.3728538
Input	Starter	Enriched	R2	0.325	26.10	9.50	0.3728538
Input	Starter	Enriched	R3	0.348	26.10	9.50	0.3728538
Input	Starter	Enriched	R4	0.381	26.10	9.50	0.3728538
Input	Starter	Enriched	R5	0.335	26.10	9.50	0.3728538
Input	Starter	Enriched_Digesta	R1	0.34	26.10	9.50	0.3728538
Input	Starter	Enriched_Digesta	R2	0.358	26.10	9.50	0.3728538
Input	Starter	Enriched_Digesta	R3	0.346	26.10	9.50	0.3728538
Input	Starter	Enriched_Digesta	R4	0.324	26.10	9.50	0.3728538
Input	Starter	Enriched_Digesta	R5	0.345	26.10	9.50	0.3728538
Input	Starter	Control	R1	0.315	26.10	9.50	0.3728538
Input	Starter	Control	R2	0.316	26.10	9.50	0.3728538
Input	Starter	Control	R3	0.336	26.10	9.50	0.3728538
Input	Starter	Control	R4	0.338	26.10	9.50	0.3728538
Input	Starter	Control	R5	0.336	26.10	9.50	0.3728538
Output	Larvae	Enriched	R1	8.213	30.04	6.11	0.64376
Output	Larvae	Enriched	R2	8.23	31.01	4.76	0.635662
Output	Larvae	Enriched	R3	8.111	30.88	4.40	0.63235
Output	Larvae	Enriched	R4	7.729	31.43	5.23	0.6116575
Output	Larvae	Enriched	R5	8.199	31.11	5.97	0.6081595
Output	Larvae	Enriched_Digesta	R1	7.812	27.77	6.86	0.614241
Output	Larvae	Enriched_Digesta	R2	7.621	28.14	7.03	0.613929
Output	Larvae	Enriched_Digesta	R3	7.929	27.67	7.18	0.6110035
Output	Larvae	Enriched_Digesta	R4	7.922	27.58	6.61	0.618179
Output	Larvae	Enriched_Digesta	R5	8.081	26.74	7.03	0.615363
Output	Larvae	Control	R1	8.601	29.56	5.50	0.372852
Output	Larvae	Control	R2	8.098	31.41	5.58	0.3730465
Output	Larvae	Control	R3	8.047	29.75	5.71	0.3727705
Output	Larvae	Control	R4	8.623	30.49	5.05	0.3727735
Output	Larvae	Control	R5	8.745	29.75	5.56	0.3728265
Output	Residual_material	Enriched	R1	42.767	32.10	1.97	0.483299
Output	Residual_material	Enriched	R2	44.369	32.00	1.63	0.486747
Output	Residual_material	Enriched	R3	43.209	32.15	1.81	0.484665
Output	Residual_material	Enriched	R4	42.458	33.03	1.98	0.478417
Output	Residual_material	Enriched	R5	43.192	30.98	1.85	0.4772775
Output	Residual_material	Control	R1	43.583	31.60	1.87	0.3742815
Output	Residual_material	Control	R2	43.799	32.38	2.03	0.37434
Output	Residual_material	Control	R3	43.525	31.20	1.90	0.374323
Output	Residual_material	Control	R4	43.794	32.08	1.88	0.375372
Output	Residual_material	Control	R5	42.43	33.44	1.88	0.3720915

Table S4. Total nitrogen and 15N data to calculate the allocation of 15N in the larvae as dialysed protein N and insoluble nitrogen. * Yield = Percentage (%) of DM recovered after the extraction of the insoluble and soluble dialysed N fractions

Type	Sample	Treatment	Replicate	Yield (%)	N %	^{15}N (at %)
Output	Insoluble_N	Enriched	R1	75.66	5.69	0.6333985
Output	Insoluble_N	Enriched	R2	68.40	5.37	0.6244795
Output	Insoluble_N	Enriched	R3	71.26	5.62	0.6246345
Output	Insoluble_N	Enriched	R4	76.68	5.94	0.6119425
Output	Insoluble_N	Enriched	R5	80.72	5.52	0.6110065
Output	Insoluble_N	Enriched_Digesta	R1	69.02	6.16	0.60515
Output	Insoluble_N	Enriched_Digesta	R2	68.78	6.03	0.6054775
Output	Insoluble_N	Enriched_Digesta	R3	70.24	5.90	0.594294
Output	Insoluble_N	Enriched_Digesta	R4	71.34	6.44	0.615104
Output	Insoluble_N	Enriched_Digesta	R5	70.21	6.09	0.6113395
Output	Insoluble_N	Control	R1	82.10	6.04	0.37284
Output	Insoluble_N	Control	R2	75.47	5.25	0.372937
Output	Insoluble_N	Control	R3	77.21	6.21	0.3728355
Output	Insoluble_N	Control	R4	77.94	5.66	0.372872
Output	Insoluble_N	Control	R5	79.61	5.16	0.3727755
Output	Soluble_dialysed_N	Enriched	R1	11.28	7.69	0.6335855
Output	Soluble_dialysed_N	Enriched	R2	13.95	7.70	0.6251415
Output	Soluble_dialysed_N	Enriched	R3	12.49	8.04	0.6245415
Output	Soluble_dialysed_N	Enriched	R4	10.52	8.61	0.610187
Output	Soluble_dialysed_N	Enriched	R5	10.62	6.84	0.609675
Output	Soluble_dialysed_N	Enriched_Digesta	R1	11.76	9.44	0.612706
Output	Soluble_dialysed_N	Enriched_Digesta	R2	12.26	9.24	0.6133935
Output	Soluble_dialysed_N	Enriched_Digesta	R3	12.00	9.40	0.617788
Output	Soluble_dialysed_N	Enriched_Digesta	R4	12.59	8.60	0.622029
Output	Soluble_dialysed_N	Enriched_Digesta	R5	12.41	9.10	0.6178735
Output	Soluble_dialysed_N	Control	R1	7.58	6.85	0.3734445
Output	Soluble_dialysed_N	Control	R2	12.09	7.70	0.3735465
Output	Soluble_dialysed_N	Control	R3	9.75	8.15	0.3733905
Output	Soluble_dialysed_N	Control	R4	9.58	7.47	0.373295
Output	Soluble_dialysed_N	Control	R5	13.19	6.42	0.3732615

Table S5. Dry matter, total nitrogen and ammonia-nitrogen of manure, larvae and residues in 100 g dry matter (mean ± std. error)

Type	Sample	Treatment	DM %	N %	N_NH_3 %
Input	Manure		24.79 ± 0	2.64 ± 0	2.09
Output	Larvae	Control	30.19 ± 0.34	5.48 ± 0.11	-
Output	Larvae	Enriched	30.89 ± 0.23	5.29 ± 0.33	-
Output	Larvae	Enriched_Nfree	27.58 ± 0.23	6.94 ± 0.1	-
Output	Residual material	Control	32.14 ± 0.38	1.91 ± 0.03	0.74 ± 0.01
Output	Residual material	Enriched	32.05 ± 0.33	1.85 ± 0.06	0.75 ± 0.01

Table S6. Quantification of ammonia-nitrogen, supernatant-nitrogen and total-nitrogen in pig manure.

Variable	Method	Nitrogen (%)	Relative contribution (%)
$N\text{-}NH_3$	Colorimetric determination	2.389207126	90.4
N-supernatant	Dumas	0.418006006	15.8
Total-N	Dumas	2.642575191	

Chapter 6

Black soldier fly larvae dietary preferences

This chapter is based on:

A. Parodi, K. Van Dijk, J. J. A. Van Loon, I. J. M. De Boer, J. Van Schelt, and H. H. E. Van Zanten (2020b). "Black soldier fly larvae show a stronger preference for manure than for a mass-rearing diet". *Journal of Applied Entomology* 144.7, 560–565. DOI: 10.1111/jen.12768

Abstract

The attention for black soldier fly larvae (BSFL) as an alternative ingredient for food and feed products is on the rise. While many studies have reported the efficiency of BSFL to bio-convert a wide range of organic waste streams into larval biomass, so far, it is unknown whether BSFL prefer certain waste streams over others when they have the possibility to choose. Here, we performed a choice-test experiment to explore the preference of BSFL when exposed to pig manure and a mass-rearing diet consisting of plant by-products currently used for industrial BSFL production. We found that after 1 hour of exposure to both feeds, BSFL strongly preferred pig manure over the mass-rearing diet. The preference for manure became stronger as larval age increased. Our results provide the first evidence that BSFL express a distinct diet preference. Understanding the reasons for the strong preference for manure is relevant for a diverse array of practical applications and to inform the discussion on insect welfare.

6.1 Introduction

The mass-rearing of *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae, hereafter called black soldier fly larvae (BSFL), is expanding worldwide. BSFL have become one of the main species for mass-rearing operations due to their ability to quickly convert a wide range of organic side streams into larval biomass that, if safe and allowed by law, can be used as an alternative source of food and feed. Together with the growth of industrial mass-rearing production, biological research on BSFL has also increased over the last years. Contemporary research topics include the determination of BSFL nutritional requirements (Barragan-Fonseca et al., 2017), the estimation of feed efficiencies for different waste-stream diets (Lalander et al., 2019), the role of microbiota in growth and fitness of larvae and flies (De Smet et al., 2018), the exploration of environmental impacts associated with BSFL production (Mertenat et al., 2019; Smetana et al., 2019), food safety (Bosch et al., 2017; Lalander et al., 2015) and mating and oviposition optimization in artificial conditions (Giunti et al., 2018; Hoc et al., 2019).

Although current research topics are diverse, a question that so far remained underexplored is: do BSFL prefer certain types of feeds over others? Usually, in both industrial and experimental conditions, BSFL are provided with only one type of feed, and therefore, it remains unknown whether BSFL prefer consuming certain feeds over others when they have the possibility to choose. Here, we performed a choice-test experiment in which larvae could choose between animal manure, a material in which BSFL naturally grow (Newton et al., 2005), and a mass-rearing diet consisting of plant by-products currently used for industrial BSFL production. The results reported here could be beneficial for the design of new feeding strategies and contribute to the discussion on insect welfare (Van Huis, 2019) which is gaining interest.

6.2 Material and methods

6.2.1 Larvae rearing

Seven-day-old BSFL (hereafter called starter larvae) that had been reared on a mix of wheat bran and water at the facilities of Bestico B.V, the Netherlands, were packed and shipped to the facilities of Wageningen University & Research, where the choice test was conducted. A group of starter larvae was separated to perform the first choice test (see Section Choice test), whereas the rest of the starters were reared in two independent crates, one containing fresh pig manure and the other containing the mass-rearing diet. These two diets were provided to the starters to assess a potential effect of larval diet on preference behaviour. In each crate, 5 kg of either pig manure or the mass-rearing diet

was added together with 5,000 starter larvae (for details on the feeds, see Section 2.2). Both crates were located in a climate-control room at 27°C, 70% relative humidity and a 12-hour light–dark cycle. At ages 9, 12, 14 and 16 days, 10 groups of 50 larvae each were collected randomly from the pig manure crate and from the crate containing the mass-rearing diet. The groups of 50 larvae were used as replicates to perform the choice tests (see Figure 6.1).

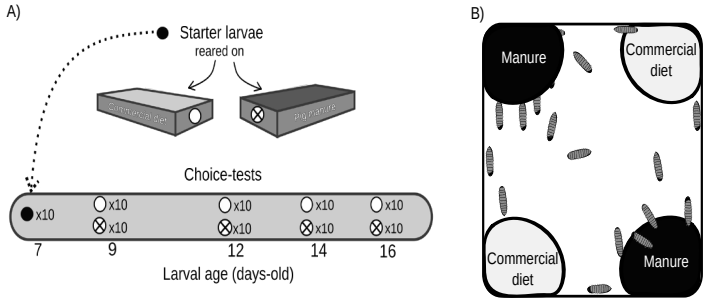

Figure 6.1: (a) Experimental design. Circles represent a group of 50 larvae. The black circle represents starter larvae, the white circle represents larvae reared on mass-rearing diet, and the crossed circle represents larvae reared on pig manure. Every choice test was replicated 10 times. (b) Schematic representation of the test arena.

6.2.2 Feeds

The fresh pig manure, collected on the first day of the experiment, had a dry matter content of 24.1%. The manure was produced by 20-week-old sows of the breed Topigs 20 fed with the feed "Uniek Start," from AgruniekRijnvallei, Wageningen, the Netherlands. The mass-rearing diet used by Bestico B.V. was shipped on the first day of the experiment and had a dry matter content of 28.6% dry matter. The feed was composed of a mixture of three commercial products: ProtiWanze® (by-product from bioethanol production from wheat starch, which contains wheat protein, sugar beet syrup and yeast), DB-blend (made of wheat starch and potato leftovers) and a binding agent. The proportion of each ingredient (fresh matter basis) was ProtiWanze 47%, DB-Blend 47% and binding agent 6%. Both feeds were stored in a refrigerator at 5°C upon arrival. On every choice-test day, refrigerated samples of both feeds were collected to perform the choice test. These samples were allowed to reach room temperature (27°C) before the start of the choice tests.

6.2.3 Choice test

For the choice test, two diagonally opposite corners of a cubic rounded-corner plastic container (20 cm × 20 cm × 20 cm) were filled each with a full 15-ml spoon of pig manure

whereas the other two diagonally opposite corners were filled each with a 15-ml spoon of the mass-rearing diet (Figure 6.1a). Subsequently, a group of 50 larvae (which were randomly collected from each crate as indicated in Section Larvae rearing) was placed in the middle of the plastic container and left for one hour in a climate-control room at 27°C and 70% relative humidity under complete darkness. Plastic lids were placed on top of each container to prevent escapes. After one hour, the container was removed from the climate-control room, and straightaway, each of the four piles of feed was collected in separated flasks for counting of larvae using water and sieves of different mesh sizes (0.25–3 mm). If larvae were found outside the piles (i.e. in the middle of the container, climbing the walls of the container or between piles), these were scored as "no choice." Larvae were removed from the experiment after the quantification of the number of larvae in each pile. Ten replicates were done for each choice test.

Exploratory videos were recorded at different larval ages to the purpose of visual illustration of BSFL behaviour in our test arena (see Video S1 and Figure S1 for details). The choice test that was recorded was not considered as a replicate and therefore was not included in our results.

6.2.4 Statistical analysis

Repeated G tests were performed to test whether there was an overall deviation from an equal proportion of larvae found in manure and the mass-rearing diet for each larval age. For each replicate, the numbers of larvae found in both manure piles were summed and the numbers found in both mass-rearing diet piles were also summed. In addition, log-linear regression models were performed to assess the effect of the following parameters: larval age (i.e. 7, 9, 12, 14 and 16 days old), initial diet (i.e. mass-rearing diet or manure) and replication (i.e. from 1 to 10), on the degree of preference of the larvae for manure or the mass-rearing diet.

6.3 Results

In all replicates, most of the larvae were found in the piles of pig manure, and only a few or sometimes not a single larva was found in the piles with the mass-rearing diet (Figure 6.2, Table S1). The video records, however, confirmed that during the choice test BSFL moved actively within the container and tried both feeds (see Video S1). The proportion of larvae scored as "no choice" was negligible (Table S1), with the exception of day 14 for the larvae growing on manure, in which for some replicates, groups of larvae ranging from 5 to 10 individuals were found climbing the walls of the containers.

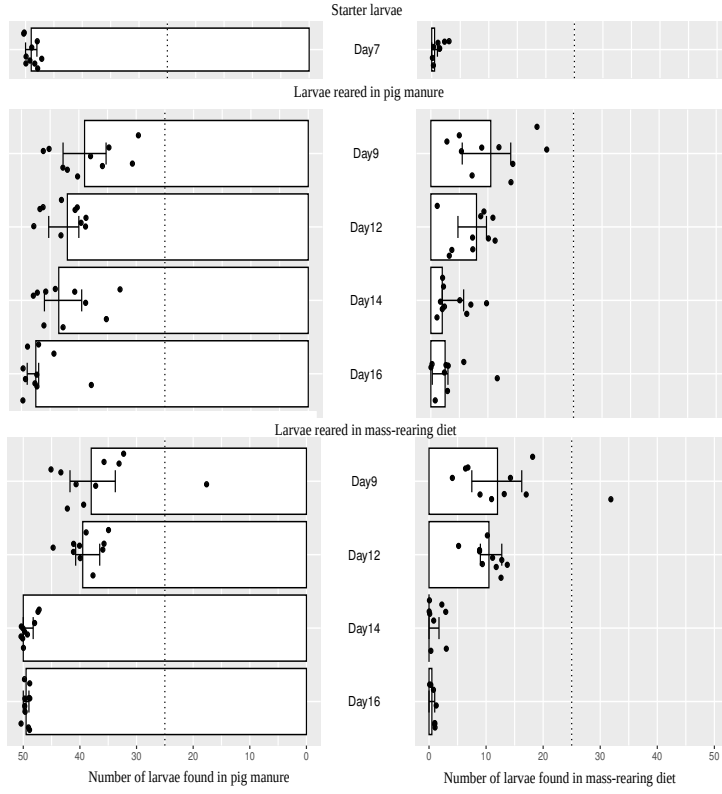

Figure 6.2: Summarized results of the choice test. Bars show the median value of the 10 replicates, and error bars show the 25% and 75% quartiles. It should be noticed that in case of an equal distribution of larvae between the mass-rearing diet and manure, both bars should be close to the dashed line, which marks the values for an equal distribution of 25 larvae in both substrates

The preference of BSFL for manure over the mass-rearing diet was observed at all ages and regardless of whether the larvae were previously fed with pig manure or with the mass-rearing diet (Table 6.1). However, the preference for manure became stronger as larval age increased (estimate = 0.04, z = 5.54, p <0.0001). Although larvae were found in both piles of manure (see Table S1), these tended to aggregate in one pile rather than being equally distributed in both piles (estimate = -0.08, z = -2.49, p = 0.01). Lastly, the type of diet to which the larvae were exposed prior to the preference test did not have any effect on the observed preference (see Table S2).

Table 6.1: Total number of larvae that preferred the mass-rearing diet and pig manure. Total G-values and p values were calculated using repeated G tests of goodness of fit. The abbreviation df refers to degrees of freedom.

Reared on	Day	Preferred		No choice	Total G	df	P value
		Manure	Mass-rearing diet				
Any	7	489	8	3	620.1262	10	8.56E-127
Manure	9	386	108	6	203.9185	10	2.46E-38
Manure	12	426	72	2	299.7459	10	1.76E-58
Manure	14	422	39	39	393.2478	10	2.57E-78
Manure	16	469	30	1	505.2718	10	3.30E-102
Mass-rearing diet	9	366	131	3	173.3952	10	5.49E-32
Mass-rearing diet	12	391	105	4	184.2149	10	3.12E-34
Mass-rearing diet	14	491	9	0	621.1553	10	5.15E-127
Mass-rearing diet	16	495	5	0	644.1276	10	6.12E-132

6.4 Discussion

The results of the choice tests demonstrate that BSFL strongly prefer pig manure over a mass-rearing diet, regardless of their age and the type of feed the larvae were reared on. While it was not the aim of this research to elucidate the underlying factors driving the preference of BSFL for manure, here we discuss three potential factors that could have influenced the observed preference: microbial diversity of the substrate, pH and chemosensory cues.

While pH is an important factor influencing the microbial community of a medium (Zhang et al., 2012), the microbial diversity of the substrate in which BSFL grow is a key factor for larval development. Microbes not only directly affect the capacity of the larval gut to digest certain compounds (Bruno et al., 2019; Gold et al., 2018), but those present in the substrate, if beneficial for the larvae, can supply bacterial-digested compounds that help to achieve higher bioconversion efficiencies in BSFL bioconversion systems (Jiang et al., 2019; Rehman et al., 2017; Xiao et al., 2018; Yu et al., 2011). Considering that the bacterial loads, richness and diversity in the mass-rearing diet were lower compared to chicken manure (Wynants et al., 2019), it is likely that these were also lower compared to pig manure. Future research efforts should focus on elucidating if BSFL are attracted to feeds with higher bacterial loads and/or higher bacterial diversity and if this attraction is related to the presence and abundance of bacterial-digested compounds. If BSFL larvae are attracted due to higher microbe-produced compounds, the observed preference would likely be a result of an attraction to manure rather than a disgust to the mass-rearing diet.

