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# Bioconversion efficiencies, greenhouse gas and ammonia emissions during black soldier fly rearing – A mass balance approach

Alejandro Parodi [a, *], Imke J.M. De Boer [a], Walter J.J. Gerrits [b], Joop J.A. Van Loon [c], Marcel J.W. Heetkamp [d], Jeroen Van Schelt [e], J. Elizabeth Bolhuis [d], Hannah H.E. Van Zanten [a]

[a] Animal Production Systems Group, Wageningen University & Research, P.O. Box 338, 6700, AH Wageningen, the Netherlands
[b] Animal Nutrition Group, Wageningen University & Research, P.O. Box 338, 6700, AH Wageningen, the Netherlands
[c] Laboratory of Entomology, Wageningen University & Research, P.O. Box 16, 6700, AA Wageningen, the Netherlands
[d] Adaptation Physiology Group, Wageningen University & Research, P.O. Box 338, 6700, AH Wageningen, the Netherlands
[e] Bestico BV, Veilingweg 6, 2651, BE Berkel en Rodenrijs, the Netherlands

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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 $CO_2$-equivalents 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.


## 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 is to replace protein-rich feed ingredients with high environmental footprints, such us 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., 2019a). 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

* Corresponding author.
*E-mail addresses:* alejandro.parodiparodi@wur.nl, aparodi312@gmail.com (A. Parodi).

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et al., 2011; Myers et al., 2008; Xiao et al., 2018a), vegetable waste (Diener et al., 2011; Lalander et al., 2014; 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., 2019a), 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 biowaste 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.

## 2. Materials and methods

### 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) (Fig. 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 (Fig. 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.

### 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 (Fig. 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 2.3.3).

### 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 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 (ISO 6496, 1999), nitrogen and carbon (Dumas method, ISO 1634-1, 2008), gross energy (oxygen bomb method, ISO 9831, 1998), phosphorus (spectrophotometry method, ISO 6941, 1998), potassium (CP-OES method, ISO 21033, 2016), and crude fat (hydrolysis method, ISO 6492, 1999). Samples of substrate and residues were also analysed for contents of starch (Amyloglucosidase method, ISO 14914, 2004). Samples of condensed water and acid were analysed for nitrogen (Kjeldahl method, ISO 5983-2, 2005).

### 2.4. Gas measurements and calculations

#### 2.4.1. $CO_2$, $CH_4$ and metabolic heat losses

Concentrations of $O_2$, $CO_2$ and $CH_4$ were measured in a cycle

**Table 1**
Nutrient composition of the substrate, starter larvae, 14-day old larvae and residual substrate (mean ± standard deviation). Except for DM, all values are expressed per 100 g of dry matter product.

