Assessment of the N- and P-Fertilization Effect of Black Soldier Fly (Diptera: Stratiomyidae) By-Products on Maize

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Abstract
To meet the growing demand for an alternative animal protein source, the Black Soldier Fly (BSF) (Hermetia illucens) industry is expanding. Thus, the valuation of its byproducts, foremost BSF frass, is getting more economic and ecological weight. Three different residues, BSF frass, larval skins, and dead adult flies, were compared with a mineral and an organic commercial fertilizer in a pot trial with maize (Zea mays L., [Poales: Poaceae]). byproducts were applied in three nutrient-based application rates (180; 215 kg N/ha; 75 kg P2O5/ha), and plant nutrients, physiological and yield parameters were measured at harvest date. Ground flies had the highest N-fertilizing effect of all byproducts, similar to commercial mineral and organic fertilizers used as controls, whereas its proportion of the BSF production systems’ output is low. Frass as the abundant byproduct showed comparably low N-fertilization effects. Its low N availability was attributed to volatilization losses, mainly driven by high pH and ammonium contents. BSF frass as the main byproduct output is more suited as a basic fertilizer or potting substrate amendment than as a short-term organic fertilizer. Postprocessing of frass seems reasonable. For a profound assessment of frass as fertilizer, several aspects (e.g., the overall impact of postprocessing, plant strengthening and plant protection potential, effects on microbial processes) must be clarified.

Key words: frass, plant, nutrition, residue, corn

Agriculture industry more than doubled its production since the year 2000 and provides nearly 50 % of the fish globally consumed nowadays (FAO 2018). In contrast, fishmeal and fish oil production decreased by 50% between 1994 and 2016 due to overfishing (FAO 2018). As 73 % of fishmeal of fish oil produced is used for fish feed in aquaculture (FAO 2018), the demand for an alternative protein source is immense. Black Soldier Fly (BSF) (Hermetia illucens) is a promising substitute for fishmeal. Depending on the fish species, between 25 and 100% of the fishmeal utilized in aquaculture can be replaced with BSF larvae meal (Belghit et al. 2019); promising studies have also been conducted on poultry, pigs and crustaceans. The protein-rich larvae of this insect can be reared on many substrates, e.g., food waste (Pastor et al. 2015) or even manure (Sheppard et al. 1994, Diener et al. 2015). As insect production companies put much effort in the automation of the processes, BSF rearing will become more cost-effective, especially in developed countries. A massive growth of the production capacities in temperate climates is to be expected (Derrien and Boccuni 2018).

With the increasing importance of the insect production sector, its often-promoted ecological sustainability needs to be assessed as an entity (Berggren et al. 2019), thereby contributing to a basis for decision making in politics and the industry itself. Unfortunately, there is a lack of knowledge about many aspects of insect production. One area that needs deeper research is the use of frass as potential soil amendment, regarding its effects on plant growth, its nutrient composition and its influencing factors on variation, and the ecological aspects of its application (Berggren et al. 2019). When agricultural sustainability and value-added environment-friendly application in agriculture or horticulture are explored, it is of essential importance to determine the actual fertilizing effect of frass.

European insect producers are mostly small or medium-sized companies, of which about 80% are currently rearing BSF; most of them for the use in pet food and aquaculture (Derrien and Boccuni 2018). Present production sums up to a few thousand tonnes per year, but production will expand substantially in the coming years (Derrien and Boccuni 2018). Frass will emerge toward a considerable byproduct as the industry grows rapidly; it represents 80–95% of the total output of a BSF production system (Devic 2016).

So far, only a few plant nutrition studies with BSF frass have been carried out, and its fertilizing effects remain unclear (Berggren et al. 2019). Choi et al. (2009) tested BSF frass against a commercial fertilizer on plots of cabbage, with no differences in growth and volume...
increase after 4 wk. In spring onions, BSF frass from two different rearing substrates improved yields to the same extent as the inorganic control (Devic 2016). Frass from BSF reared on pig slurry increased plant growth for basil and Sudan grass in a pot trial (Newton et al. 2005). Temple et al. (2013) compared a merchantable BSF frass fertilizer with worm compost and poultry manure in incubation, starter, and field trials using four experimental crops (potato, lettuce, Chinese cabbage, green bean). Frass had high N, P, and K concentrations and high availability of these, revealing its relative superiority to poultry manure and compost by the significantly strongest positive yield response.