Besides the microbial diversity of the substrate, the pH of the substrate is another factor that needs to be explored for its potential to affect BSFL choices. While the pH of fresh pig manure used in the experiment ranged between 6 and 7, the pH of the mass-rearing diet feed ranged between 3 and 4 (this pH is intentionally used to stabilize the feed in the storage tanks). Even though BSFL are able to survive and grow on diets with a wide range of pH (pH 4–9.5) (Ma et al., 2018; Meneguz et al., 2018), it has been found that in a batch feeding system, BSFL grew heavier on slightly acidic (pH 6) or slightly basic conditions (pH 8–10). The exploratory video records confirmed that during the choice test BSFL moved actively within the container and tried both feeds (Video S1). After 1 hr, however, most larvae were found in the pig manure (Table 6.1). Hence, more research is needed to (a) understand whether BSFL choices are sensitive to pH differences in feeds but without the influence of confounding variables (e.g. feed type, moisture levels, microbial loads) and (b) whether the response to pH is mediated by disgust to acid substrates as this could have implications for insect welfare.

Finally, even though there are no studies on chemosensory cues for BSFL, research on other fly species shows that flies in both larval and adult stages are able to recognize different substances. *Drosophila* larvae, feeding on yeasts on rotting fruit substrates, for instance, are able to detect fruit compounds through 90% of its olfactory receptors (Dweck et al., 2018). Specific compounds isolated from fermented wheat bran were found to cause a physiological response in adult housefly antennae and to increase both substrate attractiveness and oviposition (Tang et al., 2016). Thus, considering that adult BSF flies naturally lay eggs in decomposing material, including animal manures (Newton et al., 2005; Oliveira et al., 2015), and that pig manure emits a variety of volatiles (Schiffman et al., 2001), future research should focus on finding if olfactory receptors in both BSFL and adult flies are stimulated when exposed to animal manures or any other decomposing material, and how olfactory receptor activation patterns relate to attraction.

Even though we evaluated the preference over a short period of time (1 hr) and it cannot be excluded that the larvae will switch between diets over a longer period as a mechanism to balance the intake of different nutrients (Rho and Lee, 2014; Simpson and Raubenheimer, 2012), the mechanisms behind the initial strong preference for manure reported here could be of particular interest to the insect industry and related stakeholders. On the one hand, if the preference is mainly caused by specific compounds in the manure to which the larvae are attracted, these compounds could be used for a diverse array of practical applications, including the design of alternative feeding strategies, the prevention of larval escapes from rearing crates and the establishment of alternative methods for harvesting larvae. On the other hand, if the preference for manure is predominantly driven by the physical (e.g. moisture, texture) and/or biochemical (e.g. pH) properties of the medium, these conditions, when possible, could be either mimicked if they increase preference or avoided in case they cause avoidance. In that way, producers could make their diets more appropriate for BSFL development. The latter is relevant for the emerging discussion on

insect welfare. While it is still uncertain if insects are sentient beings (organisms that feel, perceive and experience subjectively) (see discussion in Van Huis (2019)), the International Platform of Insects for Food and Feed (IPIFF) has adopted the precautionary principle and has called upon all insect producers to implement high standards of animal welfare and care to promote insect well-being (IPIFF, 2019). Our results give a first indication that BSFL have a feed preference. Research efforts are needed to evaluate whether discomfort, distress and the freedom to express normal behaviour are factors that drive the observed preference and whether they are related to animal welfare and productivity.

In all cases, before any implementation is done, potential trade-offs with food safety (i.e. increasing pH could foster the proliferation of pathogen bacteria in storage tanks), environment (i.e. increasing the pH could lead to higher ammonia emissions) and production costs should be first assessed to avoid undesirable consequences.

6.5 Supporting Information

Video S1. Time lapse of choice tests of at different larval ages. Video can be seen in the following repository: https://doi.org/10.4121/uuid:087b8581-71b6-4bac-914b-1807ebcf90b4

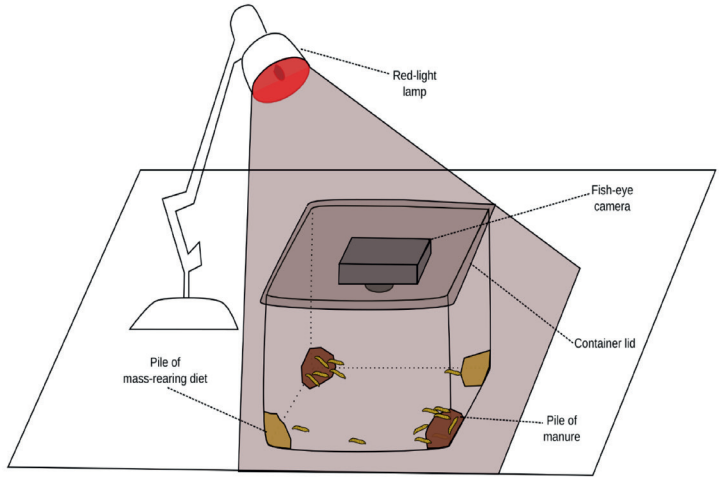

Figure S1. Schematic representation of the video records. Videos were recorded at different larval ages (7, 9, 12, 14 and 16 days-old) with a fish-eye camera (GoPro HERO4 silver edition). The camera was placed on the lid of a plastic container where the choice-tests took place. We used red light to be able to record the larvae movement.

Table S1. Raw data of the choice-test experiment. This table was too large and therefore was not included in this thesis. The table can be accessed through the online version of the publication at https://onlinelibrary.wiley.com/doi/10.1111/jen.12768

Table S2. Results of the log-linear models. Model: glm(formula = frequency ∼age + start_diet + replicate + pile, family = poisson (link="log"))

Model	Variables	Estimate	Std.error	z value	p value
Model for larvae found in manure piles	Intercept	2.640138	0.097223	27.155	<2e-16
	Age	0.037016	0.006684	5.538	3.07E-08
	Start_diet_Manure	-0.02322	0.034072	-0.681	0.4956
	Replicate	0.001126	0.005931	0.19	0.8495
	Pile M2	-0.08479	0.034101	-2.486	0.0129
Model for larvae found in mass-rearing diet piles	Intercept	4.32716	0.235219	18.396	<2e-16
	Age	-0.26163	0.018631	-14.043	<2e-16
	Start_diet_Manure	-0.00401	0.08953	-0.045	0.96429
	Replicate	0.005466	0.015586	0.351	0.72583
	Pile B2	-0.23758	0.090162	-2.635	0.00841

Chapter 7

Assessing the global warming potential of using black soldier fly larvae for food, feed and manure management

This chapter is based on:

A. Parodi, H. H. E. Van Zanten, J. Fledderus, T. Veldkamp, J. J. A. Van Loon, A. J. A. Aarnink, and I. J. M. De Boer (2022a). "Assessing the global warming potential of using black soldier fly larvae for food, feed and manure management". *(In preparation)*

Abstract

BSFL are envisioned to be fed with organic residual streams and be used as a sustainable source of food and feed. However, organic streams envisioned for BSFL rearing can have different valorization pathways in the circular economy. It is unclear whether using the organic residual streams for BSFL will result in environmental benefits compared to their current uses. The aim of this study was to quantify global warming potential (GWP) of BSFL reared on agri-food residues and pig manure using a life-cycle analysis, and to compare how BSFL bioconversion performs compared to competing valorization pathways in a Dutch context. The GWP of BSFL reared on agri-food residues and pig manure was determined mainly by emissions from frass and electricity use. We found that when reared on agri-food residues, BSFL can bring GHG benefits only if used as food but not if used as feed. Using agri-food residues directly as pig feed would lead to the production of finishing pigs with lower GWP. Furthermore, the GWP of manure bioconversion with BSFL was lower than for conventional slurry manure management, but higher than for manure liquid-solid separation. While further research on frass-related emissions is needed to improve GWP estimations, our results suggest that BSFL bioconversion could bring environmental benefits if properly utilized in the circular economy.

7.1 Introduction

The current global food system is a major driver for environmental degradation, including land use change and biodiversity loss (Benton et al., 2021; Leclère et al., 2020), alteration of nitrogen and phosphorus biogeochemical cycles (Leip et al., 2015; Uwizeye et al., 2020), climate change (Gerber et al., 2013; Tilman et al., 2011) and freshwater depletion (Wada et al., 2010). Environmental impacts occur in along the entire food system. Sourcing of resources, for example, results in deforestation and overfishing, whereas overfertilization of crops results in the emission of greenhouse gas (GHG), and pollution of air, water and soils. To avoid further environmental degradation and prevent irreversible environmental change, it has been suggested that food systems need to be restructured through a synergistic combination of measures that tackle food waste and losses, implement technological changes in agricultural practices, and promote dietary changes towards foods with a lower environmental impact (Campbell et al., 2017; Springmann et al., 2018; Van Zanten et al., 2018).

Insect farming is seen as an emerging agricultural sector with potential to contribute to the restructuration of food systems (Van Huis, 2020). Among farmed edible insects, the black soldier fly is one of the most promising species (Tomberlin and Van Huis, 2020). The potential of black soldier fly is rooted in the ability of its larvae (BSFL) to upcycle nutrients contained in a wide variety of residual organic streams (e.g., agri-food residues, manure, food waste) into food or feed (Bessa et al., 2020; Surendra et al., 2020; Van Raamsdonk et al., 2017). Hence, it is expected that BSFL fed on residual organic streams could not only reduce the wastage of resources, but also reduce the environmental impact of human diets if the insect biomass is used to replace resource-intensive animal-sourced foods (ASF) and feeds (e.g., soybean and fish meal). While there are still uncertainties regarding the environmental impacts associated with the use of farmed insects for food and feed (Berggren et al., 2019), different life cycle assessments (LCAs) showed that per mass unit of product or protein, BSFL fed on residual organic streams have generally a lower global warming potential (GWP) and land use than most ASF and resource-intensive feed ingredients such as soybean and fish meal (Parodi et al., 2018; Salomone et al., 2017; Smetana et al., 2015). It has also been reported that valorizing food waste via BSFL could reduce the GHG emissions compared to valorizing food waste via composting (Mertenat et al., 2019).

Although the current available evidence suggests that BSFL could contribute to improve the sustainability of food systems, broader assessments using different frameworks are needed to elucidate in which context BSFL as food and feed would be beneficial or detrimental for the environment. For instance, BSFL destined for feed are sometimes fed on agri-food residues that are suitable and commercialized as feed for conventional livestock, for example, pigs. While current LCA comparisons show that larvae fed on

these agri-food residues perform similarly or better than current feed ingredients (Bosch et al., 2019; Smetana et al., 2016), they do not show if the pigs fed with these BSFL would have a lower environmental impact compared to pigs fed the agri-food residues directly. Furthermore, no assessments have quantified what would be the environmental impact of using larvae fed on agri-food residues directly as human food compared to pigs fed with the same agri-food residues. Additionally, while there is increasing attention to the use of insect larvae to reduce the environmental impact of manure management by upgrading the nutrients as feed and fertilizers (Chen et al., 2019; Parodi et al., 2022b; Van Huis, 2019), no assessments have compared the performance of manure bioconversion with BSFL compared to current manure valorization practices (e.g., used as fertilizer).

In this study we first quantify the GWP of producing 1000 kg of dried BSFL reared on two residual organic streams, namely a mix of agri-food residues currently used to industrially produce BSFL in the Netherlands and pig manure, using an LCA. Then, we used the LCA outcomes to assess under which scenarios rearing BSFL with those residual organic streams can bring GHG benefits compared to the existing valorization pathways for the same streams in a Dutch context. Specifically, we assessed the following scenarios:

I) Producing human edible protein from BSFL fed on agri-food residues (S1) compared to pigs fed with a compound feed containing the same agri-food residues (S2).

II) Producing pigs fed with a compound feed containing agri-food residues (S3) compared to pigs fed with a compound feed containing BSFL reared on agri-food residues (S4).

III) Managing pig manure via BSFL bioconversion to produce feed and fertilizers (S5), compared to using manure as fertilizers via conventional slurry manure management (S6) and liquid-solid segregation of manure (S7).

7.2 Methods

7.2.1 LCA of BSFL reared on agri-food residues and pig manure

The goal of the LCA was to estimate the GWP emissions associated to the production of 1000 kg of dried BSFL when reared on two different residual organic streams. One of the streams was a mix of agri-food residues with 28% DM (i.e., potato leftovers, residual starch and dried distillers' grains) currently used to industrially produce BSFL in the Netherlands. The other stream was pig manure with 24% DM, which consisted mainly of solid feces that had contact with urine. The system boundary of the system studied is showed in Figure 7.1.

Larvae were assumed to be reared on agri-food residues in a production system with the same inputs, and process flows as the Dutch large-scale BSFL producer Bestico B.V. (see

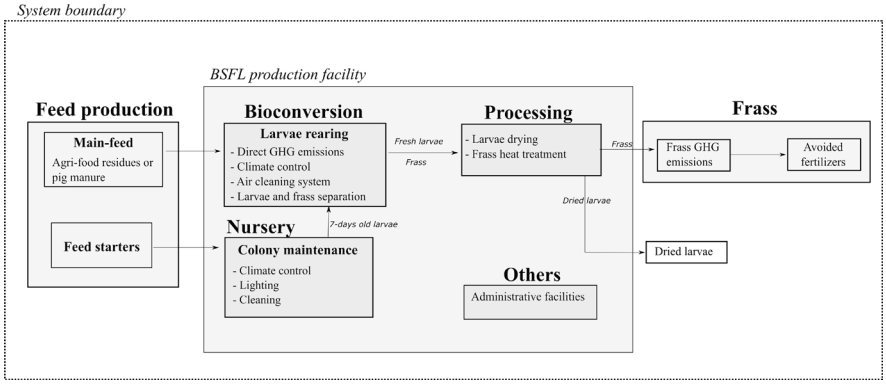

Figure 7.1: Chapters of this thesis and their connection with sustainable food systems themes.

Table S1 for the life cycle inventory, and Supplementary methods for a detailed description of the system). The estimation of GHG emissions of producing dried BSFL included emissions associated with feed production, colony maintenance, bioconversion process, processing (i.e., drying), and frass management (Figure 7.1). To avoid allocation, system expansion was used for frass, assuming that it replaced mineral fertilizers. The flows of biomass and nutrients are presented in Table 7.1.

For BSFL reared on manure, we assumed that larvae were produced in a similar production system as those fed on agri-food residues. However, as inputs in the life cycle inventory were given for 1000 kg of dried larvae fed on agri-food residues, and larval yields differ between rearing on agri-food residues and manure, we scaled most inputs by a factor of 2.62 (see Table S2 for values). This factor indicated how much additional substrate was needed to obtain 1000 kg of dried larvae when fed on manure (see Supplementary Methods for details on how the factor was calculated). The flows of biomass and nutrient flow data are presented in in Table 7.1.

Assumptions

The GWP associated with the production of the agri-food residues used to formulate the larvae diet were obtained from Feedprint and are presented in Table S3. The GWP associated with the production of manure were assumed to be zero. For both cases, transport-related impacts (e.g., from agri-food processing plants or pig farm to the insect farm) were excluded, as distances can vary depending on production plants and farms. All environmental impacts associated with the production of technosphere-inputs for BSFL production (e.g., gas, electricity, water, transportation) were obtained from Ecoinvent 3.8 (Wernet et al., 2016), and are presented in Table S4. Biogenic methane and nitrous oxide emissions produced during the bioconversion activity as well as the nutrient content

Table 7.1: Biomass flows for producing 1000 kg dry matter larvae with agri-food residues and pig manure.

Parameter	Unit	Agri-food residues	Manure
Substrate input	kg	12682[a]	33261[c]
Substrate DM	%	28.3[b]	24.9[c]
Fresh larvae yield	kg	2793[a]	3623[c]
Dry larvae yield	kg	1000	1000
Frass yield	kg	2793[a]	19311[c]
Frass DM	%	79.6[b]	32.5[c]
GHG emissions bioconversion	kg CO_2 eq	17 ± 8.6[b]	344± 43[c]
N in frass	kg	65[b]	151[c]
P in frass	kg	13[b]	132[c]
K in frass	kg	45[b]	163[c]
NH_4NO_3 (avoided)[d]	kg	116	267
P_2O_5 (avoided)[e]	kg	29	302
K_2O (avoided)[f]	kg	54	197

[a] Data provided by Dutch BSFL producer.

[b] Parodi et al. (2020a).

[c] Parodi et al. (2021).

[d] Calculated using a N fertilizer replacement value of 68% and a N to NH_4NO_3 conversion factor of 2.9.

[e] Calculated using a P to P_2O_5 conversion factor of 2.3.

[f] Calculated using a K to K_2O conversion factor of 1.2.

in larvae and frass, were obtained from Parodi et al. (2020a) and Parodi et al. (2021). Emissions from frass management are scarce in the literature, but we used those recently reported by Rummel et al. (2021), who reported that on average 5.38% of the N in frass directly applied to soils is lost as N_2O. It is worth to mention that frass can be composted prior to its use as a soil amendment, or directly applied to the soil without any prior composting. For our system, we assumed the later as it represented the measurements reported by Rummel et al. (2021). We assumed that frass replaced the mineral fertilizer ammonium nitrate (NH_4NO_3 for N, triple superphosphate for phosphorus pentoxide (P_2O_5), and potassium chloride for potassium oxide (K_2O). The impacts for the production of the fertilizers were obtained from Ecoinvent 3.8 (Wernet et al., 2016) (see Table S4). The quantity of mineral fertilizers substituted by frass was calculated using the specific frass nutrient contents as reported by Parodi et al. (2020a) and Parodi et al. (2021). As there are data gaps regarding the fertilizer replacement values of BSFL frass relative to mineral fertilizers, we assumed that the N, P and K fertilizer replacement values were equal to

those used for pig manure by De Vries et al. (2013) (i.e., 62% for N, and 100% for P and K).

7.2.2 Comparison of BSFL bioconversion versus existing valorization pathways

We used a life cycle analysis to compare the GHG of using organic residual streams to produce BSFL as food and feed versus existing valorization pathways to utilize those streams. For the agri-food residues, the valorization pathway was their direct use as pig feed while for manure as fertilizer. Comparisons were done using different functional units. For the comparison of using agri-food residues to produce BSFL or pig meat destined for food, the functional unit was 1 kg of human edible protein (Figure 7.2). For the comparison of pigs fed with BSFL and pigs fed with agri-food residues directly, the functional unit was 1 finishing pig (Figure 7.2). Lastly, for the comparison of manure bioconversion with BSFL and current manure management methods, the functional unit was 1000 kg of slurry manure (Figure 7.2).

7.2.3 Scenario definition

S1 – Human edible protein from BSFL

Scenario 1 consisted of producing BSFL reared on 1000 kg of agri-food residues and estimating the GWP per kg of human-edible protein. The impacts associated with BSFL production were obtained from the LCA previously described (section 7.2.1). To express the GWP per kg of human edible protein, we assumed that dried BSFL were 100% edible. This is a valid assumption given that the whole larvae are processed. The assumed protein content of the larvae on a DM basis was 32.4%. This value was calculated by multiplying the total N content in BSFL reported by Parodi et al. (2020a) with the nitrogen-to-protein factor of 4.76 reported by Janssen et al. (2017). The used nitrogen-to-protein factor allowed the exclusion of non-protein nitrogen such as chitin, from the estimation of the larval protein content.