| | 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 |

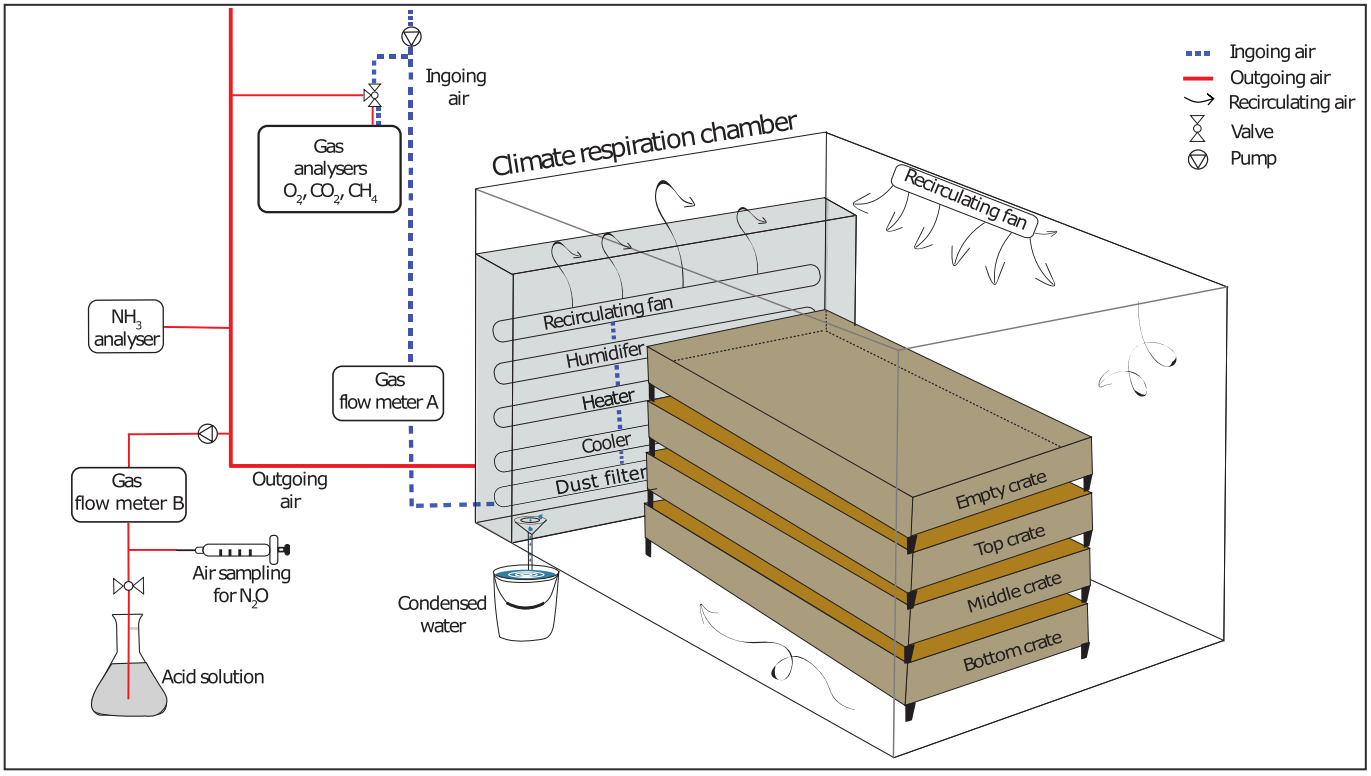


**Fig. 1.** Schematic representation of the respiration chamber: air flows, climate control unit (shaded box), gas analysers and rearing crates. The shaded box was separated from the "animal space" in which the crates were located.

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 information 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):

$$Q = 16.175\ VO_2 + 5.021 VCO_2 - 2.167 VCH_4 \quad (1)$$

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):

$$RQ = \frac{VCO_2}{VO_2} \quad (2)$$

### 2.4.2. 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 Fig. 1). Total nitrogen emissions were determined using the following equation:

$$TN = \left[\frac{N_{acid}}{1000} \left(\frac{Vt\ x\ As}{Vf}\right)\right] + \left[\frac{N_{cond}}{1000} D\right] \quad (3)$$

where $TN$ is the total nitrogen emissions (grams) 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), $Vt$ (measured by gas flowmeter A, see Fig. 1) is the total air ventilated volume (in $m^3$), $As$ is the grams of acid (in g), $Vf$ (measured by gas flowmeter B) is the total air ventilated volume (in $m^3$) that went through the acid bottle, $N_{cond}$ 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 Fig. 1) using a calibrated $NH_3$ sensor (Dräger Polytron® 8100 EC with sensor type NH3-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.

#### 2.4.3. 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):

$$TN_2O = \sum g_i \times 10^{-4} \times W_i \times \frac{44}{0.0224} \quad (4)$$

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 %, $Wi$ 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.

#### 2.4.4. Nutrient bioconversion efficiency

To determine the nutrient bioconversion efficiency, we adapted the bioconversion efficiency equation of Bosch et al. (2019a) as follows:

$$NUE_n = \frac{(B_m\ Y_m - B_s Y_s)_n}{B_d Y_d} \quad (5)$$

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), $Y_m$ 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. 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.

### 3.1. 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 (Fig. 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.

Between 37 and 53% of the starch provided with the substrate was recovered in residues (Fig. 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 3.5). 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 $CO_2$ (Fig. 3A), and nearly no $CH_4$ (Fig. 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 (Fig. 3A). A trial parallel to our