However, high frass application rates can lead to growth inhibition (Newton et al. 2005, Temple et al. 2013, Alattar et al. 2016) and yield reduction (Newton et al. 2005, Temple et al. 2013), which often is attributed to NH\textsubscript{4}\textsuperscript{+}-N toxicity (Temple et al. 2013, Alattar et al. 2016). This could be a reproducible phenomenon at high application rates of BSF frass. Negative effects of frass on plant growth and germination were found for other insect species as well (Silander et al. 1983, Kagata and Ohgushi 2012) but were associated to allelopathic effects.

The feeding substrate influences frass in its microbial composition as well as in its nutrient content (Poveda et al. 2019). Nitrogen is mainly excreted as urea, uric acid, and allantoin (O’Donnell and Donnini 2017). The nitrogen fraction in the frass of terrestrial insects contains 9–27% of ammonium (Kagata and Ohgushi 2011), but most insects mainly excrete uric acid (Cochran 1985, Halloran et al. 2018). Allantoin breaks down into urea which then is converted into NH\textsubscript{4}\textsuperscript{+} by urease, leading to a high ammonium content in frass (Green and Popa 2012). The conversion of the mentioned nitrogen compounds of frass to ammonia should be relatively low due to its low moisture (Halloran et al. 2018). Although it is likely that ammonia emissions will increase if the moisture content of frass rises, e.g., due to changes in the production system or during fertilizer application, no measurements have been conducted yet (Green and Popa 2012, Halloran et al. 2018).

BSF frass has a higher pH value than most comparable fertilizers. Ma et al. (2018) stated that the maximum growth performance of BSF larvae can be reached on substrates with pH 6.0 or higher, while the larvae adjusted the substrate pH in these treatments to values from 8.0 to 8.5. A similar pH range from 8 to 9 was found by Green and Popa (2012) and Alattar (2012). Gärttling and Schulz (2019) reported a mean pH of 7.75 when averaging 14 frass analyses from different sources. The pH shift during the processing of frass, unprocessed larval skins, frozen flies) was examined for quality parameters (pH (0.01 M CaCl\textsubscript{2}), dry mass, organic matter, residue on ignition—N, NH\textsubscript{4}\textsuperscript{+}-N, P\textsubscript{2}O\textsubscript{5}, K\textsubscript{2}O, CaO, MgO, Na, S—microbial load (Enterobacteriaceae, Salmonella) (Supp Table 1 [online only]).

To assess fresh matter (FM) and organic matter (OM) obtained, insect substrates were dried at a low temperature of 39°C in order to minimize ammonia emissions. Larval skins were ground with a hammer mill at a sieve width of 0.5 mm, adult flies at 1.2 mm. The control fertilizers (granules) and the insect frass (powdery substance) retained their original grain size.

Materials and Methods

Fertilizer Sampling

Three byproducts of the BSF production were examined in the form in which they arise in the production plant. Frass (FR) samples were taken during the sieving process. The larval skins (LS) were separated from the larvae by winnowing. Adult flies (AD) were frozen and emptied directly into lockable buckets. A fraction of each material used for the fertilization experiment (unprocessed frass, unprocessed larval skins, frozen flies) was examined for quality parameters (pH (0.01 M CaCl\textsubscript{2}), dry mass, organic matter, residue on ignition—N, NH\textsubscript{4}\textsuperscript{+}-N, P\textsubscript{2}O\textsubscript{5}, K\textsubscript{2}O, CaO, MgO, Na, S—microbial load (Enterobacteriaceae, Salmonella) (Supp Table 1 [online only]).

Experiment Set-up

In the pot trial, the three fertilizers were applied each in three levels of 180 and 215 kg N/ha as well as 75 kg P\textsubscript{2}O\textsubscript{5}/ha, which was based on a soil surface area of 225 cm\textsuperscript{2} (15 × 15 cm) per pot. The variation of nontargeted nutrients due to different nutrient profiles was not adjusted, leading to application rates displayed in Table 2. Two control treatments were set up on the level of 180 kg N/ha. All treatments were replicated four times. For the organic-fertilized control (ORG), Phytogrieb® GOLD and Provita Haarmehl-Pellets were mixed in the ratio 7:1. The mineral-fertilized control (NPK) contained commercial NPK fertilizer ‘COMPO Rosen Langzeit-Dünger’ (COMPO GmbH, Muenster, Germany). Because of their advantageous N:P ratios (Table 1), the controls also were comparable to the P-fertilized treatments (Table 2). The weighted fertilizer quantities were between 2.79 g (NPK, 180 kg N/ha) and 16.37 g (FR, 215 kg N/ha) per pot, corresponding to a range of 1.24–7.28 t/ha.