S2 – Human edible protein from pigs fed on agri-food residues

Scenario 2 consisted of producing pigs fed with the same mixture of agri-food residues used to produce BSFL in S1 and estimating the GWP per kg of human-edible protein. However, unlike BSFL producers who rear BSFL fed solely with the mix of agri-food residues, under current commercial conditions, Dutch feed manufacturers and finishing pig producers would mix the agri-food residues with other ingredients to have a nutritionally balanced pig feed for optimal pig growth. To make a comparison that represented the existing pig production

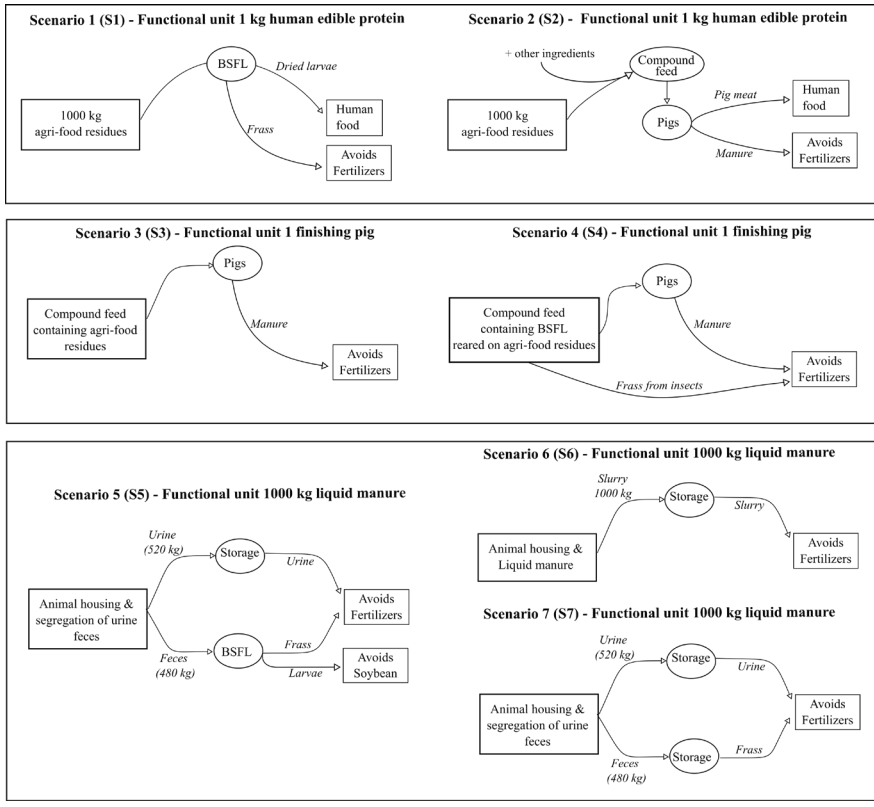

Figure 7.2: Schematic representation of the scenarios. Scenarios within the same rectangle were compared to each other.

systems, we assumed that pigs were fed with a nutritionally balanced compound feed that contained the mix of agri-food residues and additional ingredients (Table 7.2 and Table S6). The estimation of the GWP of pig production accounted for the impacts associated with piglet production, feed production, enteric fermentation, housing, manure management, manure application and avoidance of mineral fertilizers. All assumptions regarding pig growth, feeds and impacts of the different production phases of pig production (e.g., piglet, manure, etc.) are explained in the Supplementary Methods.

For this scenario we considered that 746 kg of compound feed (Table 7.2) were used as input, as this is the amount of compound feed needed to equal the quantity of agri-food residues assumed for S1 (i.e., 1000 kg at 28% DM). As we assumed that each finishing pig consumed 226 kg of compound feed, the 746 kg of compound feed led to the production of 3.3 pigs (see Supplementary Methods for details). We estimated the total content of protein contained in the 3.3 pigs assuming that 60% of the body mass of a finishing pig

Table 7.2: Compound pig diets for scenarios S2, S3 and S4. The first two columns show the inclusion of each ingredient (%) on a fresh matter basis. The last column shows the quantity (kg) of each ingredient assumed for S2, in order to include 1000 kg of agri-food residues at 28% DM.

Ingredient	Diet for S2 and S3 (% of each ingredient)	Diet for S4 (% of each ingredient)	Diet for S2 (kg of each ingredient)
Wheat	26.01	18.821	194
Barley		40	0
Maize	10		75
Wheat bran	15	15	112
Rapeseed meal	2.61	2	19
Sunflowerseed meal	3.88	5	29
Beet molasses	2	3	15
Limestone	1.28	0.46	10
Lysin	0.48	0.28	4
Salt	0.23	0.51	2
Premix vitamins and trace elements	0.2	0.2	1
Threonin	0.12		1
Methionin	0.09		1
Tryptophan	0.01		0
Agri-food residues (mix at 88% DM)*	38.07		284
Dried larvae (reared on agri-food residues)		14.75	
Total	100	100	746

*The 284 kg of agri-food residues at 88% DM are equivalent to 1000 kg at 28% DM. The water contained in the agri-food residues was assumed to contribute to the pigs' intake of fresh water.

was edible, 72% of the edible part consisted of DM and that the edible part had a protein content of 40.7% on a DM basis (Parodi et al., 2018). The GWP was expressed then per kg of human edible protein.

S3 – Pigs fed with agri-food residues

Scenario 3 consisted of producing one finishing pig fed with a nutritionally balanced compound feed containing agri-food residues (Table 7.2, Table S6). This scenario was similar to S2, with the exception that the functional unit was not 1 kg of protein, but 1 finishing pig. Thus, we assumed that all impacts associated to the different phases of pig production (i.e., piglet production, feed production, enteric fermentation, housing, manure management, manure application and avoidance of mineral fertilizers) were the same to those estimated for S2.

S4 – Pigs fed on BSFL reared on agri-food residues

Scenario 4 consisted of producing one finishing pig fed with a nutritionally balanced compound feed containing BSFL produced from agri-food residues (Table 7.2 and Table S8). BSFL were assumed to be produced in the same production system described for scenario S1. Given that the compound feed had a similar nutrient composition as that of scenario S2 and S3, pigs were assumed to have the same inputs, growth performance and outputs. Thus, while the GWP associated with feed production was different than in S2 and S3, the GWP of all other stages (i.e., piglet production, enteric fermentation, housing, manure management and manure application) were assumed to be equal.

S5 – Manure bioconversion with BSFL

Scenario 5 consisted of feeding pig manure to BSFL and using the obtained larval biomass as animal feed and the frass as fertilizer. To make the scenarios S5, S6 and S7 comparable, the starting point for all of them was 1000 kg of liquid manure. However, we assumed that BSFL consumed manure from a farm equipped with liquid-solid V-belt segregation system (see De Vries et al. (2013) for details). In such farms, 1000 kg of liquid manure would be equivalent to 480 kg of solids composed mainly of feces and 520 kg of liquids composed mainly of urine (De Vries et al., 2013). Thus, for every 1000 kg of liquid manure, we assumed that BSFL consumed 480 kg of solids, and the 520 kg of liquids were used as fertilizers. The processes associated with the production of BSFL reared on manure were the same as those described in section 7.2.1. For 480 kg of manure solids, the dried larval yield was 14.4 kg and the fresh frass yield was 279 kg. The obtained larval biomass was assumed to substitute soybean meal based on crude protein content. The nutrient content of the frass, and solid and liquid fraction of segregated manure is presented in Table S9.

S6 – Conventional slurry manure management

Scenario 6 consisted of conventional liquid manure management in the Netherlands, which involves the storage of mixed urine and feces (i.e., called slurry) under the slatted floor of the farm for months, and the subsequent application of slurry in agricultural soils via injection. The nutrient composition of manure is presented in Table S9. Manure-related impacts included CH_4, direct and indirect N_2O from manure management (i.e., storage) and N_2O emissions for manure application to agricultural soils via injection. All emissions were estimated using IPPC guidelines and country-specific data when possible (Table S10). The applied manure into agricultural soils was assumed to substitute NH_4NO_3 for N, triple superphosphate for P_2O_5, and potassium chloride for K_2O. The impacts associated with their production were obtained from Ecoinvent 3.8 (Wernet et al., 2016)(see Table S4).

The amount of mineral fertilizers substituted were calculated following the same approach as De Vries et al. (2013), and assuming that after storage losses, the remaining slurry manure applied to the soil contained 6.99 kg N, 4.7 kg of P_2O_5 and 6.8 kg of K_2O, and that the N, P and K fertilizer replacement values were 62%, 100% and 100%, respectively.

S7 – Manure management via liquid-solid segregation

Scenario 7 consisted of segregated liquid and solid fractions of manure obtained from a farm equipped with a V-belt system. Both fractions were assumed to be stored, and then used as fertilizers, via injection for the liquids, and surface application for the solids. Similarly to S4, we assumed that 1000 kg of slurry manure would be equivalent to 480 kg of solids, and 520 kg of liquids (De Vries et al., 2013). The nutrient composition of each fraction is presented in Table S9. Impacts for both solid and liquid fractions included CH_4 and direct and indirect N_2O emissions during storage, and N_2O after injection. For the liquid fraction, nitrogen losses, direct and indirect N_2O emission factors from storage, and N_2O emissions from urine injection to agricultural soils, were assumed to be equal to those reported for slurry manure (see Table S10). For the nitrogen losses, and direct and indirect N_2O emissions during the storage of the solid fraction we used emission factors specific for solid manure storage (see Table S10). Similarly, N_2O emission factors from solid manure application to the soil were different than for slurry manure under the assumption that feces were applied via surface spreading. In both cases, we assumed that slurry manure and its solid and liquid fraction substituted the same type of fertilizers as described in S5. Emissions resulting from manure transport from the farm to the field were excluded, as for all the other treatments.

7.2.4 Uncertainty analysis

To quantify the uncertainty for the outcome for each scenario we used Monte Carlo simulations. Simulations were made based on the variability of the input parameters for which we were able to obtain a distribution (i.e., number of data points, mean and standard deviation). The number of Monte Carlo simulations for each scenario was based on the advice by Heijungs (2020). The upper limit to the number simulations was based on the minimum number of data points (i.e., n) for the parameters with a distribution in each of the scenarios.

Table 7.3: Global Warming Potential (GWP) to produce 1000 kg of dry matter BSFL reared on agri-food residues and pig manure (mean ± std. deviation). The avoided emissions due to the use of frass as a fertilizer could reduce the GWP by 6% when BSFL are reared on agri-food residues, and 10% when reared on manure.

Impacts	GWP (kg CO_2 eq) when BSFL are reared on	
	Agri-food residues	Manure
Direct impact	3737 ± 16	7042 ± 24
Avoided impact (fertilizers)	-211	-679
Total	3526 ± 16	6363 ± 24

7.3 Results and discussion

7.3.1 GWP of BSFL production

The GWP emissions to produce 1000 kg of DM BSFL reared on agri-food residues and pig manure is presented in Table 7.3. The GWP is shown for the direct emissions occurring during the production of BSFL and avoided emissions due to the assumed substitution of chemical fertilizers with frass. For BSFL reared on agri-food residues, 91% of the total GHG emissions were linked to frass, electricity and feed production (Figure 7.3A). Within these three processes, the direct GHG originating from frass application to agricultural soils was the main contributor to the GWP with 44%, followed by electricity use with 27% and feed production with 20%. The direct GHG emissions occurring during BSFL rearing and which were measured by Parodi et al. (2020a) (chapter 3) were 17 ± 9 kg CO_2 eq and only accounted for the 0.5% of the overall GWP. These results confirmed that for this specific rearing substrate, measuring the direct GHG emissions during BSFL rearing is not crucial to improve GWP estimations. Instead, the generation and collection of better data for frass emissions, electricity and feed production should be prioritized.

For BSFL reared on manure, frass and electricity use also contributed most to the GWP (Figure 7.3B). In this case, however, the impacts associated with feed production were lower than when reared on agri-food residues as the only feed-related impact considered was wheat bran used to feed the starter larvae. In addition, the direct GHG emissions during BSFL rearing on manure measured by (Parodi et al., 2021)(chapter 4) were 345 ± 43 kg CO_2 eq and accounted for 5% of the overall GWP (Figure 7.3B). Although this is a much higher contribution compared to the 0.5% of BSFL reared on agri-food residues, it again shows that direct GHG emissions are not a major contributor of GHG from BSFL production.

After accounting for differences in rearing conditions, inputs and assumptions, our estimations fall in the range of other LCA values. For instance, Mertenat et al. (2019)

reported 690 kg CO_2 eq per tonne of fresh larvae in an Indonesian system with no heating system and no impacts allocated to the substrate as the larvae were reared on food waste. The mean impact per tonne of fresh larvae calculated in this study was 1151 kg CO_2 eq, but if heating and feed impacts are excluded to mimic the conditions of the Indonesian system assessed by Mertenat et al. (2019), this results in a GWP of 720 kg CO_2 eq per tonne of fresh larvae. Smetana et al. (2019) reported 1157 kg CO_2 eq per tonne of fresh larvae reared in an industrial Dutch system. Their value is nearly equal to our estimation, however, given the limited information provided, it is not possible to do a detailed analysis of the similarities between the two systems. Guo et al. (2021) reported 2904 kg CO_2 eq per tonne of dried larvae reared on food waste mixed with rice hulls in a Chinese industrial production system. Considering that Guo et al. (2021) did not account for feed related impacts because the larvae were fed mainly on food waste, our estimations are similar if the 741 kg CO_2 eq associated with feed production are excluded (Figure 7.3A). Bosch et al. (2019) reported a GWP of 3 kg CO_2 eq per kg of protein sourced from BSFL reared on agri-food residues in the Netherlands. Considering that Bosch et al. (2019) did not account for any emissions resulting from frass, our estimations give 3.6 kg per kg of protein when excluding all frass emissions. Finally, (Salomone et al., 2017) reported a GWP of 1020 kg CO_2 eq per tonne of dried BSFL reared on food waste in an Italian pilot plant. In that study, the impacts associated with feed production and frass were not included. Even correcting for these two processes (i.e., feed and frass emissions), our impacts are still higher by 830 kg CO_2 eq, suggesting that other variables such as feed conversion ratio, or electricity use differed and likely caused the gap.

Mitigation potential and the effect of alternative assumptions

Frass and electricity use were the main contributors to GHG emissions of BSFL production (Figure 7.3). To increase the understanding of how the overall GWP is affected with methodological assumptions for frass-related emissions and electricity impacts, we estimated the GWP using different input parameters for these two processes. In addition to the assumed 5.38 ± 2.8 % of the N contained in frass lost as N_2O when frass was directly applied to agricultural soils (Rummel et al., 2021), the overall GWP was estimated using two different values for frass-related emissions. One was based on the latest IPCC emission factor for N_2O emissions resulting from the application of organic amendments to agricultural soils (i.e., 1% of N lost as N_2O) (Hergoualc'h et al., 2019). The other was a fixed emission factor of 116 kg CO_2 eq per tonne of frass which has been used in previous LCA studies on BSFL production (Guo et al., 2021; Mertenat et al., 2019). This value corresponds to the GHG emissions originating from the composting of 1 tonne of household and municipal waste as recommended by the United Nations Framework Convention on Climate Change (UNFCC, 2017). Unlike the IPCC emission factor which is based on the N content of the applied amendment, the UNFCC emission factor is just based on

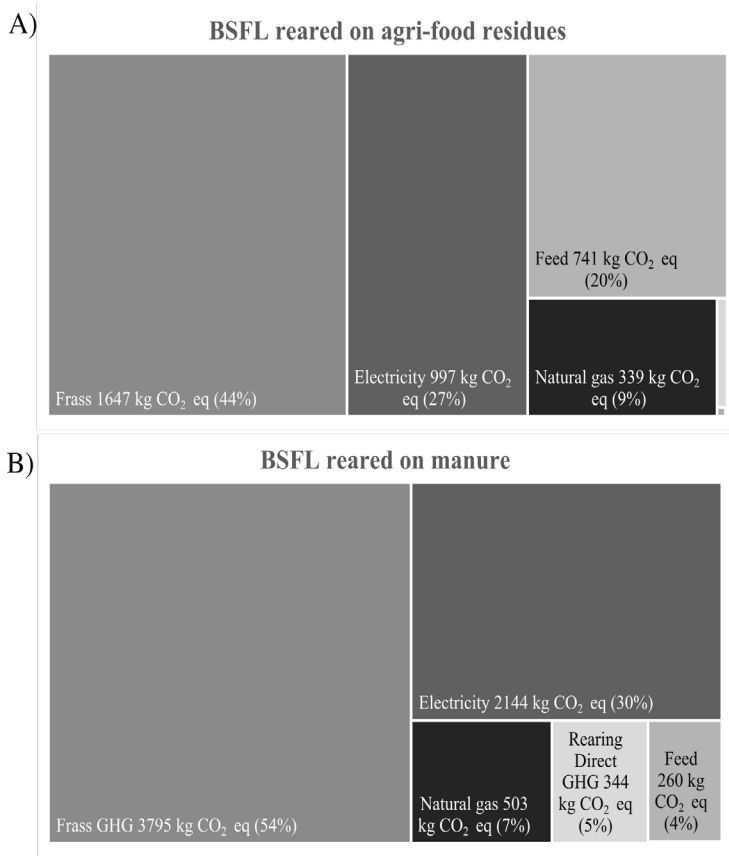

Figure 7.3: Contribution of the different processes to the overall GWP. The relative contribution of each process is given in parenthesis. A) For BSFL reared on agri-food residues. B) For BSFL reared on manure.

mass. We found that the overall GWP varies largely depending on assumptions made for frass-related emissions. Relative to our original assumption, when BSFL was reared on food residues the GWP was reduced by 40% with the IPCC and UNFCC emission factors (Figure **??**). When BSFL was reared on pig manure, the GWP was reduced by 25% and 50% with the UNFCC and IPCC emission factors, respectively. These results show the large influence that assumptions made for frass-related emissions can have in the GWP estimations and the outcomes of the comparisons made with other products and systems. It is therefore crucial to perform more analytical measurements on the GHG emissions associated to frass. Such studies should account for the different management methods currently practiced (i.e., storage, composting and direct application).

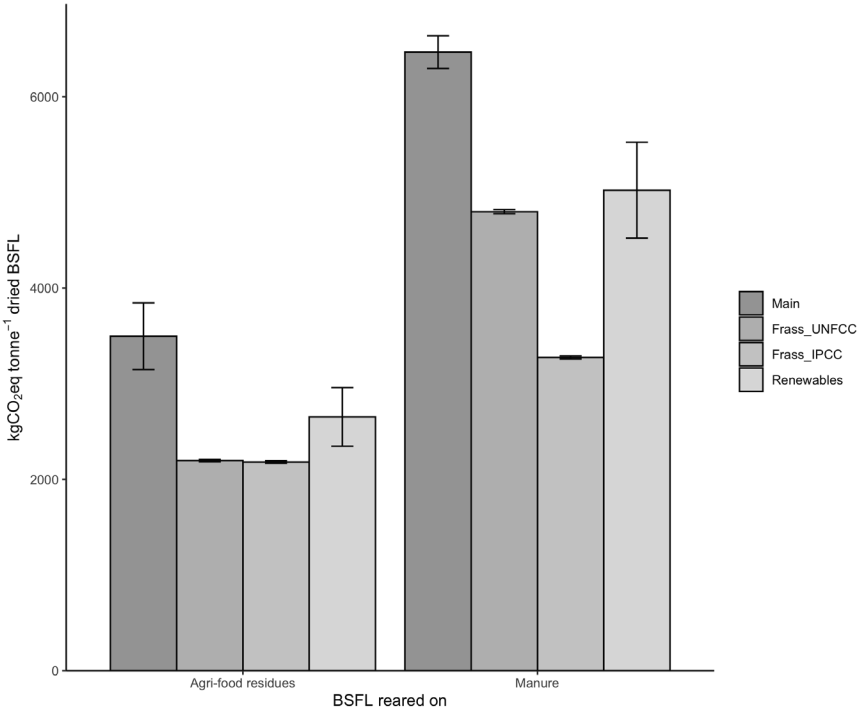

Figure 7.4: GWP of BSFL reared on agri-food residues and pig manure, using different input parameters for frass-related emissions and electricity production. The "Main" category assumes that 5.38 ± 2.8 % of the N contained in frass was lost as N_2O when used directly as a soil amendment. This value was used for Table 7.2 and Figure 7.3.

For electricity, our original assumption was that it was provided by the Dutch mix energy grid, which includes different sources of energy (natural gas, coal, wind, etc). When assuming that all electricity use was supplied by photovoltaic energy in the Netherlands, the GWP was reduced by 25% (Figure 7.4). This mitigation potential of renewable energies has also been reported for other BSFL production systems in the Netherlands (Smetana et al., 2019). The mitigation potential of renewable energies in our system is substantial, as electricity was used for different processes (e.g., climate control, drying). However, lower mitigation potentials might be attained with renewable energies in tropical production systems where no or less heating is needed, or in energy-efficient insect production systems that utilize residual heat, or that aim to use the larval metabolic heat for climate control.

7.3.2 Comparisons with other valorization pathways for residual streams

Human edible protein from BSFL (S1) versus pigs (S2)

The GWP of the production of 1 kg of human edible protein derived from BSFL fed on agri-food residues (S1) was 10.2 ± 1 kg CO_2 eq. For pigs (S2), the GWP was 25.8 ± 0.4 kg CO_2 eq with conventional slurry manure management and 19.3 ± 0.06 kg CO_2 eq with manure management via liquid-solid manure separation (Figure 7.5A). These results suggest that under current production systems, BSFL fed on agri-food residues can bring GHG benefits when used as source of human food instead of pig meat. While for BSFL most GHG emissions originated from frass, electricity use and feed production (Figure 7.3A), for the assumed pig production systems with conventional manure management and liquid-solid manure separation, piglet production accounted for 23 and 32% of the GWP, feed production for 33 and 45% and manure management for 34 and 9%, respectively. Feed production had a substantial contribution to the overall GWP of pigs, as observed in previous studies (De Vries and De Boer, 2010; Van Zanten et al., 2015a). It would have been ideal to estimate the GWP of pigs solely fed on agri-food residues for a fairer comparison with BSFL which was only fed with agri-food residues. However, this was not possible due to the lack of real data and the still unusual practice to use such feeding strategies under commercial conditions. Although circular pig diets made of only agri-food residues will reduce the GWP per kg of protein derived from pigs and reduce the gap with BSFL, it is unlikely that they will perform better (see Figure 7.6). BSFL has the unquestionable advantage to be a food with 100% edible mass, while the edible mass of pigs is 60% (Parodi et al., 2018). Furthermore, BSFL have higher reproduction rates and shorter maturation periods, which leads to a lower use of resources compared to those demanded by sows and piglets.