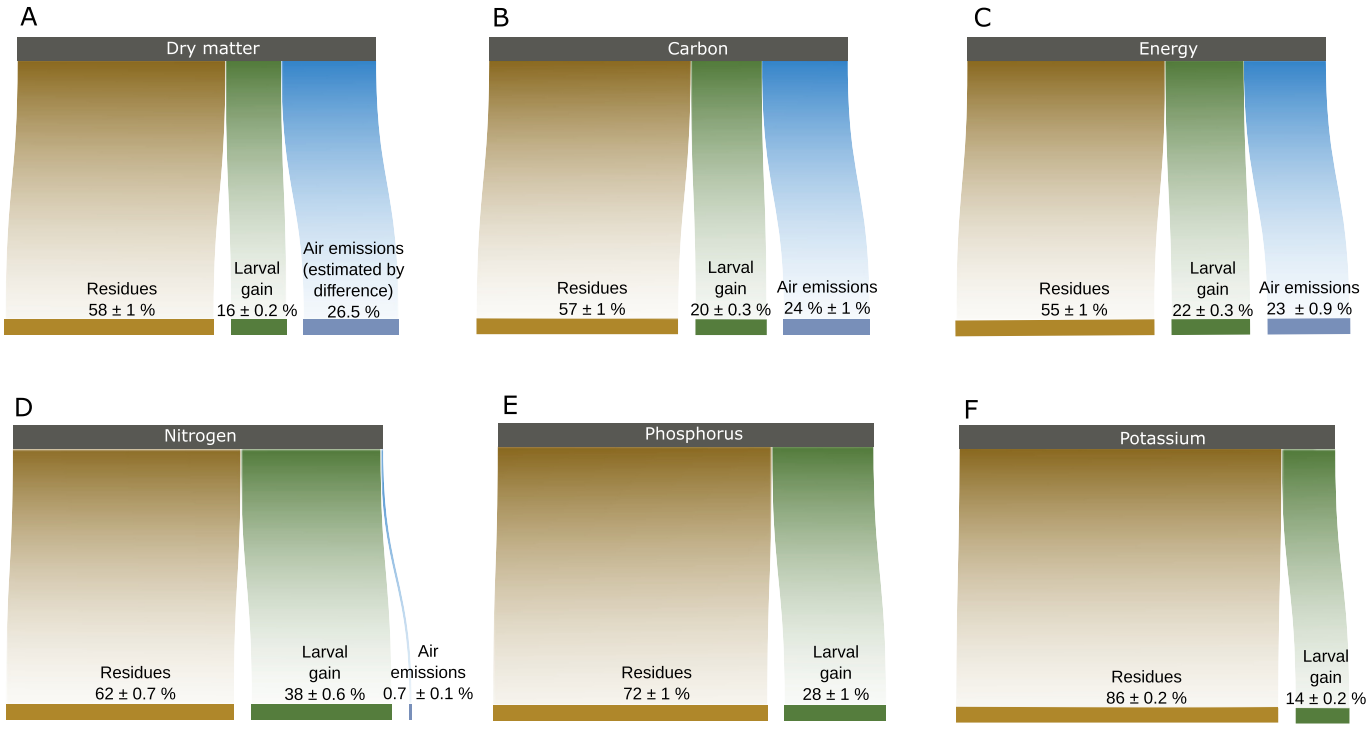


**Fig. 2.** Dry matter, carbon, energy, nitrogen, phosphorus and potassium balances. All balances are expressed in percentage of each output ± standard error of the mean. Substrate was considered as the only input and larval gain shows how much of each input was incorporated as larval biomass (after subtraction of the inputs contained in the starter larvae). See Table S1 for the basic mass values.