Pigs fed with agri-food residues (S3) versus pigs fed with BSFL (S4)

Pigs fed with a compound feed containing agri-food residues (S3) had a lower GWP than pigs fed with a compound feed containing BSFL reared on agri-food residues (S4) (Figure 5B). The GWP for the pigs of S3 was 29 to 36% lower than for the pigs of S4, depending on the type of manure management assumed. These results suggest that using BSFL reared on agri-food residues as pig feed will not bring GHG benefits, and that the agri-food residues should be directly used as feed. In this specific comparison, the difference in GWP among the two scenarios was fully related to the different impacts of the pig diets, as all other parameters (i.e., piglet production, enteric fermentation, manure management and housing) were kept equal. While the assumed GWP per kg of feed for the S3 scenario was 0.4 kg CO_2 eq, for S4 it was 1 kg CO_2 eq, with BSFL contributing 52% to the overall impact. Even compared with the 0.5 to 0.8 kg CO_2 eq per kg of concentrated feed for high-input finishing production systems (Van Der Werf et al., 2005), the compound feed

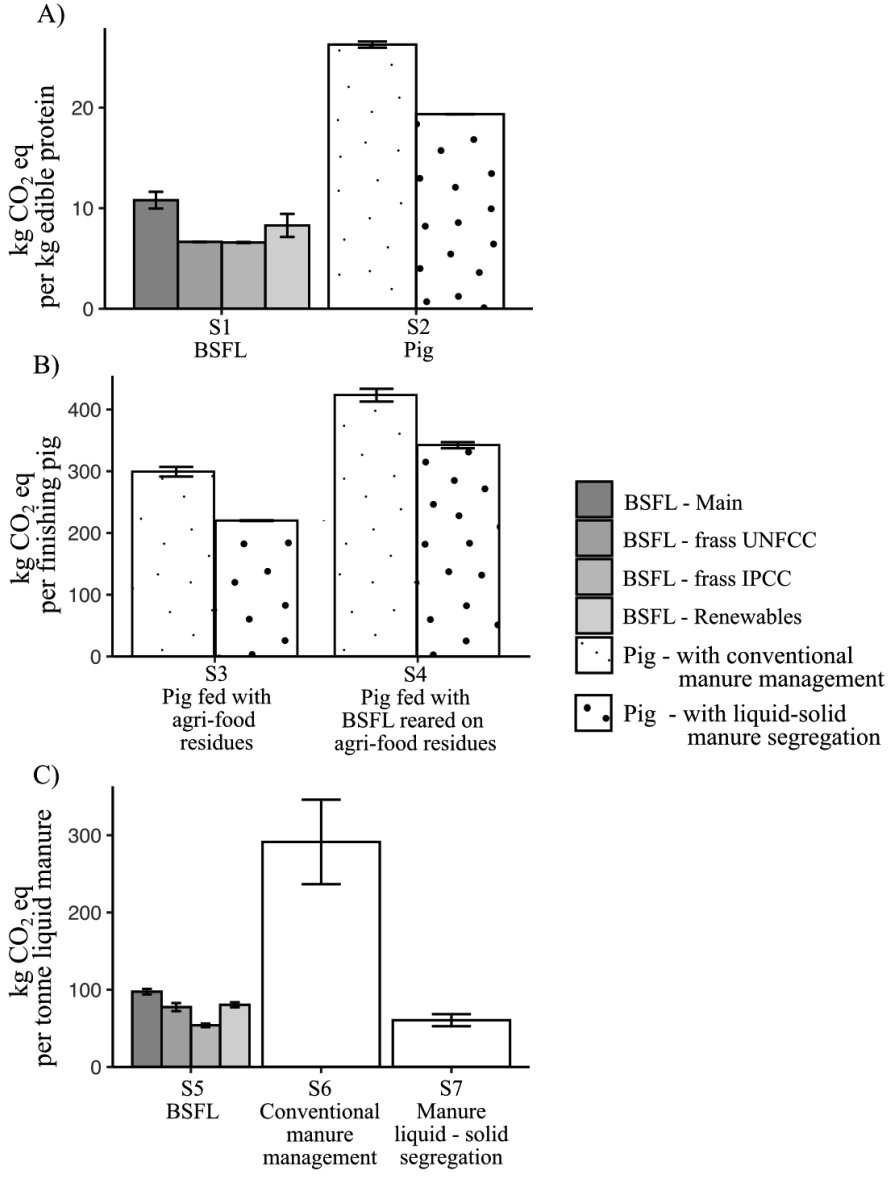

Figure 7.5: GWP of the use of BSFL versus different valorization pathways. A) GWP when BSFL fed on agri-food residues are used as food. B) GWP when BSFL fed on agri-food residues are used as feed. C) GWP of manure bioconversion with BSFL.

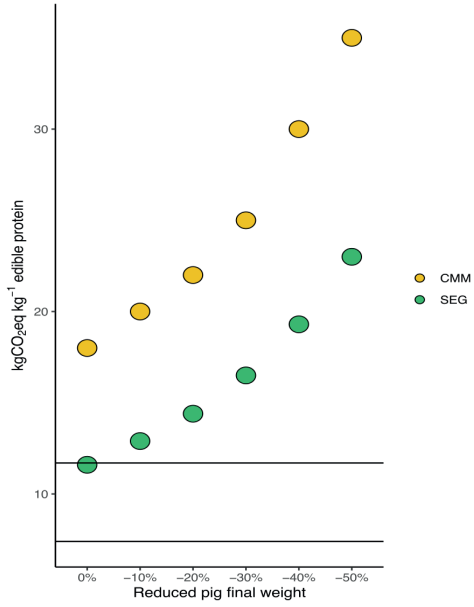

Figure 7.6: GWP per kg of human edible protein sourced from pigs with two manure management systems. The GWP was estimated assuming that GHG emissions per kg of pig feed were equal to food residues (i.e., 0.06 kg CO_2 eq) and for different final weights of finishing pigs (i.e., 0% = pig of 116 kg, while -50% = pig of 58 kg). The lines and shaded area show the GWP for BSFL reared on agri-food residues as described for scenario S1.

containing BSFL reared on agri-food residues had higher GWP. This demonstrates that even with low-inclusion rates of 14% (see Table 1), the use of BSFL reared on agri-food residues as feed can increase the GWP of livestock diets.

Manure bioconversion with BSFL (S5) versus conventional manure management (S6) and liquid-solid manure segregation (S7)

Manure management via BSFL bioconversion had a substantially lower GWP than conventional slurry manure management, but a higher GWP than liquid solid manure segregation (Figure 7.5C). The GWP associated with the management of 1000 kg of liquid manure via conventional slurry manure management was 291 ± 54 kg CO_2 eq, for manure bioconversion it was 97 ± 4 kg CO_2 eq and for liquid solid manure separation it was 61 ± 8 kg CO_2 eq. While the use of renewable energies for BSFL production could reduce the GWP to 80 kg CO_2 eq, this is not enough to equal liquid solid manure segregation. It should be noticed, however, that if we would have assumed the IPCC emission factor to estimate the emissions resulting from frass, our conclusion would have been that manure bioconversion

with BSFL could offer GHG benefits compared to liquid-solid manure segregation. It is therefore key to experimentally quantify frass-related emissions for more robust LCA estimations. Our estimations for slurry manure management and liquid solid separation are in line with previous estimates. For instance, De Vries et al. (2013) estimated that the GWP of 1000 kg of liquid manure was 320 kg CO_2 eq for conventional slurry manure management, and 56 kg CO_2 eq for liquid-solid manure segregation. While for conventional slurry manure management nearly 90% of the GWP was associated with CH_4 emissions during manure storage, for liquid-solid manure separation, CH_4 emissions were reduced by 10-fold. For manure bioconversion with BSFL there are no studies available for comparison, but most impacts were associated with N_2O emissions resulting from frass and fossil CO_2 due to electricity use (Figure 7.3). The manure bioconversion system in the current study was modelled by correcting the input data of a production system in which BSFL were reared on agri-food residues (see section 7.2.1). However, it is key to elaborate LCAs using data from real BSFL production systems in which BSFL are reared on manure, and such assessments should also include other relevant impact categories such as N losses.

Despite the large GHG benefits that manure bioconversion with BSFL and solid-liquid manure separation can have compared to conventional slurry manure management, the implementation of such systems will require drastic changes in the existing animal housing infrastructure to allow the separated collection of manure and feces. Manure solid-liquid separation systems exist already for decades (Kempen et al., 2003; Sheppard et al., 1994) and have been used successfully in the poultry industry for years (Koger et al., 2014). However, so far, they have not been extensively adopted for pig and dairy production, likely due to the initial costs. Overcoming this infrastructure barrier is therefore crucial to lower the GWP of manure management with methods such as manure bioconversion or liquid-solid manure separation.

7.4 Conclusions

The GWP of BSFL reared on agri-food residues is mainly determined by the GHG emissions originating from frass and electricity use. So far, the GHG emissions associated with frass have only been quantified by one study and should be further explored given its substantial contribution to the overall GWP. Furthermore, the use of BSFL reared on agri-food residues is only environmentally beneficial if used as food but not if used as feed. Thus, as long as BSFL reared on agri-food residues are not used for human consumption, the agri-food residues should be used directly as livestock feed. Finally, we found that manure bioconversion with BSFL had a GWP three times lower than conventional slurry manure management, but slightly higher than liquid-solid manure separation. Although further research on frass-related GHG emissions are needed for refined estimations, our results suggest that BSFL bioconversion could bring environmental benefits if properly

utilized in the circular economy. However, that will only be realized if consumer barriers for BSFL consumption and infrastructure barriers for manure segregation are successfully overcome.

7.5 Supplementary Information

The supporting information of this chapter includes:

Appendix A - Supplementary Methods

Appendix B - Supplementary Tables (S1-S10)

Appendix A - Supplementary Methods

Production system for BSFL reared on agri-food residues

The LCA of BSFL reared on agri-food residues was based on the production system of the Dutch BSFL producer Bestico B.V. In their system, just-hatched larvae are fed with wheat bran and water for 7 days, and subsequently reared for 7 additional days in a rearing substrate consisting of a mix of three different agri-food residues (i.e., potato leftovers, residual starch and dried distillers' grains) with a dry matter content of 28%. The specific proportion of each ingredient, the number of larvae per unit of diet, and the rearing time have been defined to have a profitable production system. Larvae are then separated from the frass (i.e., mix of larval excreta, exuviae and unconsumed feed) at 14-days old, and dried in a heater until most water is lost. The frass also passes through a heat treatment, but only for 25 minutes at 117 °C. For every 1000 kg of agri-food residues, 79 kg of dried larvae, and 220 of frass are obtained.

Scaling inputs for BSFL reared on manure

To estimate the environmental impact of producing BSFL reared on pig manure, we re-scaled some of the inputs given in the life cycle inventory of BSFL reared on agri-food residues (Table S1). In Table S1, all inputs are given per 1000 kg of dried BSFL when reared on agri-food residues, but as bioconversion efficiencies and final yields are different between BSFL reared on agri-food residues and manure, some of the inputs had to be re-scaled. We based the re-scaling in the extra amount of fresh substrate needed to obtain 1000 kg of dried larvae. For such re-scaling we did the following calculation:

$$ScalingFactor = \frac{S_{ma}}{S_{fr}}$$

Where, S_{ma} is the amount of fresh manure needed for 1000 kg of dried BSFL and S_{fr} is the amount of fresh agri-food residues needed for 1000 kg of dried BSFL.

$$\frac{33261}{12682} = 2.62$$

A way to interpret this factor is that if 200 rearing crates filled with agri-food residues were needed to obtain 1000 kg larvae, when BSFL are reared on manure 524 crates will be needed to obtain the same quantity of larvae. As the number of crates is increased by 2.62 times, inputs such as electricity, gas, feed for starters are also increased by 2.62 times. The re-scaling factor was applied to most processes, except larval drying, frass emissions and direct GHG emissions during rearing. Larval drying was not re-scaled given that the impact is independent of the type of substrate used.

Assumptions for pig production (S2, S3 and S4)

Pig compound feeds for S2, S3 and S4 were formulated using commercial linear programming software that aimed at minimizing the cost price of the pig diet while meeting nutrient requirements of the pig (see Table S5 for the list of nutrient constraints). The nutrient compositions of the feeds are presented in Table S6. For scenario S4 the model was set to have maximum inclusion of dried BSFL equal to 100% (see Table S5 for the list of constraints). The price of BSFL was set to a minimum to maximize its inclusion in the feed.

The estimations were based assuming that a pig of 70 days old and 23.6 kg body weight consumed 226 kg of the compound feed in 110 days, and reached a final body mass of 226 kg at 180 days old. These parameters were based on Van Zanten et al. (2015a), who modelled the growth of finishing pigs fed with a compound feed with the same net energy (NE) and standardized ileal digestible (SID) lysin content as assumed by us. The overall amount of compound feed needed to include 1000 kg of agri-food residues at 28% DM was 746 kg (Table 7.2). As we assumed that each finishing pig consumed 226 kg compound feed, an availability of 746 kg compound feed led to the production of 3.3 finishing pigs. The environmental impact of the ingredients that made the compound feeds were obtained from Feedprint and presented in Table S7.

We used fixed values for the impacts associated with piglet production (i.e., 19.3 kg CO_2 eq per finishing pig) and housing (i.e., 19.3 kg CO_2 eq per finishing pig). These were obtained from Van Zanten et al. (2015a). Enteric fermentation emissions per finishing pig (i.e., 73 kg CO_2 eq per finishing pig) were estimated by adjusting the default emission factor of 1.5 kg of CH_4 per finishing pig per year (Gavrilova et al., 2019) to 110 days.

Impacts associated with manure management were based on conventional slurry manure management in the Netherlands and liquid and solid manure segregation. Impacts for both solid and liquid fractions included CH_4 and direct and indirect N_2O emissions during storage, and N_2O after injection. We assumed that the production of manure was 1100 kg

of slurry manure per pig space per year (RIVM, 2013). Thus, considering that three pigs are produced in a pig space per year, we assumed a slurry manure production of 367 per finishing pig.

Supplementary Tables

Table S1. Life-cycle inventory for BSFL production on agri-food residues by a Dutch BSFL producer. All values are given per tonne of dried BSFL.

Stage	Process	Input	Unit	Value	Dispersion (%)	Source
Starters feed	Feed	Bran	kg	223	5	Producer
Starters feed	Feed	water	kg	578	5	Producer
Main feed	Feed	Ingredient 1	kg	5573	5	Producer
Main feed	Feed	Ingredient 2	kg	5573	5	Producer
Main feed	Feed	Ingredient 3	kg	1520	5	Producer
Nursery and colony	Starters feed cleaning	Water	kg	0.06	50	Producer
Nursery and colony	Lighting	Electricity	kWh	87	20	Producer
Nursery and colony	Climate control	Electricity	kWh	237	20	Producer
Nursery and colony	Climate control	Natural gas	m^3	70	20	Producer
Nursery and colony	Cleaning	Water	kg	0.04	50	Producer
Bioconversion	Climate control	Electricity	kWh	570	20	Producer
Bioconversion	Climate control	Natural gas	m^3	244	20	Producer
Bioconversion	Direct emissions	CH_4 and N_2O	kg CO_2 eq	17	50	Parodi et al. 2020
Bioconversion	Air clearing system	Water	kg	4078	20	Producer
Bioconversion	Air clearing system	Electricity	kWh	285	20	Producer
Bioconversion	Air clearing system	Sulfuric acid	kg	14	20	Producer
Processing	Frass heat treatment	Natural gas	m^3	22	50	Producer
Processing	Frass storage	Area	m^2	0.003		Producer
Processing	Frass output	Frass	kg	2793		Producer
Processing	Killing and drying	Electricity	kWh	500	30	Producer
Processing	Killing and drying	Natural gas	kWh	800	30	Producer
Others	Administrativ facilities	Electricity	kWh	45	50	Producer
Others	Administrativ facilities	Natural gas	m^3	2	50	Producer
Others	Factory area	Area	m^2	3		Producer

Table S2. Life cycle inventory for BSFL reared on manure. All values are given per tonne of dried BSFL. Most values were obtained by re-calculating the inputs in Table S1 using the scaling of 2.61. An explanation of the re-scaling factor is given in the Supplementary Methods.

Stage	Process	Input	Unit	Value per tonne dried larvae	Dispersion (%)	Soure
Starters feed	Feed	Bran	kg	585.47	5	Calculated
Starters feed	Feed	water	kg	1514.92	5	Calculated
Nursery and colony	Starters feed cleaning	Water	kg	0.15	50	Calculated
Nursery and colony	Lighting	Electricity	kWh	227.60	20	Calculated
Nursery and colony	Climate control	Electricity	kWh	622.07	20	Calculated
Nursery and colony	Climate control	Natural gas	m^3	182.96	20	Calculated
Nursery and colony	Cleaning	Water	kg	0.11	50	Calculated
Bioconversion	Climate control	Electricity	kWh	1494.42	20	Calculated
Bioconversion	Climate control	Natural gas	m^3	640.36	20	Calculated
Bioconversion	Direct emissions	CH_4 and N_2O	kg CO_2 eq	344.00	12.5	Calculated
Bioconversion	Air clearing system	Water	kg	10684.92	20	Parodi et al 2021
Bioconversion	Air clearing system	Electricity	kWh	747.21	20	Calculated
Bioconversion	Air clearing system	Sulfuric acid	kg	36.59	20	Calculated
Processing	Frass heat treatment	Natural gas	m^3	58.55	50	Calculated
Processing	Frass storage	Area	m^2	0.01	0	Calculated
Processing	Killing and drying	Electricity	kWh	500.00	30	Producer
Processing	Killing and drying	Natural gas	kWh	800.00	30	Producer
Processing	Frass output	Frass	kg	19311.00	48	Parodi et al 2021
Others	Administrativ facilities	Electricity	kWh	117.09	50	Calculated
Others	Administrativ facilities	Natural gas	m^3	5.85	50	Calculated
Others	Factory area	Area	m^2	2.79	0	Calculated

Table S3. Environmental impact of each of the agri-food residues used to formulate the insect diet.

Input	Environmental impact per kg product	
	GWP unit (kg CO_2 eq)	Land use (m^2)
Bran	0.444	0.58
Potato peels	0.006	0
DB-blend[a]	0.037	0.037
DDGS	0.264	0

[a] DB-blend is a feed ingredient that consists of a mixture of pre-fried potato cuttings (37%), wheat starch (37%) and water (26%). The environmental impacts of the ingredients were obtained from Feedprint (i.e., potato cuttings and wheat starch) and Ecoinvent 3.8

Table S4. Environmental impact of technosphere inputs obtained from Ecoinvent 3.8. RER refers to Europe.

Location	Name	Unit	kg CO_2 eq	m^2
NL	market for natural gas, high pressure	cubic meter	0.297779581	0.003232
NL	market for electricity, high voltage	kilowatt hour	0.578109723	0.034126
RER	sulfuric acid production	kilogram	0.018663986	0.003494
Europe without SW	tap water production, conventional treatment	kilogram	0.000253926	0.0000303
RER	transport, freight, lorry 16-32 metric ton, EURO6	ton kilometer	0.162555291	0.021537
RER	market for transport, freight, lorry >32 metric ton, EURO6	ton kilometer	0.086776106	0.020789
RER	market for transport, freight, lorry 3.5-7.5 metric ton, EURO6	ton kilometer	0.509221923	0.047219
RER	ammonium nitrate production	kilogram	1.40117067	0.06646121
RER	triple superphosphate production	kilogram	0.64077876	0.51733421
RER	potassium chloride production	kilogram	0.56758909	0.13342515
RER	soybean meal — market for soybean meal — RoW — kilogram — ecoinventcutoff38	kilogram	3.0091	

Table S5. Parameters used to formulate the pig diets for scenarios S2, S4 and S4.

Nutrient	unit	MIN	MAX
moisture	g/kg		130
crude protein	g/kg	135	
Ca	g/kg	5.2	6.5
P	g/kg	4	5.5
digestible P	g/kg	2.1	
Na	g/kg	1.8	2.5
starch	g/kg	340	
met sid/lys sid		0.33	
m+c sid/lys sid		0.62	
thr sid/lys sid		0.64	
trp sid/lys sid		0.18	
val sid/lys sid		0.66	
sid Lysin	g/kg	7.24	
NE-value	MJ/kg	9.5	9.51

Table S6. Nutrient composition of pig diets for scenarios S2, S3 and S4.

Nutrient	Unit	Compound feed for S2 and S3 (including agri-food residues)	Compound feed for S4 (including insects)
moisture	g/kg	111.37	109
dry matter			
crude ash	g/kg	54.76	49.9
crude fiber	g/kg	48.15	66.9
crude protein	g/kg	155.24	183.7
Lysin	g/kg	8.48	8.96
Ca	g/kg	6.5	6.5
P	g/kg	4.44	5.1
digestible P	g/kg	2.51	2.4
Na	g/kg	2.5	2.5
K	g/kg	11.11	8.6
C18:2	g/kg	10.12	14.7
Ca/vP		2.59	2.7
starch	g/kg	340	340
crude fat	g/kg	40.84	65.1
met sid/lys sid	ratio	0.37	0.38
m+c sid/lys sid	ratio	0.62	0.66
thr sid/lys sid	ratio	0.64	0.69
trp sid/lys sid	ratio	0.18	0.25
iso sid/lys sid	ratio	0.5	0.72
val sid/lys sid	ratio	0.66	0.97
sid Lysin	g/kg	7.24	7.24
NE-value	MJ/kg	9.5	9.51

Table S7. Environmental impacts of ingredients used for the compound pig feeds obtained from Feedprint. Impacts are expressed per kg of each ingredient.

Ingredient	GHG (g CO_2 eq)	Land use (m^2)	Source
Wheat	586.0	1.2	Feedprint
Triticale / Rye	658.0	1.9	Feedprint
Barley	557.0	1.4	Feedprint
Maize	667.0	1.5	Feedprint
Wheat bran	512.0	0.6	Feedprint
Rapeseed meal	800.0	2.2	Feedprint
Sunflowerseed meal	810.0	3.2	Feedprint
Beet molasses	143.8	0.2	Feedprint
Biscuit meal	12.0	0.0	Feedprint
Limestone	32.2	0.0	Feedprint
Lysin	2735.7	2.1	Feedprint
Salt	100.3	0.0	Feedprint
Premix vitamins and trace elements	395.3	0.2	Feedprint
Threonin	2730.0	2.1	Feedprint
Methionin	2334.6	0.0	Feedprint
Tryptophan	5471.4	4.3	Feedprint
DBblend + Potato peals + DDGS	50.6	0.0	Calculated
Larvae - food residues - full fat	3143	0.4	Own calculation

Table S8. Nutrient content and sources used for the assumed chemical composition of dried black soldier fly larvae fed on agri-food residues. These values were used to formulate the pig diets for scenario S3. Except moisture, all values, are presented on dry matter basis.

Nutrient	Units	Value	Source	Notes
Cr.Protein	g/kg	433.7	Parodi et al., (2020)	
Cr.Ash	g/kg	82.5873303	Crosbie et al. (2020); Enterra, (2021) Liu et al. (2017); Spranghers et al. (2016)	For Spranghers et al (2016) we selected the values for larvae fed on chicken feed and vegetable waste
Moisture	g/kg	83	Crosbie et al. (2020) and Enterra (2021)	
Cr.Fiber	g/kg	86.199095	Crosbie et al. (2020); Enterra, (2021)	
Cr.Fat (HCl)	g/kg	264	Parodi et al. (2020)	
Stach (total)	g/kg	9.04977376	Crosbie et al. (2020)	
K	g/kg	12.9	Parodi et al. (2020)	
Cl	g/kg	1.65546689	Biasato et al. (2019)	
P	g/kg	8.9	(Parodi et al., 2020)	
Na	g/kg	0.73718326	Crosbie et al. (2020); Liu et al. (2017); Spranghers et al. (2016)	For Spranghers et al (2016) we selected the values for larvae fed on chicken feed and vegetable waste
Ca	g/kg	23.5898824	(Crosbie et al. (2020); Enterra, (2021); Liu et al. (2017); Spranghers et al. (2016)	For Spranghers et al (2016) we selected the values for larvae fed on chicken feed and vegetable waste
dig.P	g/kg	6.23	It was assumed that 70% of P was digestible	
NE pigs	MJ/kg	13.8042336	Calculated	((11.7 * 0.85 * crude protein) + (35.74*0.9*crude fat) + (14.14*1*Starch) + (12.75*1*Sugar) + (9.75*0.7*NSP))/1000
Lysin	g/kg	22.4707692	Crosbie et al. (2020); Enterra (2021); Liu et al. (2017); Spranghers et al. (2016)	For Spranghers et al (2016) we selected the values for larvae fed on chicken feed and vegetable waste
Threonine	g/kg	18.1800905	Crosbie et al. (2020); Enterra, (2021); Liu et al. (2017)	
Tryptophan	g/kg	5	Enterra, (2021)	
Isoleucine	g/kg	16.525	Enterra, (2021); Liu et al. (2017); Spranghers et al. (2016)	For Spranghers et al (2016) we selected the values for larvae fed on chicken feed and vegetable waste
Methionine + Cysteine	g/kg	10.4		
Valine	g/kg	23.1202112	Crosbie et al. (2020); Enterra, (2021); Liu et al. (2017)	
Methionine	g/kg	10.2168326	Crosbie et al. (2020); Enterra (2021); Liu et al. (2017); Spranghers et al. (2016)	
sidLYS	g/kg	16.8530769		
sidTHR	g/kg	13.6350679	It was assumed that 75% of each aa was digestible	(Amino acid * 0.75)
sidTRY	g/kg	3.75		
sidILE	g/kg	12.39375		
sidM+C	g/kg	7.8		
sidVAL	g/kg	17.3401584		
sidMET	g/kg	7.66262443		
Sugar	g/kg	0		
NSP	g/kg	127.662896	Calculated	(1000 − crude protein − crude ash − moisture − crude fat − starch − sugars)
C18:2	g/kg	32.2563	Enterra, (2021); Liu et al. (2017); Spranghers et al. (2016)	
C18:3	g/kg	3.892675	Enterra, (2021); Liu et al. (2017); Spranghers et al. (2016)	
Lauric acid	g/kg	186.399475	Enterra, (2021); Liu et al. (2017); Spranghers et al. (2016)	

Table S9. Nutrient content of different manure and frass types. Slurry manure content was obtained from De Vries et al. (2013). N, P, K values for the solid fraction of manure and the two types of frass were obtained from Parodi et al. (2021). Values for urine were calculated by difference, considering that 1000 kg of slurry manure contained 480 kg of feces and 520 kg of urine.

Item	DM (kg/t)	OM (kg/t)	N (kg/t)	P_2O_5 (kg/t)	K_2O (kg/t)	NH_4NO_3 (kg/t)	OM corrected
1000 kg slurry manure	111.0	80.9	10.6	4.7	6.8	18.78	110
1000 kg urine (kg)	2.1	7.0	12.9	0	7.1	22.82	9.46
1000 kg feces	229.0	161.0	8.1	9.9	6.3	14.39	218.91
1000 kg frass from manure-fed larvae	325	-	8	16	10	13.82	-
1000 kg frass from agri-food residues-fed larvae	796	-	23	10	19	41.46	-

Table S10. Parameters used for the estimation of GHG associated to manure management and manure application. Values are given for slurry manure, solid manure (feces) and frass.

Item	Stage	Parameter	Unit	Value	std dev	n	Source	Note
Slurry manure	-	Manure production	kg pig place yr	1110.00	-		RIVM (2013)	The latest GHG inventory in the Netherlands (2021) refers to this report.
	-	N content in excreted manure	kg finishing pig	11.60	-		Bikker et al. (2019)	Value reported in Table 4 for slurry manure of finishing pigs.
	-	Volatile solids in slurry manure	kg ton manure	110.00	-		Groenestein et al. (2016)	Value reported in page 12
	Storage	Maximum CH_4 production capacity	m^3 CH_4 per kg volatile solid	0.31	0.1	23	Groenestein et al. (2016)	Value given in Table 2
	Storage	Methane conversion factor	% of maximum CH_4 production capacity	38.00	20	5	Groenestein et al. (2016)	Average given in table 4. We calculated the std. deviation of the selected studies under the category "Gemiddelde van overig"
	Storage	N losses (as NH3 and NO_x)	% of N in excreted manure	34.00	-		Bikker et al. (2019)	Value reported in Table 4 for slurry manure of finishing pigs. No variation
	Storage	Emission factor for direct N_2O emissions	kg N_2O-N per kg of nitrogen excreted	0.000	-		(Gavrilova et al., 2019)	Value reported in Table 10.21 (page 90) for liquid/slurry without natural crust cover
	Storage	Emission factor for indirect N_2O (due to N volatilization)	kg N_2O-N per kg of N volatized	0.010	0.008	8	Hergoualc'h et al. (2019)	Value reported in Table 11.3 (page 26). Std. deviation was calculated based on uncertainty ranges (value-min uncertainty) N_2O emissions from leaching were omitted given that during manure storage the fraction of N leached is 0% according to IPCC 2019 (Table 10.22). As 8 studies were cited we assumed that n=8
	Field application	Emission factor for N_2O emissions	% of N lost as N_2O per N suplied	1.10	1.06	28	Velthof and Mosquera (2011)	Value reported in Table 5. Standard deviation was calculated based in the std error (0.2) an number of studies (n=28)
Pig urine	Storage	N losses (as NH3 and NO_x)	% of N in excreted manure	34.00	-		Bikker et al. (2019)	Assumed to be the same as for slurry manure
	Storage	Emission factor for direct N_2O emissions	kg N_2O-N per kg of nitrogen excreted	0.00	-		Gavrilova et al. (2019)	Assumed to be the same as for slurry manure
	Storage	Emission factor for indirect N_2O (due to volatilization)	kg N_2O-N per kg of nitrogen volatized	0.010	0.008	8	Hergoualc'h et al. (2019)	Assumed to be the same as for slurry manure
	Application	Emission factor for N_2O emissions	% of N lost as N_2O per N suplied	1.10			Velthof and Mosquera (2011)	Assumed to be the same as for slurry manure
Pig feces	-	Volatile solids in slurry manure	kg ton manure	110			Groenestein et al. (2016)	Assumed to be equal to slurry manure and that 100% of the volatile solids were in the solid fraction
	Storage	Maximum CH_4 production capacity	m^3 CH_4 per kg volatile solid	0.31	0.1	23	Groenestein et al. (2016)	Value obtained from Table 5
	Storage	Methane conversion factor	% of maximum CH_4 production capacity	2.00	-		Groenestein et al. (2016)	Value obtained from Table 6. No variation given
	Storage	N losses (as NH_3 and NO_x)	% of N in excreted manure	45.00	-		Bikker et al. (2019)	Value reported in Table 4 for solid feces
	Storage	Emission factor for direct N_2O emissions	kg N_2O per kg N excreted	0.01	-		(Gavrilova et al., 2019)	Value reported in Table 10.21 for solid storage
	Storage	Emission factor for indirect N_2O (due to N volatilization)	kg N_2O-N per kg of N volatized	0.01	0.008	8	Hergoualc'h et al. (2019)	Value reported in Table 11.3 (page 26). We selected the aggregated default value (EF4).
	Application	Emission factor for N_2O emissions	% of N lost as N_2O per N suplied	0.40		11	Calculated from Velthof and Mosquera (2011)	Value reported in Table 5. Standard deviation was calculated based in the std error (0.2) an number of studies (n=11)
Frass	Application	Emission factor for N_2O emissions	% of N lost as N_2O per N suplied	5.38	2.82	12		Average of all treatments

Chapter 8

Principles for the responsible use of farmed insects as livestock feed

This chapter is based on:

A. Parodi*, A. F. Ipema*, H. H. E. Van Zanten, J. E. Bolhuis, J. J. A. Van Loon, and I. J. M. De Boer (2022). "Principles for the responsible use of farmed insects as livestock feed". *Submitted as a comment paper.*

* Authors contributed equally to this chapter.

Insect farming is a new and rapidly expanding agricultural sector with the potential to make livestock production systems, and therefore human diets, more sustainable. However, to realize this, a responsible and effective use of farmed insects as livestock feed is needed.

Main

It is widely acknowledged that food systems need to be transformed to achieve the Sustainable Development Goals (SDGs) (Loboguerrero et al., 2020). Production systems of conventional livestock (including farm animals and fish) are among the systems in dire need for transformation. Livestock plays a key role for global food security, however, intensive livestock production is currently not congruent with many of the SDGs (e.g., SDG 12 Responsible consumption and production) as it harms the environment and impairs animal welfare. In the context of making conventional livestock production more sustainable, the use of farmed insects as a source of feed for livestock is gaining traction (Dicke, 2018). The idea is to feed farmed insects with the massive quantities of by-products, food losses and waste (here referred to as organic waste streams) we produce in current food systems, and to use the resulting insect biomass as livestock feed. The insect biomass, rich in valuable proteins, fats, vitamins, and minerals, is expected to replace conventional feed ingredients that have high environmental footprints, such as soybean and fishmeal. Using farmed insects as feed not only has the potential to contribute to sustainable and circular livestock production, but could also improve livestock welfare by facilitating natural foraging behaviour and promoting good health (e.g., by reducing broiler leg problems)(Ipema et al., 2020).

However, just feeding insects with organic waste streams and then feeding these insects to livestock will not by default solve the environmental and welfare problems of current livestock production systems. Here, we present seven key principles to guide the responsible use of farmed insects as livestock feed. These principles are based on our view of the current developments in the sector integrated with the outcomes of a four-year research project aimed at holistic assessment of the effects of farmed insects on the environmental, welfare and productivity dimensions of conventional livestock production systems.

0. Prioritize waste reduction

The environmental benefits of using insects are founded on their potential to turn organic waste streams, such as manure, food industry waste or household waste, into valuable feed or food. However, from an environmental point of view it is always more efficient to avoid the generation of these waste streams. Preventing food waste is out of the scope of insect producers, but should be the first priority for policy makers, governmental agencies, the

private sector and civil society organizations engaged with rerouting food systems towards a sustainable and food secure future (Muscat et al., 2021). It is important to focus efforts on creating and enabling an environment for food systems that generate less waste, where insects and other circular interventions are used to upcycle only those nutrients contained in unavoidable waste streams.

1. Avoid unnecessary feed competition between farmed insects and conventional livestock

In the circular economy, organic residual streams such as crop residues, food waste, livestock manure and food by-products can be used for different purposes such as food, feed, fertilizers, biomaterials, and pharmaceuticals. From these purposes, basic human needs such as food, and by extension feed, are generally considered most important (Muscat et al., 2021), and therefore recovering and reusing waste streams into edible biomass should be prioritized over other uses. In this context, it is key to select the optimal pathways to utilize the streams to maximize the production of human edible biomass with the lowest environmental impact. While conventional livestock such as ruminants consume different crop residues, and monogastric animals such as pigs have been traditionally fed on by-products from the food industry and swill (Ermgassen et al., 2016; Mottet et al., 2017), now farmed insects are becoming a new pathway to do so. If we use farmed insects as feed, we should not feed them with residual streams that can be directly fed to livestock as this will create unnecessary competition for feedstocks and higher environmental footprints. For instance, feeding pigs with insects fed on food-by-products that are also consumed by pigs (e.g., dried distillers' grains, potato leftovers) can increase the GHG emissions and land use per kg of pork by more than 30% compared to feeding these by-products directly to pigs (Chapter 7. TThus, it is more responsible and effective to concentrate efforts on rearing insects destined for feed with streams non-edible for livestock, such as food waste and manure. Still, in contexts where the available residual streams are not suitable for the local livestock or where streams are disposed directly into landfills, insects could play a role in upcycling nutrients and making them suitable for local and overseas livestock consumption.

2. Ensure farmed insects are safe as livestock feed

Many of the abundant streams non-edible to conventional livestock that could be consumed by insects, such as manure and food waste, pose safety risks for livestock and humans. Potential hazards in low-value rearing substrates include heavy metals, (myco)toxins, pathogenic microorganisms, pesticides, parasites, and in case of manure, veterinary drugs and hormones (Imathiu, 2020; Van der Fels-Klerx et al., 2018). While in some cases it is known that particular components can accumulate in the insect body mass when present

in high concentrations in the feed substrate (e.g., heavy metals) (Van der Fels-Klerx et al., 2018), in other cases insects can break down or excrete the unsafe components (i.e., mycotoxins) resulting in concentrations below maximum residue limits or even below detection limits (Bosch et al., 2017). For some hazards (e.g., parasites) the accumulation potential is still unknown and must be investigated to assess the safety risks of using insects as livestock feed. To overcome safety risks, we must invest in developing strategies for waste segregation (i.e., separation of plant and animal waste), pre-treatment of residual streams (e.g., pasteurization, fermentation) and larval processing to eliminate hazards (e.g., fasting, drying, boiling, blanching, acidification, fermentation, and/or freezing). These strategies should be evaluated for unwanted consequences for the environment (e.g., high energy use) and insect welfare. If feed safety cannot be guaranteed, then it is important to evaluate the environmental consequences of using the insect biomass for applications outside the food chain (e.g., pet food, biofuels, and pharmaceuticals) versus not producing insects and using residual waste streams for other purposes in the bioeconomy (e.g., biomaterials, bioenergy, fertilizers).

3. Revise legislation based on experimental evidence

For an efficient use of insects as feed in which unnecessary competition with conventional livestock is avoided, we need an enabling legislation landscape. In regions such as the European Union (EU), strict regulatory frameworks for food and feed safety exist, and therefore farmed insects can often only be fed with the same feedstocks currently allowed for conventional livestock. Residual streams demonstrated to be suitable to feed insects, such as food waste and manure, are not allowed as insect feed due to potential feed safety concerns in the EU, but in other world regions they are. Without changing regulatory frameworks in the EU and other regions with similar restrictions for use of insect as livestock feed, insect producers will be constrained and motivated to use feedstocks that inevitably promote feed competition between insects and conventional livestock. We must therefore revise and update legislation frameworks based on the experimental evidence available, in order to promote responsible insect farming for feed purposes.

4. Optimize performance of insects fed low-value streams

Farming insects fed on low-value streams such as vegetable waste and manure tends to have lower yields and performance parameters compared to those fed on nutrient-rich and more digestible streams (Spranghers et al., 2016). Driven by the current legislation and motivated by the market profits, many large-scale insect producers are attaining high insect yields by feeding insects with customised diets made of a mix of different livestock-edible side streams that complement each other nutritionally (e.g., dried distillers' grains, by-products from the potato industry). Although this would not be a problem

if insects are destined for food, it is a problem when insects are destined for feed, as it promotes feed competition with conventional livestock and the non-responsible use of insects. To avoid this, it is key that the insect production sector and relevant stakeholders (e.g., regulatory agencies) also focus on optimizing low-quality feeds to attain higher yields. Examples of strategies to optimally use low-quality streams include pre-treatment and co-treatment methods (i.e., physical, chemical and biological) (Peguero et al., 2022), combining streams to overcome nutrient deficiencies in pure streams, and genetically improving the performance of larvae and their gut microbiome on specific feeds (Fowles and Nansen, 2019).

5. Balance livestock productivity, welfare, and environmental goals of insect feeding strategies

Alongside aiming for positive environmental consequences of feeding insects to conventional livestock, we should also aim to improve livestock productivity and/or welfare by feeding them insects, though there will be trade-offs between these goals. Important variables determining which goals are favoured include insect species, rearing method, degree of processing (e.g., alive, dead, or as meal) and level of inclusion in livestock diets. Generally, livestock productivity can be increased by including low levels (¡10%) of processed insects (i.e., meal), while higher inclusion levels or providing whole insects maintains or decreases productivity (Ipema et al., 2020; Moula et al., 2018). Concerning livestock welfare, many insects contain immunomodulatory components such as lauric acid and antimicrobial peptides which can benefit livestock health, but their presence and availability depends on insect rearing substrates and processing methods (Dorper et al., 2020). In contrast to processed insects, whole insects (i.e., fresh or dried without further processing) have been found to stimulate natural behaviours and activity in poultry (e.g., pecking and scratching litter) and pigs (e.g., rooting), which has clear health and welfare benefits. For instance, low activity levels of broilers cause leg problems such as lameness and foot lesions, and by promoting active foraging behaviour providing whole insects reduced broiler leg problems, and these welfare benefits increased when the larvae inclusion level increased from 5 to 10% (Ipema et al., 2022; Ipema et al., 2020). Similarly, pigs that received larvae spent less time biting other pigs (Ipema et al., 2021).