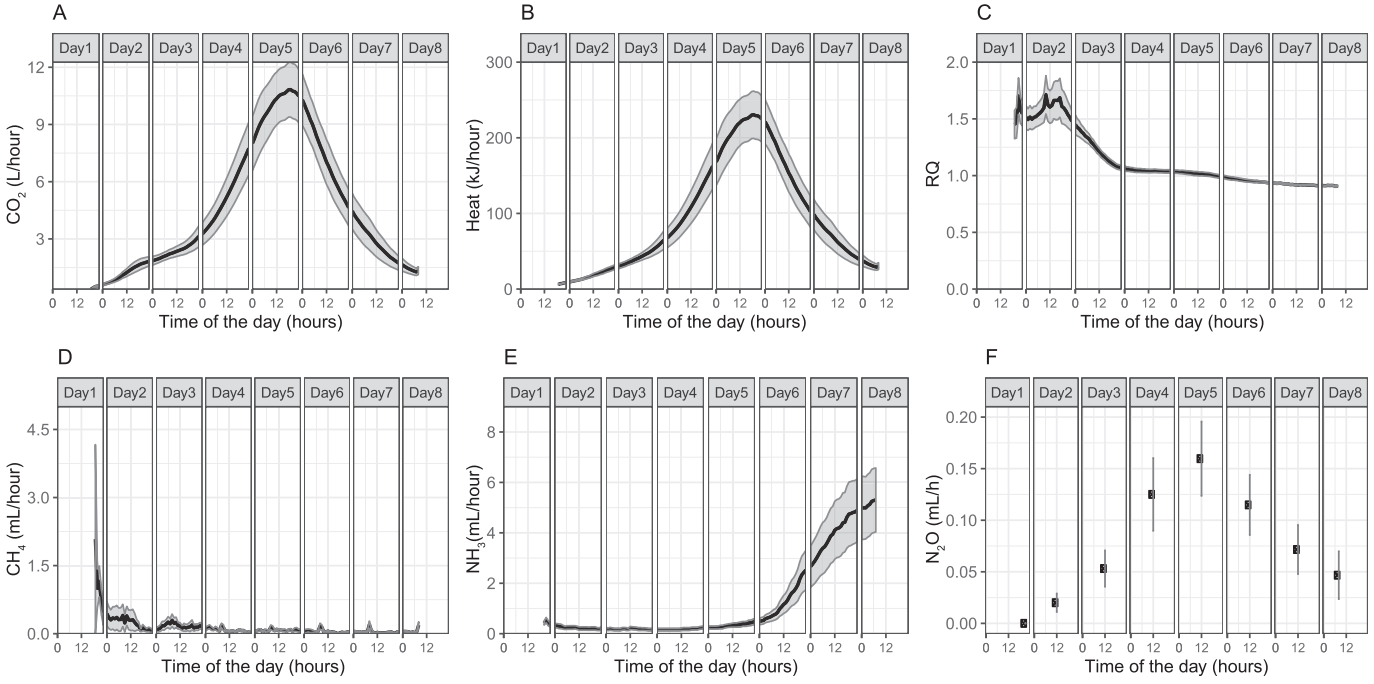


**Fig. 3.** Parameters registered over time in the climate respiration chambers for A) $CO_2$, B) heat, C) RQ, D) $CH_4$, E) $NH_3$ and F) $N_2O$. The black lines show the mean value obtained from 11 replicates (except for $NH_3$ which were 9), and the grey shaded area and error bars (panel F) the standard error of the mean. $N_2O$ was measured once per day. See Fig. S3 for figures per replicate.

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 Fig. 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., 2018b; 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 (Fig. 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 (Fig. 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.

### 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 (Fig. 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. (2019b) 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 (Fig. 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 (Groot 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 (Groot 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 (Groot 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 (Fig. 3E). This pattern is more evident when $CO_2$ and $NH_3$ emissions are observed per replicate (Fig. 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 (Fig. 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 $CO_2$ (Fig. S3; Fig. 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. 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 (Fig. 2E and 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.

### 3.4. 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 2. Overall, the direct emissions from $CH_4$ and $N_2O$ per kg of fresh larval gain were $6 \pm 3.23$ g $CO_2$eq per kg of fresh larvae and $16.8 \pm 8.6$ g $CO_2$eq per kg of dry larvae (see Fig. 3 A-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 2). All studies listed in Table 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 larvae$^{-1}$) 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., 2019, 2015) 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 preprocessing, 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 De 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 $7.58 \pm 2.29$ g $CO_2$eq, 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 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.

### 3.5. 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

**Table 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 - $CO_2$eq (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 (section 1.4).
[b] Standard deviation was large because in one of the repetitions (#11) much more methane 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).

GHG and nitrogen per kg of larvae gain lower.

## 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 $CO_2$eq 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.

## Declaration of competing interestCOI

None.

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## Data statement

The data supporting the findings of this study are available in this paper and its Supplementary data. Raw data and custom R scripts developed for the analyses and visualizations are available at https://doi.org/10.4121/uuid:3a6fa855-4637-4207-be93-eff1436a430f.

## CRediT authorship contribution statement

**Alejandro Parodi:** Conceptualization, Investigation, Formal analysis, Visualization, Writing - original draft. **Imke J.M. De Boer:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Walter J.J. Gerrits:** Conceptualization, Writing - review & editing, Supervision. **Joop J.A. Van Loon:** Conceptualization, Writing - review & editing, Supervision. **Marcel J.W. Heetkamp:** Software, Data curation, Writing - review & editing. **Jeroen Van Schelt:** Writing - review & editing, Resources. **J. Elizabeth Bolhuis:** Writing - review & editing, Project administration, Funding acquisition. **Hannah H.E. Van Zanten:** Conceptualization, Writing - review & editing, Supervision.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.122488.

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