Currently, including low levels of processed insects is often favoured over whole insects as this is considered safer and can increase livestock productivity. However, as it is expected that insect production systems and feeding strategies will be optimized over time, in the future feeding higher amounts of whole insects to livestock could increase the benefits for livestock welfare and the environment. Throughout time, insect feeding strategies should be continuously re-evaluated and adjusted to balance livestock productivity, welfare, and environmental goals of livestock production systems, in order to avoid unexpected trade-offs and to optimally benefit from using farmed insects as livestock feed.

6. Account for insect welfare

There is increasing evidence that many insect species, including those farmed, possess cognitive and emotional abilities and sentience (Lambert et al., 2021). For example, black soldier fly larvae will actively move towards a preferred substrate when they have the possibility to choose (Parodi et al., 2020b), showing they possess agency. Ethicians argue that, just as for conventional livestock species, the intrinsic value of insects must be acknowledged and warranted in commercial production systems (Baracchi and Baciadonna, 2020). In these systems, insect welfare can be compromised by for example suboptimal stocking densities and feed quality, starvation, and during killing (e.g., by boiling, freezing, or being fed live to conventional livestock). As insects must be reared and killed to benefit conventional livestock welfare, there is a clear trade-off between insect and livestock welfare that should be evaluated. In this evaluation, we must also consider the use of insects directly as food, as this could reduce the overall impact on insect welfare compared to when used as feed. While some organizations are promoting the adoption of good practices for the ethical production of insects (IPIFF, 2019), a change in ethical perspectives on insect production is required to guide and create legislation that assures good insect welfare in both commercial rearing systems and research.

To conclude, farmed insects have the potential to improve the environmental sustainability and welfare of livestock production systems, but for that to happen an enabling environment is needed. Insects must be optimally fed with residual streams unsuitable for livestock, be safe to use as feed, and, if proven safe, legislation needs to be revised and an optimal balance must be found between livestock productivity, livestock and insect welfare, and environmental sustainability. For a responsible use of insects as feed, the principles we outlined should be adhered to now that the sector is starting to grow. Mistakes made in the past (i.e., food-feed competition, compromised livestock welfare) should not be repeated in this emerging agricultural sector.

Chapter 9

General Discussion

Main

Food systems need to be reconfigured towards more sustainable futures to safeguard planetary health and food security (Loboguerrero et al., 2020). A cornerstone for such reconfiguration is the reduction of the environmental impact associated with the production and consumption of animal-source food (ASF), such as beef, pork and chicken meat. Among the different upcoming innovations to reduce the environmental impact of ASF, the use of farmed insects is gaining attention. Insect farming for food, feed and waste management purposes is rapidly expanding worldwide, and although the expectations to improve the sustainability of food systems are high, there is a need for broad-scale sustainability assessments to quantify their real transformative potential. **The aim of this thesis was to provide a better understanding of the contribution of farmed insects to a sustainable food system when reared on residual organic streams**. This was achieved by using experimental and modelling approaches. Most of the evidence generated in this thesis focus on the black soldier fly larvae (BSFL) as they are the main farmed insect worldwide.

This chapter is divided in two main sections (Figure 9.1). In the first section "Synthesis" I integrate the main findings of this thesis into one storyline. I explain how the specific research objectives were achieved with the findings of each of the chapters, and discuss the limitations encountered and the prospects for future research. In the second section "Outlook" I address the societal relevance of the topics covered in this thesis and discuss the societal and institutional changes that are needed to make farmed insects a building block for sustainable food systems.

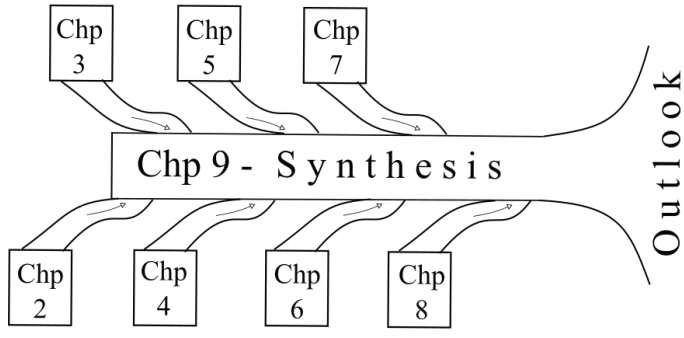

Figure 9.1: Graphical representation of the architecture and objective of chapter 9.

9.1 Synthesis

9.1.1 Farmed insects and their potential for healthy and sustainable diets

Given the increasing awareness of the environmental benefits that edible insects could have for food systems if adopted as a source of food and feed, the first research objective of this thesis was to compare the nutrient composition and environmental footprints of farmed insects with other foods. **These comparisons, covered in chapter 2, were based on the available literature and showed that farmed insects contained the complete array of essential macro- and micronutrients, and that these nutrients were present in similar or higher concentrations than in ASF. Moreover, it was found that farmed insects could provide most of the macro- and micronutrients with a substantially lower land use than most ASF, and lower or similar GHG emissions to the best performing ASF**. The assessment confirmed that farmed insects could not only provide protein, but also a range of other valuable macro- and micronutrients in an environmentally sustainable way, especially when reared on residual organic streams. These promising results revealed that it was worth to further investigate the potential of farmed insects fed on residual organic streams by overcoming knowledge gaps and exploring new lines of research. And so we did, and we specifically focused on BSFL reared on agri-food residues and pig manure. Agri-food residues were selected for being already a stream used in western Europe to industrially produce BSFL. Pig manure was selected for being an abundant stream considered an environmental burden in livestock-dense regions, and for which BSFL bioconversion is being envisioned as a circular strategy to upgrade the nutrients contained in manure as feed.

9.1.2 Overcoming knowledge gaps – quantification of the nutrient flows occurring during BSFL rearing

The second objective of this thesis was to quantify the nutrient flows during BSFL reared on agri-food residues and manure. Although previous life cycle assessments (LCAs) assessed the environmental impact of BSFL production, these studies either did not account for the direct gaseous emissions occurring during larvae rearing (Salomone et al., 2017; Smetana et al., 2019) or used data generated for other species and reared on substrates different than the ones evaluated (Smetana et al., 2016). As it was not fully clear whether methane (CH_4) emissions during BSFL rearing would reach similar levels as those observed for enteric fermentation in cows, or if nitrous oxide (N_2O) and ammonia (NH_3) emissions would be increased or decreased compared to the emissions naturally released from manure, the accurate quantification of these nutrient flows was needed for better environmental impact assessment of BSFL production.

In chapters 3 and 4 we performed complete and matching nutrient balances for carbon, nitrogen (N), energy, phosphorus and potassium, and quantified GHG emissions when BSFL was reared on agri-food residues and pig manure. **In chapter 3 we found that when BSFL were reared on agri-food residues, direct GHG and NH_3 emissions occurring during the bioconversion process were low, with minimum CH4, total N and N2O emissions (although see section for limitations). In chapter 4, we found that when BSFL were reared on manure GHG and NH_3 emissions resulting from the bioconversion process were much larger than those reported in chapter 3. However, the fact that CH_4 and N_2O emissions during the bioconversion process on manure hardly differed from those occurring from manure without larvae, indicated that the rearing substrate caused the observed difference between the bioconversion of the two residual streams**. These findings confirmed that the BSFL bioconversion activity itself does not lead to the production of CH_4 as observed for ruminants, and that GHG emissions during BSFL rearing are substrate-specific. The latter suggests that exploring which streams can lead to BSFL production with the lowest GHG emissions is not what matters, as such studies only focus on differences between substrates. Instead, the focus should be on finding ways to minimize the GHG emissions when larvae are reared on a specific stream, and whether the bioconversion activity can bring environmental benefits compared to the disposal or other valorization pathways that those streams have in the food system.

The outcomes of chapters 3 and 4 provided a reliable quantitative basis of GHG emissions and mass flows occurring during BSFL rearing. These measurements were later used in chapter 7 to perform an LCA of BSFL production. Furthermore, the findings of chapters 3 and 4 helped to gain novel insights in the temporal dynamics of gaseous emissions occurring during the bioconversion activity. For instance, in chapter 3 we found that when BSFL were reared on agri-food residues, NH_3 was consistently produced from the sixth day of rearing and only after the peak of CO_2 production had been reached (Figure 3.3). These results suggest that if BSFL reared on agri-food residues were harvested before the CO2 emission peak was reached, NH_3 emissions during the bioconversion could have been avoided. Even with such timing for larvae harvesting, it is anyway important to implement good post-harvest management practices to avoid NH_3 emissions from frass. For larvae reared on pig manure, NH_3 was emitted from the very beginning, as emissions originated from the manure itself (Figure 4.4). The dissimilarities in the temporal pattern of gaseous losses between the bioconversion process with the two rearing substrates suggested that management interventions aimed to minimize nutrient losses during BSFL rearing might have to be tailored according to the type of substrate used. **Furthermore, in chapter 4 we found that BSFL reared on manure incorporated nutrients in their body mass and substantially reduced the nutrient content initially present in fresh manure. Nonetheless, the bioconversion activity led to larger gaseous losses compared to manure without larvae**.

To further understand the influence of BSFL bioconversion on the N flows observed in chapter 4 (Figure 4.2), especially those related to ammonia-N, in chapter 5 we used the staple isotope ^{15}N in ammonium chloride to verify if ammonia-N could be incorporated into the BSFL body mass during pig manure bioconversion. **Our findings confirmed that 13% of the labelled material was incorporated into BSFL body mass, including proteins**. Although it is unknown if 13% is the maximum level of ammonia-N that can be incorporated in larval body mass, or if higher incorporation rates can be achieved, our results showed that the bioconversion of manure with BSFL can upgrade ammonia-N contained in manure as protein and therefore contribute to a circular protein supply.

Based on the outcomes of chapter 4 and 5 on the N flows during BSFL rearing on manure, I concluded that BSFL bioconversion can simultaneously trigger and mitigate manure ammonia-N emissions. Elucidating how this balance comes about is key for the goal of designing manure BSFL bioconversion systems in which the N gaseous losses are minimized and ammonia-N retention in larval body mass is maximized. Such systems should also simultaneously aim to minimize the pathogenic content of manure in order to reduce the risks of disease transmission when the larvae and frass are used as feed and soil amendment.

9.1.3 Insights on BSFL dietary behavior, an unexplored research theme

Despite the increasing diversity of research topics associated with BSFL farming, behavioral studies of BSFL are scarce (Giannetti et al., 2022; Shishkov et al., 2019). During the experimental work realized in chapter 3, I consistently observed that when the 7-day old BSFL were initially placed in a crate containing the mix of agri-food residues, these tended to escape from the rearing crates during the first hours of exposure (Figure 9.2). This escaping behavior was not observed when BSFL were reared on manure. These observations motivated the formulation of the third research objective of this thesis, which was to verify if BSFL displayed dietary preferences when exposed to contrasting rearing substrates. In chapter 6, we designed and used a novel choice-test to quantify the BSFL dietary preference after 1h of exposure to the two diets (i.e., pig manure and a mix of agri-food residues currently used for industrial BSFL production), and at different larval ages. The results showed that BSFL strongly and consistently preferred pig manure over the agri-food residues. **The preference for manure was present at all larval ages, but it became stronger as the larval age increased**. These results are not only relevant to inform the discussion on farmed insect welfare (see Van Huis (2019) with novel insights about BSFL feeding behavior, but could also be used as a starting point for the development of practical applications. For instance, while novel and effective methods using larval discomfort as a trigger are being proposed to harvest BSFL pre-pupae (Giannetti et al., 2022), harvesting methods using attractants are still underexplored. To

use manure as an attractant, however, we first need to elucidate if the preference for manure is mediated by chemical cues, or if it is linked to repulsion caused by certain physicochemical properties of the agri-food residual streams.

Figure 9.2: Starter larvae (7-day old) escaping from the rearing crates filled with the mix of agri-food residues.

9.1.4 (Re)Assessing the GHG emissions of BSFL production

The fourth and last objective of this thesis was to assess the sustainable use of BSFL as food and feed. To achieve this, we quantified the global warming potential (GWP) of BSFL reared on agri-food residues and pig manure using a life cycle analysis (chapter 7). But unlike as in chapter 2, where LCAs of insects were based on literature data, we here used a life cycle inventory provided by a Dutch BSFL producer. In addition, we included the direct GHG emissions occurring during BSFL rearing as quantified in chapter 3 and 4 in our life cycle inventory. Environmental impact assessments of BSFL, especially when fed on residual organic streams, should be based on comparing the bioconversion activity with the current uses of the streams envisioned as BSFL feed. Therefore, in addition to quantifying the GWP associated with the production of BSFL reared on agri-food residues and pig manure, we also compared the GWP of BSFL bioconversion when used as

food, feed and manure management, with the GWP associated with existing valorization pathways for those streams (chapter 7). All comparisons were based on a Dutch context. Based on the LCA, **we found that direct GHG emissions occurring during larval rearing in agri-food residues represented a very small portion (i.e., 0.5%) of the overall GWP associated with BSFL production, while for manure, these accounted for up to 6%. In both cases, the majority of the GWP emissions caused mainly by the emissions from frass, followed by electricity use**. While we found that 24% of the overall GHG emissions of BSFL production could be mitigated if photovoltaic energy is used for electricity supply, the mitigation potential for frass-related emissions is unknown. So far, the GHG emissions associated with frass have only been quantified by one study and should be further explored given its substantial contribution to the overall impact.

The comparisons between using agri-food residues to produce BSFL versus using them directly as pig feed, showed that **BSFL reared on agri-food residues could only be environmentally beneficial in terms of GHG emissions if used as food, but not if used as pig feed. This is due to the fact that feeding the agri-food residues directly to pigs is less GHG intense than feeding the agri-food residues to BSFL and then feeding these to pigs. For manure management, manure bioconversion with BSFL can offer GHG benefits compared to conventional slurry manure management, but not necessarily compared to manure management with liquid and solid segregation**.

Overall, results of chapter 7 suggest that BSFL bioconversion could bring GHG benefits if properly utilized, but for that to happen different social, institutional, and also infrastructural changes are needed. For instance, to take advantage of the GHG benefits that BSFL bioconversion reared on agri-food residues can offer, the larvae (whole or processed) will have to be consumed directly by humans, and for that, consumer acceptance barriers first need to be overcome. Furthermore, although manure bioconversion can offer GHG benefits compared to slurry manure management, BSFL can grow on pig feces mixed with some urine but cannot properly grow in slurry manure. As in most pig housing systems slurry manure storage is the standard, the use of BSFL for manure bioconversion will require changes in housing infrastructure for the separate collection of urine and feces.

To properly utilize insect farming, we need to implement this enabling environment. Although the changes needed might be seen far away, we should make them part of the innovation agenda for the transformation of food systems towards more sustainable, resilient and food secure futures. **To contribute to this agenda, in chapter 8 we proposed seven principles needed for a responsible use of farmed insects as livestock feed in the format of a comment paper. These principles touch upon different food system subjects such as food waste reduction, food-feed competition, safety, and animal welfare**. The principles are not mentioned in this

synthesis section, as they are already synthesized in chapter 8. Finally, and to further contribute to the discussion around the "enabling environment", in the outlook section of this chapter, I discuss some of the essential elements for accelerating the systemic transformation of food systems proposed by Herrero et al. (2020), adapted to the context of insect farming.

9.1.5 Limitations

Data gaps on environmental and nutritional attributes

As happens with all scientific research, the research performed in this thesis was not exempt from limitations. In chapter 2, our analysis was based on existing literature, and therefore any data limitation in the LCAs reviewed (e.g., lack of data on accurate GHG emissions during insect rearing and frass management) became a limitation of our assessment as well. For instance, some of the papers reviewed for BSFL did not account for frass-related emissions (Salomone et al., 2017), and therefore the environmental impacts were underestimated. Nonetheless, these data gaps were overcome in the LCA performed in chapter 7. Furthermore, our analysis in chapter 2 was based on the environmental impact categories of land use and global warming potential, and our analysis in chapter 7 only included global warming potential. In these two studies we did not account for other relevant impact categories associated with food production, such as nitrogen (N) pollution, biodiversity loss and freshwater use. Therefore, it remains key to also quantify those impact categories for future environmental assessments of insect farming, as it is unclear whether insect farming will perform positively for those environmental issues as well. Such assessments should be transparent in terms of assumptions and reproducible. Moreover, from a nutritional point of view, our analysis covered in chapter 2 was based on raw nutrient contents and did not account for bioavailability of nutrients nor for potential nutrient losses occurring during processing needed to make them edible (e.g., heating). Both nutrient bioavailability and processing losses should be accounted for in future analyses, as they might not only negatively affect the nutritional quality of farmed insects, but also increase the environmental footprint per unit of bioavailable nutrient.

Experimental challenges

Chapters 3, 4, 5 were based on controlled experiments, which also had limitations. To obtain representative data for BSFL production in commercial conditions, we tried to replicate the rearing conditions of the Dutch BSFL producer in Chapter 3. These rearing conditions included no water provisioning additional to that already initially present in the substrate, specific ratios of larvae and substrate, and a rearing period of 7 days. However, we observed that larval yields were 30% lower than those usually attained in the commercial

large-scale production facility. These reduced yields might have been related to water scarcity, especially in the last days of the rearing trial. Water scarcity was likely caused by the higher speed of circulating air inside the experimental chambers than in commercial rearing conditions. It is hypothesized that the circulating air inside the chamber dried out the substrate at higher rates than in commercial conditions, as fans were close to the rearing crates. This experimental factor likely affected the nutrient conversion efficiencies of the larvae. Without this limitation, lower GHG per kg of larvae might be expected for BSFL. Thus, although we accurately quantified the GHG emissions within the respiration chambers, these may not be fully representative for those occurring under commercial conditions. Another limitation faced in chapters 3 and 4 was the quantification of N_2O. While the other gases (e.g., CO_2, CH_4, O_2, NH_3) were measured at 9-minute intervals, N_2O gas sampling was done by taking 60 mL air samples once every 24 h. As we only had one data point per day, it is uncertain whether the N_2O concentrations recorded were representative for the time period between two consecutive samples. Given the high GWP of N_2O (i.e., 298 CO_2 eq.) it is important to have improved gas sampling protocols. This could be done either by increasing frequency of gas sampling or using alternative methods (e.g., vessels) to obtain daily gas samples representative for longer time periods.

Refined environmental impact assessments

The quantification of the environmental impact of manure bioconversion with BSFL was based on the life cycle inventory of a BSFL production facility that used agri-food residues as a rearing substrate. Even though we re-scaled the inputs by correcting for the differences in feed conversion ratio between BSFL fed with agri-food residues and pig manure, primary data from existing production systems are needed for more robust estimates. Furthermore, the comparisons made in chapter 7, especially those that involved pig production, were based on current commercial production models, but as more data become available and production models adopt more circular practices, these should be refined. For instance, pigs were assumed to be fed with nutritionally optimized diets, containing agri-food residues and crops, while BSFL were fed solely on agri-food residues which were not necessarily nutritionally optimized. Comparisons in which both pigs and BSFL are solely fed on optimized agri-food residues would be more just, and likely show a reduction of the environmental impact gap between insects and pigs. While the availability of data in the well-studied field of pig nutrition allows the modelling of pig growth fed with such diets (e.g., for instance see Van Hal et al. (2019a)), more knowledge on nutrient requirements for optimal larval growth is needed to do the same for BSFL. Previous modelling efforts to simulate digestion in BSFL have failed to match the results of feeding experiments (Gold et al., 2020), and just recently experimental evidence on the optimal concentrations of specific nutrients for BSFL is becoming available (Koethe et al., 2021). Despite these limitations, the data collected and the framework developed for chapter 7 can be used as

a starting point to build more robust assessments to explore the environmental potential of insect farming in circular economy.

9.2 Outlook

9.2.1 Societal relevance

This thesis has generated novel knowledge for topics with high societal relevance within the domain of sustainable food systems. The experimental chapters on nutrient flows and GHG emissions, and the subsequent environmental impact assessment, have direct implications for relevant topics within the theme of environmental sustainability, such as reduction of the environmental impact of animal production systems, sustainable diets, improved waste management systems and transition towards circular food systems. Furthermore, this thesis also included chapters that explored the crossroads between environmental sustainability, food security and animal welfare (Figure 9.3). In chapter 2, food security was addressed by assessing the potential nutritional contribution of farmed insects and other novel foods to healthy diets. In chapters 6 and 8 animal welfare was addressed by studying the unexplored field of BSFL larval behavior and highlighting the relevance of accounting for insect and livestock welfare in the discussion associated with the use of insects as feed.

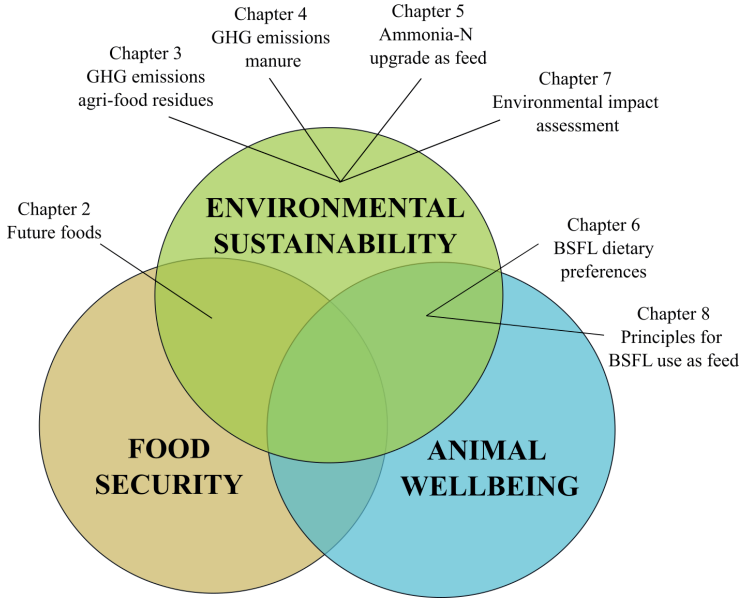

Figure 9.3: Chapters of this thesis and their connection with sustainable food systems themes.

Furthermore, the work of this thesis was developed having societal outreach in mind. For instance, this thesis was conducted following the F.A.I.R. data principles (Findable, Accessible, Interoperable, Re-usable). All data and the code for the data analysis and visualization of each of the chapters published as scientific papers are stored at https://data.4tu.nl/search?q=alejandro%20parodi&licenses=8 and are open access. This was done with the intention to support knowledge discovery, allow the reproducibility of our research, and contribute to educational initiatives through the provisioning of data and code. Furthermore, an example of the societal outreach of the work developed in this thesis, includes the recent citation of the publication associated with chapter 2 (Parodi et al., 2018) in the latest IPCC report Climate Change 2022: Impacts, Adaptation and Vulnerability (IPCC, 2022). Our publication was mentioned to highlight the potential of insects as alternative food and feed sources.

9.2.2 Themes for future research

Achieving each of the research objectives of this thesis provided new perspectives and unlocked topics for future research. Addressing these topics is important to further understand but also utilize the potential of farmed insects for the transition towards sustainable food systems.

Understand and predict N emissions during BSFL rearing

Our results showed that the overall N gaseous emissions occurring during BSFL rearing were heavily influenced by the rearing substrate. However, independent of the rearing substrate, the time-course measurements showed that the larvae affected the timing of the N emissions, especially during the phase of exponential growth. Future research should aim to understand the underlying causes for bioconversion-mediated N emissions and predict when and how much N will be emitted during BSFL rearing with any given substrate, just by knowing the N initial content. This could be approached by using experimental designs in which diverse parameters of the substrate such as pH, temperature, moisture, concentration of uric acid, ammonia-N and total nitrogen, are recorded over time, simultaneously with N gaseous losses.

Furthermore, when BSFL are reared on a substrate that contains ammonia-N (i.e., manure), the larvae can simultaneously trigger ammonia emissions and prevent them by retaining ammonia-N in their body mass. It is therefore key to elucidate how to maximize incorporation of ammonia-N into the larval body mass, while minimizing N emissions. For that it is important to perform more studies with stable isotopes, and to measure the ^{15}N flows not only in the larvae and frass, but also in the gaseous losses.

BSFL and N use efficiency

Nitrogen use efficiency (NUE) is a widely used indicator that shows the efficiency with which an N input into an agricultural production system is converted to N in agricultural products. The N contained in manure is considered a loss, as it decreases the NUE of livestock systems. As BSFL bioconversion can incorporate the nutrients contained in manure and upgrade them as animal feed, future assessments could quantify how much the NUE can be improved when manure-fed larvae are used as animal feed. Such assessments could demonstrate the environmental potential of BSFL for manure valorization using a widely known indicator for N efficiency.

Quantify frass-related GHG emissions

The LCA developed in chapter 7 showed that frass-related emissions are one of the main contributors to the overall GHG emissions of BSFL production. Up to date, only one study experimentally quantified frass emissions (Rummel et al., 2021). To account for frass emissions, LCAs (Guo et al., 2021; Mertenat et al., 2019) have relied on standard emission factors for food waste composting (UNFCC, 2017), while other LCAs apparently omitted them (Bosch et al., 2019; Smetana et al., 2019). The GHG emissions associated with frass application in agricultural soils reported by Rummel et al. (2021) are only based on BSFL reared on a few substrates. Future research should therefore quantify the GHG emissions derived from frass coming from different rearing substrates, and account for the different ways to utilize the frass (i.e., composting previous to soil application, or direct application to agricultural soils).

Environmental impacts of making insects safe

BSFL should be ideally used as feed only when the larvae are reared on residual streams which are non-edible to livestock, such as manure. Although the farming of BSFL is expanding worldwide and regulatory agencies are starting to authorize the use of insects as feed for pigs, poultry, and fish, the use of manure as a rearing substrate for BSFL rearing is constrained by food safety risks in different parts of the world. It is therefore key to explore sustainable solutions to achieve safe manure-fed BSFL systems. Future studies should therefore review the different processing techniques with potential to minimize the presence of pathogens in the manure-fed larvae, and subsequently quantify their environmental impacts. Simultaneously, it is essential to explore different BSFL incubation conditions targeted to improve BSFL productivity when reared on manure. Such conditions could include the inoculation of beneficial bacteria (Gold et al., 2021) and the addition of low-graded ingredients to improve the nutrient composition of manure-based diets.

Compare BSFL bioconversion with competing valorization pathways

When assessing the environmental potential of farmed insects fed with residual streams, studies should look beyond product-based comparisons and focus on comparing BSFL bioconversion with different competing valorization pathways. For instance, comparing the footprint of BSFL fed with food waste versus the footprint of BSFL fed with sludge will provide insights into which larvae can be produced with lower environmental footprint, but will not shed light on the environmental benefits that BSFL bioconversion can bring if adopted to upgrade those streams in the circular economy. To tackle this, BSFL bioconversion should be compared with existing and competing valorization pathways of the same residual streams (composting, anaerobic digestion, bio-refining, feed applications). Examples of relevant research questions include: can insect bioconversion bring environmental benefits over anaerobic digestion? Or should we feed leftovers X and Y to chickens or to BSFL to maximize food provisioning with the lowest environmental impact? In that way, the potential benefits associated with the use of BSFL can be better assessed. Such analyses could be sometimes complex for researchers without expertise outside the food system domain, as they might require technical knowledge on for instance the energy and waste management sectors. It is therefore important to establish interdisciplinary collaboration and always use a systems approach to avoid undesired consequences.

9.2.3 How to create an enabling environment for insect farming?

One of the conclusions of this thesis is that insects have indeed potential to contribute to a food secure and sustainable food system, but for that to happen, an enabling environment to properly utilize them is needed. So, what is needed to achieve such enabling environment? Beyond overcoming research gaps and developing technological innovations, creating an enabling environment for insect farming for food and feed applications will also require changing social and institutional factors. In this section, inspired by the work of Herrero et al. (2020), I provide some insights on what additional elements are needed to create the enabling environment for the proper use of farmed insects in the food system.

Build trust

The introduction of a new product or service and its successful adoption needs the trust of all involved stakeholders, which in the case of farmed insects, include insect producers, livestock farmers, feed and food processing companies, regulatory environmental and safety agencies, and consumers. To create trust, we need dialogue spaces to communicate, transparently and objectively, the opportunities and risks of insect farming to all stakeholders (e.g., potential of insects to upgrade manure ammonia-nitrogen as feed, but risks for feed safety). In addition to communication, these spaces for dialogue should also aim to create

consensus among stakeholders. With consensus, it will become easier to target efforts collectively for successful transition pathways. An example of an existing organization that does such work is the International Platform of Insects for Food and Feed (IPIFF), which represents the interests of the insect production sector towards EU policy makers, European stakeholders and citizens. With their work on the science-policy interface they have helped to develop many of the recent legislative frameworks that regulate the use of farmed insects in Europe.

Transform mindsets

The effectiveness of an innovation is not a guarantee for social acceptance. In the context of farmed insects, presenting all evidence about the nutritional and environmental benefits of adopting insect consumption as part of human diets, does not mean that people will eat them. Consumers are programmed from childhood to prefer familiar foods (Tuorila and Hartmann, 2020), and these foods are preferred over others not only for their flavor, but also because they are part of the culture, identity and history of people. That is why in some world regions, entomophagy is normal. With this in mind, the focus should not be on asking consumers without an entomophagy culture to change their eating habits and include insects in their diets. Instead, we should first aim to change the way people think about insects as food, or in other words, we should aim to transform mindsets. If neophobia towards insect consumption is relieved, people (and their upcoming generations) might be more inclined to try them and perhaps adopt them as part of their diets. One way to achieve that, I believe, is through art. As researchers, we should not forget about the power of a novel, a movie, or music to transform mindsets.

Design market incentives

As an emerging agricultural sector, insect farming needs to have the right incentives to successfully position in the market. Herrero et al. (2020) mentions that incentives can come in the form of market-pull and market-push measures. An example of a market-pull measure that is working to promote insect farming in some African countries, is the agreements in which insect producers get paid by local municipalities (which before used to pay organic waste disposal companies) to use the fruit and vegetable waste from street markets. Examples of market-push measures could be tax benefits or higher custom duties for imported feeds. These measures do not necessarily have to be directed only to insect farming though, but instead could be used to incentivize the promotion of diverse emerging sectors. For instance, increasing custom duties for imported soybean, or offering tax benefits to feed manufacturers and farmers that use circular sources for animal diets, could not only help insect producers, but also biorefineries or yeast producers. Such incentives, however, should be designed to avoid undesirable indirect effects. For instance,

an undesired effect of promoting a higher demand for circular feeds, could hamper food waste reduction efforts.

Develop transition pathways

Innovations should be congruent with the Sustainable Development Goals (SDGs). Making insect farming compatible with the SDGs is therefore a must, but to do that in practice, we need to develop transition pathways. Main elements for such transition pathways (i.e., the set of plans and actions to realize transformation) include desired science targets, identification of winners and losers and strategies to minimize adverse effects (Herrero et al., 2020). For insect farming, a desired science target could be for instance, the quantification of GHG emissions associated with different frass management strategies, or to maximize the incorporation of manure ammonia-N in the larval body mass. For winners and losers, a key point for discussion is who will farm the insects in the future, and how will it be done? If we do not want big corporations with high-tech automated insect production systems outcompeting smallholders, especially in the global south, then transition pathways should be developed to make such scenarios unlikely. Lastly, transition pathways should also anticipate unforeseen scenarios, such as what will happen with producers of BSFL reared on manure if manure become less abundant (e.g., due to legal mandates to reduce the number of animals). All of this should be part of a big dialogue, which needs to start now.

9.3 Main Conclusions

The overall research aim of this thesis was to provide a better understanding of the contribution of farmed insects to sustainable food systems, especially when reared on residual organic streams. This thesis focused mainly on black soldier larvae, a species which is mostly fed with residual organic streams and farmed worldwide.

- Farmed insects, including the black soldier fly larvae, can provide, in addition to protein, different minerals, vitamins and omega-3 fatty acids with lower land use and GHG emissions than most conventional animal-source foods.

- The GHG and N emissions occurring during the rearing phase of BSFL are strongly influenced by the type of substrate on which the larvae grow. This study provided a reliable quantitative basis for these GHG and N losses when BSFL were reared on a mix of agri-food residues and pig manure.

- BSFL can reduce the initial nutrient contents present in pig manure, not only by incorporating nutrients in their body mass but also by triggering CO_2 and N emissions.

- BSFL reared on manure can incorporate at least 13% of ammonia-N contained in manure in their body mass, also in protein, and therefore contribute to a circular protein supply.

- BSFL show an early dietary preference for pig manure over agri-food residues.

- The direct GHG emissions during BSFL rearing have a small contribution to the overall global warming potential of BSFL production. Frass emissions are the main source of GHG emissions and should be better quantified.

- To offer GHG benefits, BSFL reared on agri-food residues should be utilized as food instead of feed.

- Manure bioconversion with BSFL can offer GHG benefits compared to conventional slurry manure management, but not compared to manure management via liquid-solid segregation systems.

- Farmed insects have the potential to improve the environmental sustainability and welfare of livestock production systems, but for that to happen an enabling environment is needed.

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Summary

Food systems need to be reconfigured towards more sustainable and climate-resilient futures to safeguard planetary health and food security. A cornerstone for such reconfiguration is the reduction of the environmental impacts associated with the production and consumption of animal-source food (ASF), such as beef, pork and chicken meat. Among the different upcoming innovations to tackle this issue, the use of farmed insects to reduce the environmental impact of ASF is gaining attention. Insect farming for food, feed and organic waste management purposes is rapidly expanding worldwide, and although the expectations to improve the sustainability of food systems are high, there is a need for broad-scale sustainability assessments to quantify their real transformative potential. The aim of this thesis was to provide a better understanding of the contribution of farmed insects to a sustainable food system when reared on residual organic streams. This was achieved by using experimental and modelling approaches. Most of the evidence generated in this thesis focus on the black soldier fly larvae (BSFL) as they are the main farmed insect worldwide.

Chapter 2 compares the nutritional content and environmental impact of farmed edible insect with different novel sources of food and the main animal- and plant-source foods. This was done by reviewing the available literature on nutritional composition and environmental impacts and by creating a standardized functional unit that allowed for comparison between foods. The results showed that farmed insects could not only provide protein, but also a range of other valuable macro- and micronutrients with a lower land use and greenhouse gas (GHG) emissions than ASF, especially when reared on organic-residual streams. The following chapters therefore focused on overcoming knowledge gaps and assessing with further detail the implications associated to the production of BSFL reared on residual organic streams.

Chapters 3 and 4 quantify the flows of energy and nutrients (i.e., nitrogen, carbon, potassium and phosphorus) and the emissions of carbon dioxide, methane, nitrous oxide, ammonia and total nitrogen, during BSFL rearing on agri-food residues and pig manure. This was done by rearing the larvae in climate respiration chambers and determining the chemical composition of inputs and outputs to build complete nutrient balances. It was found that the direct greenhouse gas (GHG) and ammonia emissions occurring during

BSFL reared on agri-food residues were minimal. For BSFL reared on manure GHG emissions were larger but these were mainly produced by the manure itself. Furthermore, in chapter 4 we found that BSFL reared on manure can incorporate nutrients in their body mass and substantially reduce the nutrient content initially present in fresh manure. However, the bioconversion activity can also lead to larger carbon and nitrogen gaseous losses compared to manure without larvae.

Chapter 5 quantifies the incorporation of ammonia-nitrogen into BSFL body mass and larval proteins after manure bioconversion with BSFL. This was done by rearing BSFL in manure and using the stable isotope ^{15}N as a tracer. We found that 13% of the added ^{15}N was incorporated into the BSFL larval body mass, including larval proteins. This result confirms that the bioconversion of manure with BSFL can upgrade ammonia-N contained in manure as protein feed and therefore contribute to a circular protein supply.

Chapter 6 explores the dietary preference of BSFL when exposed to two contrasting rearing substrates. For that, we designed and performed a simple and novel choice-test in which BSFL of different ages were exposed to agri-food residues and pig manure for 1 hour. We found that BSFL strongly and consistently preferred pig manure over the agri-food residues. The preference for manure was present at all larval ages, but it became stronger as the larval age increased. These results are not only relevant to inform the discussion on farmed insect welfare with novel insights about BSFL feeding behavior, but could also be used as the starting point for the development of practical applications.

Chapter 7 quantifies using a life cycle approach, the GHG associated to the production of BSFL reared on agri-foods residues and pig manure. In addition, the GHG impacts of BSFL production for food, feed and manure management are compared to those occurring from the existing valorization pathways for the same organic residual streams. We found that direct GHG emissions occurring during larval rearing in agri-food residues (measured in chapter 3) represented a very small portion (i.e., 0.5%) of the overall emissions associated to BSFL production, while for manure (measured in chapter 4), these accounted for just 6%. In both cases, the majority of the GHG emissions were linked mainly to frass utilization, followed by electricity use. The comparisons with other valorization pathways showed that BSFL reared on agri-food residues could only be environmentally beneficial in terms of GHG emissions if used as food, but not if used as pig feed. In addition, manure bioconversion with BSFL can offer GHG benefits compared to conventional slurry manure management, but not necessarily compared to manure management with liquid and solid segregation.

Chapter 8 provides seven key principles to guide the responsible use of farmed insects as feed. This was done by integrating the information generated in the previous chapters, with the most recent research outcomes on the effects of farmed insect on livestock growth, health and welfare, and insights on food waste reduction, food-feed competition and safety.

It was concluded that insects used as feed have potential to contribute to more sustainable livestock systems, but for that to happen an enabling environment is needed.

Overall, it is concluded that farmed insects reared on residual organic streams can contribute to the transition towards sustainable food systems if used properly. Beyond overcoming scientific knowledge gaps and developing technological innovations, it is key to center efforts on shaping the social and institutional conditions needed to utilized farmed insects in their full potential.

Resumen

Los sistemas alimentarios necesitan ser reconfigurados hacia futuros más sostenibles y climáticamente resilientes para salvaguardar la salud planetaria y seguridad alimentaria. Un punto clave para dicha reconfiguración es la reducción del impacto ambiental asociado a la producción y consumo de alimentos de origen animal, como lo son la carne de res, cerdo y pollo. Entre las diferentes innovaciones en el sector agroalimentario, la crianza de insectos está ganando cabida. Diferentes especies de insectos están siendo criadas con fines alimenticios, tanto para consumo humanos y de animales de granja, así como también para el manejo y tratamiento de residuos orgánicos. La crianza de insectos es una actividad que se expande rápidamente por el mundo, y a pesar de que las expectativas sobre su potencial para mejorar la sostenibilidad de los sistemas alimentarios son altas, se necesita más evidencia que permita cuantificar su verdadero potencial transformador. En esta tesis se utilizan enfoques experimentales y de modelamiento para generar un mejor entendimiento sobre la contribución de los insectos alimentados con residuos orgánicos para la transición hacia los sistemas alimentarios sostenibles. La mayor parte de la evidencia generada en esta tesis está basada en la larva de la mosca soldado negra, por ser la principal especie de insecto criada alrededor del mundo.

En el capítulo 2 se compara el contenido nutricional y el impacto ambiental de distintas especies de insectos, con alimentos alternativos (hongos, algas, carne cultivada) y los principales alimentos de origen animal y vegetal. Para ello, se revisaron los estudios nutricionales y los análisis de ciclo de vida disponibles para los distintos alimentos, y se calculó una unidad funcional estandarizada que permitió comparar los atributos nutricionales y ambientales entre los distintos alimentos. Los resultados muestran que los insectos pueden proporcionar proteínas, y distintos macro- y micronutrientes con menor uso de suelo y gases de efecto invernadero (GEI) que los alimentos de origen animal. Se observó que los beneficios ambientales fueron mayores cuando los insectos eran alimentados con residuos orgánicos. Los siguientes capítulos se enfocan en estudiar a mayor detalle las implicancias, principalmente ambientales, de producir las larvas de la mosca soldado negra alimentadas con residuos orgánicos.

En los capítulos 3 y 4 se cuantifica el flujo de energía y nutrientes (nitrógeno, carbono, fósforo y potasio) y las emisiones de dióxido de carbono, óxido nítrico, amoniaco y

nitrógeno, durante la crianza de la larva de la mosca soldado negra en residuos de la industria alimentaria y en estiércol porcino. Esto se hizo criando las larvas en cámaras climatizadas de respiración y determinando la composición química de los insumos y productos del sistema, para construir balances completos de nutrientes. Se encontró que las emisiones directas de GEI durante la fase de crianza de las larvas en los residuos agro-alimentarios fueron mínimas. Para las larvas criadas en estiércol porcino las emisiones de GEI fueron mayores, pero se observó que estas provinieron principalmente del estiércol. Además, en el capítulo 4 se encontró, que si bien las larvas criadas en estiércol pueden incorporar nutrientes en su cuerpo y así reducir sustancialmente la cantidad de nutrientes inicialmente presentes en el estiércol, su presencia también puede propiciar mayores emisiones de carbono y de nitrógeno.

En el capítulo 5 se cuantifica la incorporación de nitrógeno amoniacal en la masa corporal de las larvas de la mosca soldado negra cuando estas son criadas en estiércol porcino. Para ello se criaron las larvas en estiércol mezclado con el isotopo estable ^{15}N, que fue utilizado como trazador. Se encontró que 13% del 15N agregado fue incorporado en la masa corporal de las larvas, incluyendo proteínas. Este resultado confirma que la bioconversión de estiércol con larvas de la mosca soldado negra puede recuperar y valorizar el nitrógeno amoniacal presente en los estiércoles y, por lo tanto, contribuir a un provisionamiento de proteínas circulares para la alimentación animal.

En el capítulo 6 se explora las preferencias alimentarias de las larvas de la mosca soldado negra cuando estas son expuestas a dos sustratos distintos. Para ello, se diseño y se ejecutó una novedosa y simple prueba de elección, en la cual larvas de distintas edades fueron expuestas por una hora a residuos de la industria alimentaria y estiércol porcino. Los resultados mostraron que las larvas constantemente prefirieron al estiércol porcino, y que la preferencia se dio en las larvas de todas las edades evaluadas, no obstante, esta fue mayor con larvas de mayor edad. Estos resultados aportan nuevas perspectivas sobre el comportamiento alimenticio de las larvas que pueden servir para enriquecer la discusión sobre el bienestar animal en la crianza de insectos.

En el capítulo 7 se cuantifica, con una perspectiva de ciclo de vida, las GEI asociadas a la producción de las larvas de la mosca soldado negra criadas en residuos agro-alimentarios y estiércol porcino. Adicionalmente, compara los GEI asociados al uso de las larvas como alimento humano, alimento animal y como estrategia para el tratamiento y manejo de estiércol, con las GEI asociadas a otras alternativas para valorizar los mismos residuos. Se encontró que las emisiones de GEI que ocurren durante la fase crianza de las larvas en residuos agro-alimentarios (medidos en el capítulo 3), representaron solo el 0.5% de las emisiones totales asociadas a la producción de las larvas. Mientras que para las larvas criadas en estiércol porcino, estas emisiones aportaron al 6% del total. En ambos casos, la mayoría de las emisiones de GEI estuvieron asociadas a la utilización del "frass" (estiércol de los insectos) y al uso de electricidad. Las comparaciones con otras alternativas de

valorización de los mismos residuos orgánicos mostraron que producir las larvas alimentadas con residuos agro-alimentarios sólo brinda beneficios de GEI si es que estas se utilizan como alimento humano, pero no si se utilizan como alimento porcino. En vez de utilizar a estas larvas como alimento porcino, alimentar a los cerdos directamente con los residuos agro-alimentarios genera menos GEI. Adicionalmente, la bioconversión de estiércol porcino con la mosca soldado negra puede ofrecer beneficios en GEI comparado al manejo convencional del estiércol líquido. No obstante, estos beneficios no se dan cuando el manejo del estiércol se realiza por medio de la segregación de líquidos y sólidos.

En el capítulo 8 se proponen siete principios claves para guiar el uso responsable de los insectos utilizados como alimento animal. Los principios incluyen temas como la selección de residuos para la producción de insectos, los efectos que generan los insectos en el crecimiento, salud y bienestar de los animales de granja, la reducción del desperdicio de alimentos, la competencia entre comida y alimento animal, y la inocuidad alimentaria. Se concluye que los insectos tienen gran potencial para contribuir a construir sistemas de producción animal más sostenibles, sin embargo, para que ello suceda, se necesita un ambiente propicio para estos sean utilizados responsable y efectivamente.

En conclusión, los insectos criados en residuos orgánicos pueden contribuir a la transición hacia los sistemas alimentarios sostenibles si es que se utilizan correctamente. Además de superar brechas de conocimiento y de generar innovación tecnológica, es clave que se centren esfuerzos en crear las condiciones sociales e institucionales para utilizarlos en su máximo potencial.

Acknowledgements

I like to reflect on things using metaphors, and as cycling passionate I see my PhD journey as a 3-week world tour cycling race. Every cycling team riding a world tour race have a lead rider, who is usually the most visible person of the team. But lead riders can only perform well if they have a good team behind their backs. A cycling team consists of other cyclists who protect the captain from the wind and the attacks of other cyclists, race directors advising how to approach different racing situations, mechanics preparing the bicycles for the next day, physios facilitating a faster recovery, managers ensuring funding and arranging everything to have the best logistics, and assistants along the route that provide food, water and aid in case of eventualities. In addition to having a sound team, other key factors for a successful race include a good training season to develop physical and mental fitness, and cheerful people in the hardest parts of each stage. In my own PhD-tour, I had it all: family that supported my education, a great supervision team that provided guidance, co-authors that helped to improved the quality of the work, and family and friends that kept me motivated to give my best until the end. I did not have other contenders rather than time, as I started this PhD with the ambition to take no more than 4 years to finish it. I made it, and here are my acknowledgements to those who made it possible:

Mi primer agradecimiento va para ti Cristina. Además de ser mi compañera de vida y esposa, eres mi mejor amiga, confidente, gran pareja de viajes, deportes y mudanzas, y recientemente súper mamá. Para mí es realmente un espectáculo disfrutar la vida junto a ti y nuestra hermosa Flora. Me siento muy afortunado de tenerte y siempre estaré agradecido por el apoyo incondicional que me brindaste para empezar, ejecutar y especialmente para terminar esta tesis. Espero estar a la altura cuando me toque acompañarte en tu propio tour.

Thanks to my official and non-official supervision team. To my promotor Imke, thank you for valuing my work since my MSc times and for all the knowledge and insights shared with me via supervising meetings, manuscripts, research proposals, and informal conversations. Also, thanks for giving me room to express without hesitation my willing and concerns about my PhD process, I appreciate it. To my co-promotor and daily supervisor Hannah, thanks for your continuous guidance and support to my scientific career (also since MSc

times!), for making me part of your nice collaboration network, for your always constructive feedback and for your friendship. Looking forward to work together again! To my promotor Joop, thanks for sharing your knowledge, tips and specially passion for experimental insect research. I really enjoyed the long talks in your office about all the experiments we could do to answer exciting research questions. Thanks also to my non-official supervising team, Liesbeth thanks for managing the project smoothly and efficient, and for all your insights shared along these four years. Walter, your comments usually took some time to arrive, but the await was always worthy. The high quality feedback provided has been always inspiring to me (since the Research Master Cluster course) and key for the quality of our research outputs. I hope one day I can provide feedback in the concise and precise way you do it.

Thanks to all the members of the NWO project involved in my PhD research. Allyson, I enjoyed writing the comment paper with you and the nice chats. Jeroen and Lisa, thanks for all the logistic coordination for the deliveries of larvae and for the feedback and insights. To the rest of the users: Joost, Carla, Bas, thanks for the intellectual and material support to conduct our exiting research.

Thanks to all the people that helped me to perform the experimental work. Thanks Marcel, Tamme and Sven for your technical advice and fun coffee breaks during the experiments at Carus. Thanks Arjan for facilitating the contact with farmers and providing us with pig manure for the experimental pilots. Thanks Henk for providing the pig manure from your farm to conduct our experiments. Thanks to Jane-Martine, Michael and Erika for your always positive support during my interaction with the ANU lab. Thanks Dennis for the insights and perspectives shared during the spontaneous chats in the corridors of Zodiac.

To the APS family, thanks to each of you for making me part of it. Special thanks to my office mates Abi, Loeki, Sally, Cecilia and Ben. It was nice to share with each of you the day to day joys and struggles of the PhD life. Lia thanks for your positive energy and for all the administrative support. Fokje thanks for all the tips on how to supervise students and for your accurate and constructive feedback to make good scientific posters. Thanks to all the bachelor and master students that did their thesis under my supervision: Floor, Stefan, Kim, Qifa.

Thanks to all the beautiful people from around the world that I had the chance to meet and hang with during my time in Wageningen: Lobos, Pipe, Santiago y Pancho, que la amistad continue hasta que seamos viejos mis hermanos. Eka, Dani, Nati, Laci, Auke and Jan, son nuestra familia Wageningera muchachos. A la comunidad peruana: Chino gracias por la hospitalidad de siempre y por juntarnos a todos para comer rico y pasarla bien en la Peruvian embassy. Diana, Matias, Claudia, Mili, Milu, Xime, Deborah, Ornella, Julia, Max, Franco, Lorena, Gianfranco, y el resto de peruan@s que tuve la suerte de conocer por allá, gracias a todos por los buenos momentos. Eyal & Offir, thank you guys for the

always positive vibes and for sharing with us your amazing global grooves. Mathieu, Sara and Jasper, thanks for receiving me in your cozy home and for sharing all the tips for the parenthood life. Cata & André obrigado por las buena energia y las buenas conversaciones, especialmente las amazónicas. To my squash partners, Vic, Hans, Cornelis, Caas, Mathieu, Thiemo thanks guys for all the intense games we had along these years and the great time we spent together at the Bongerd. Thanks, Josephine, for your delicious food and for your friendship.

Gracias familia. Pa, gracias por sembrar en mí la semilla de la curiosidad de entender cómo funcionan las cosas. Ma, gracias por transmitirme ese lado afectivo y emocional que te caracteriza. A ambos, gracias por la libertad que siempre me dieron para tomar mis propias decisiones y por el apoyo a mi formación académica. Gonzalo e Ian, ambos siempre fueron motivo de ilusión para visitar Lima con más frecuencía. Me alegra que estemos cerca nuevamente. A mis suegros Leo y Mariella, y a mis cuñados Salvador y Joaquín, gracias por todo el cariño y confianza de siempre, y por hacerme parte del clan. Ese clan que más que nunca se volvió indispensable en el último año de mi doctorado. Fue una buena decisión volver a Lima y que Flora empiece su vida con ustedes (literalmente) al lado. A mi familia de elespuru312, las milkitas, Maribel, y recientemente tíos Cesar y Carola, gracias por siempre estar pendiente de nosotros y por abrirme las puertas de la casa en múltiples ocasiones. Gracias a toda la familia y amigos nos visitó en nuestro tiempo en Europa: mis papás, suegros, tío Juan, Melissa, Nati, Don Oscar, Nelly, Rosita, Isabella, Scarlet, Dani, Fabrizio, Chuck, Bruno, Mema. Sus visitas siempre nos recargaron.

Gracias a los familiares y amigos que nos recibieron en Europa. Claudia, Gerard, Tess y Laurenz, gracias por su hospitalidad de siempre y por recibirnos esa navidad del 2018. Tía Ana y Christian, primos Pedro y Bettina, son una familia hermosa, muchas gracias por recibirnos siempre con los brazos abiertos en Laumet y hacernos sentir como en casa. Tía Miriam y tío Pascal muchas gracias por recibirnos siempre con la mejor onda en Paris.

Gracias a Pepe Bárcenas y Lili Santamaría por el apoyo. Ambos aparecieron en los últimos siete meses de este camino de cuatro años, pero su ayuda fue clave para que yo pueda terminar de recorrer los últimos kilometros, los que más me costaron. Pepe me guiaste para que yo mismo empiece a entender quién soy y qué quiero. Lili, facilitaste que pueda enfocarme en terminar esta tesis sabiendo que Flora estaba en buenas manos.

Finalmente, te agradezco mucho Flora. Tu llegada, casi al final de este camino, cambió mi vida para siempre. No hay nada que me emocione más que verte crecer mi hijita linda.

About the author

Alejandro Parodi was born in Lima, Peru on February 1990. He lived for seven years in the Amazonian city of Pucallpa, where he finished high school in 2006. After the years spent in the Amazon he decided to study wildlife biology. In 2012 he obtained his BSc in Biology from Cayetano Heredia Peruvian University. For his BSc thesis Alejandro studied the activity patterns of Amazonian mammals using camera traps. Alejandro worked as a technical assistant for two years at the Wildlife Conservation Society in Peru, where he assisted in the implementation of biodiversity monitoring plans for Peruvian natural protected areas. Realizing that many of the activities threatening natural ecosystems were linked to food production, Alejandro decided to pursue his postgraduate studies in sustainable agriculture. In 2016 he moved to the Netherlands and started an MSc in Organic Agriculture with specialization in Agroecology at Wageningen University & Research. During his MSc studies he got interested in using a systems approach to evaluate the environmental impact of innovations in the food system. In 2018 he was offered a PhD position at the Animal Production Systems group at Wageningen University & Research. For his PhD, he assessed the sustainability of farmed insects, focusing mainly on the black soldier fly larvae. The result of his PhD work is presented in this thesis. Alejandro will continue working on sustainable and circular food systems as a postdoctoral researcher at the Farming Systems Ecology group of Wageningen University & Research. Beyond the academic world, Alejandro likes to sport, play music, cook, and to enjoy his new family life with his wife Cristina and daughter Flora.

Peer-reviewed Journal Publications

Herrero, M., P. K. Thornton, D. Mason-D'Croz, J. Palmer, B. L. Bodirsky, P. Pradhan, C. B. Barrett, T. G. Benton, A. Hall, I. Pikaar, J. R. Bogard, G. D. Bonnett, B. A. Bryan, B. M. Campbell, S. Christensen, M. Clark, J. Fanzo, C. M. Godde, A. Jarvis, A. M. Loboguerrero, A. Mathys, C. L. McIntyre, R. L. Naylor, R. Nelson, M. Obersteiner, **A. Parodi**, A. Popp, K. Ricketts, P. Smith, H. Valin, S. J. Vermeulen, J. Vervoort, M. van Wijk, H. H. E. Van Zanten, P. C. West, S. A. Wood, and J. Rockström (2021). "Articulating the effect of food systems innovation on the Sustainable Development Goals". *The Lancet Planetary Health* 5.1, e50–e62. DOI: 10.1016/S2542-5196(20)30277-1.

Herrero, M., P. K. Thornton, D. Mason-D'Croz, J. Palmer, T. G. Benton, B. L. Bodirsky, J. R. Bogard, A. Hall, B. Lee, K. Nyborg, P. Pradhan, G. D. Bonnett, B. A. Bryan, B. M. Campbell, S. Christensen, M. Clark, M. T. Cook, I. J. M. De Boer, C. Downs, K. Dizyee, C. Folberth, C. M. Godde, J. S. Gerber, M. Grundy, P. Havlik, A. Jarvis, R. King, A. M. Loboguerrero, M. A. Lopes, C. L. McIntyre, R. Naylor, J. Navarro, M. Obersteiner, **A. Parodi**, M. B. Peoples, I. Pikaar, A. Popp, J. Rockström, M. J. Robertson, P. Smith, E. Stehfest, S. M. Swain, H. Valin, M. van Wijk, H. H. E. van Zanten, S. Vermeulen, J. Vervoort, and P. C. West (2020). "Innovation can accelerate the transition towards a sustainable food system". *Nature Food* 1.5, 266–272. DOI: 10.1038/s43016-020-0074-1.

Karlsson, J. O., **A. Parodi**, H. H. E. van Zanten, P.-A. Hansson, and E. Röös (2021). "Halting European Union soybean feed imports favours ruminants over pigs and poultry". *Nature Food* 2.1, 38–46. DOI: 10.1038/s43016-020-00203-7.

Latka, C., **A. Parodi**, O. van Hal, T. Heckelei, A. Leip, H.-P. Witzke, and H. H. van Zanten (2022). "Competing for food waste – Policies' market feedbacks imply sustainability tradeoffs". *Resources, Conservation and Recycling* 186, 106545. DOI: 10.1016/J.RESCONREC.2022.106545.

Parodi, **A.**, A. Leip, I. J. M. De Boer, P. M. Slegers, F. Ziegler, E. H. M. Temme, M. Herrero, H. Tuomisto, H. Valin, C. E. Van Middelaar, J. J. A. Van Loon, and H. H. E. Van Zanten (2018). "The potential of future foods for sustainable and healthy diets". *Nature Sustainability* 1.12, 782–789. DOI: 10.1038/s41893-018-0189-7.

Parodi, **A.**, I. J. M. De Boer, W. J. J. Gerrits, J. J. A. Van Loon, M. J. W. Heetkamp, J. Van Schelt, J. E. Bolhuis, and H. H. E. Van Zanten (2020a). "Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing – A mass balance approach". *Journal of Cleaner Production* 271, 122488. DOI: 10.1016/j.jclepro.2020.122488.

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efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions". *Waste Management* 126, 674–683. DOI: 10.1016/J.WASMAN.2021.04.001.

Parodi, **A.**, K. Van Dijk, J. J. A. Van Loon, I. J. M. De Boer, J. Van Schelt, and H. H. E. Van Zanten (2020b). "Black soldier fly larvae show a stronger preference for manure than for a mass-rearing diet". *Journal of Applied Entomology* 144.7, 560–565. DOI: 10.1111/jen.12768.

Parodi, **A.**, G. Villamonte-Cuneo, A. M. Loboguerrero, D. Martínez-Barón, and I. Vázquez-Rowe (2022a). "Embedding circularity into the transition towards sustainable agroforestry systems in Peru". *Science of The Total Environment* 838, 156376. DOI: 10.1016/J.SCITOTENV.2022.156376.

Parodi, **A.**, Q. Yao, W. J. J. Gerrits, M. Mishyna, C. M. Lakemond, D. G. A. B. Oonincx, and J. J. A. Van Loon (2022b). "Upgrading ammonia-nitrogen from manure into body proteins in black soldier fly larvae". *Resources, Conservation and Recycling* 182, 106343. DOI: 10.1016/J.RESCONREC.2022.106343.

Other Scientific Publications

Parodi, **A.**, H. H. E. Van Zanten, and I. J. M. De Boer (2017). "Puzzling with proteins: towards an environmental and healthy diet". In: *3rd International Conference on Global Food Security Conference*.

Van Zanten, H. H. E., O. Van Hal, F. Ziegler, S. Hornborg, C. Latka, **A. Parodi**, T. J. Achterbosch, M. Bianchi, I. J. M. De Boer, L. Borthwick, et al. (2019). *Report on T5. 4: Sustainability impacts of potential innovations in the supply chain of livestock and fish, and fruit and vegetables, and sustainable future diets: Deliverable No. D5. 4*. Tech. rep. SUSFANS.

Zanten, H. H. E. van, **A. Parodi**, S. Hornborg, F. Ziegler, and I. J. M. De Boer (2017). *Deliverable D5. 2: Innovation pathways towards future nutrition security: Innovation pathways towards more sustainable production and consumption in the livestock-fish supply chain and their uptake in the SUSFANS models*. Tech. rep. SUSFANS.

Training and supervision plan

With the activities listed the PhD candidate has complied with the educational requirements set by the Graduate School of Wageningen Institute of Animal Sciences (WIAS). One ECTS equals a study load of 28 hours

The basic package (2 ECTS)

- WIAS introduction day (2018)
- Ethics and Animal Sciences (2021)

Disciplinary competences (15 ECTS)

- PhD proposal WIAS (2018)
- Bayesian Statistics (2018)
- WIAS/PE&RC advanced statistics course Design of Experiments (2018)
- International Advanced Course "Environmental Impact Assessment of Livestock Systems" (2019)
- Modelling Food Security (2019)
- Modelling and optimization with GAMS (2019)
- A bio-based society: from principles to practice (2021)

Professional Competences (6.3 ECTS)

- Project and time management (2018)
- Adobe InDesign (2019)
- Research Data Management (2021)
- Critical thinking & argumentation (2021)
- Lecturing (2021)
- Scientific integrity (2021)
- Writing Grant Proposals (2021)

Presentation skills (4 ECTS)

- WIAS Science day, oral (2018)
- WIAS Science day, poster (2018) (awarded as best poster voted by the public).
- WIAS Science day, poster (2019)
- International conference Insects to feed the world, oral (2020)
- Future of EU livestock sector workshop, oral (2021)

Teaching competences (6 ECTS)

- Supervising MSc major thesis (x3)
- Supervising BSc thesis

This research was funded by the Netherlands Organisation for Scientific Research (NWO) through the Toegepaste en Technische Wetenschappen (TTW) program [project number 15567].

Cover design by Alejandro Parodi using the R package generativeart developed by Katharina Brunner
Printed by ProefschriftMaken