For the experiment, a total of 44 plant pots with 15 × 15 × 22-cm dimensions were filled with 1.5 kg of preformulated low-nutrient potting soil (‘F.-E. Typ Nullerde’, Industrie-Erdwerk Archut GmbH & Co. KG, Sauensiek, Germany) for data sheet see Supp Table 2 [online only]). The fertilizers were mixed into the upper 350 g of the potting substrate. Maize seed of the variety ‘PM PRALINIA’ (Planterra, BayWa AG, München, Germany) was sterilized on the day of sowing. For this purpose, the seeds were soaked for two and a half minutes in 10% hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), then rinsed three times with water and poured off. They were then immersed in 70% ethanol for 5 min and again rinsed three times. Floating seeds were
The image analysis platform Fiji (Schindelin et al. 2012). After setting a known length (scan width or scanned ruler) as a scale, the living leaf area was separated by color threshold in a range from 165 to 215 in brightness and measured (for an example see Fig. 1). Doubled leaf areas were considered by polygon selection and added to the leaf area sum of the plant.

The remaining plants of the pot were weighed again, dried at 80°C to constant weight, and shoot dry mass content was determined to calculate DM yields from the fresh mass. Within the next 2 d, the roots were separated, washed, and dried in order to determine its dry weight. The shoots were then ground with a centrifugal mill, whereas the roots were not analyzed further.

For total carbon (C) and total nitrogen (N) analysis, 150 mg of plant powder were weighed in probition vials and analyzed via elemental analysis using the macro analyzer ‘vario MAX CHN’ (Elementar Analysensysteme GmbH, Langenselbold, Germany). For P analysis, two samples of ~0.5 g of plant powder were taken from each replicate to be reduced to ashes in the furnace (550°C for 10 h). The P analysis was carried out using method A 2.4.2.1 from Thun and Hoffmann (1991). Because of partially high P contents, the amount of filtrate used for inking was reduced to 0.5 ml.

### Table 1. Nutrient contents in fresh mass of the organic and mineral fertilizers used for the control treatments (information derived from data sheets)

<table>
<thead>
<tr>
<th>Fertilizer name</th>
<th>N</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 COMPO Rosen Langzeit-Dünger (COMPO GmbH)</td>
<td>14.5</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>2 Provita Haarmehl-Pellets (Beckmann &amp; Brehm GmbH)</td>
<td>14.1</td>
<td>0.89</td>
<td>0.24</td>
</tr>
<tr>
<td>3 Phytogries GOLD (Beckmann &amp; Brehm GmbH)</td>
<td>5.5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4 Mixture of organic fertilizers (2 and 3)</td>
<td>6.6</td>
<td>2.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

### Table 2. Overview of the experimental design of the fertilizer trial and the applied amounts of N, P\textsubscript{2}O\textsubscript{5}, and K\textsubscript{2}O

<table>
<thead>
<tr>
<th>Applied fertilizer</th>
<th>Application level</th>
<th>N (kg/ha)</th>
<th>P\textsubscript{2}O\textsubscript{5} (kg/ha)</th>
<th>K\textsubscript{2}O (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR</td>
<td>N\textsubscript{max}</td>
<td>215</td>
<td>222</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{c}</td>
<td>180</td>
<td>185</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{opt}</td>
<td>73</td>
<td>75</td>
<td>53</td>
</tr>
<tr>
<td>LS</td>
<td>N\textsubscript{max}</td>
<td>215</td>
<td>125</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{c}</td>
<td>180</td>
<td>105</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{opt}</td>
<td>129</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td>AD</td>
<td>N\textsubscript{max}</td>
<td>215</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{c}</td>
<td>180</td>
<td>38</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{opt}</td>
<td>353</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>ORG</td>
<td>N\textsubscript{c}</td>
<td>180</td>
<td>75</td>
<td>49</td>
</tr>
<tr>
<td>NPK</td>
<td>N\textsubscript{c}</td>
<td>180</td>
<td>87</td>
<td>186</td>
</tr>
</tbody>
</table>

Among the applied fertilizers, FR is frass, LS is larval skins, AD is adult flies, ORG is organic mixed-fertilizer and NPK is mineral fertilizer. For the application levels, N\textsubscript{max} is equivalent to 215 kg N/ha, N\textsubscript{c} to 180 kg N/ha and P\textsubscript{opt} to 75 kg P\textsubscript{2}O\textsubscript{5}/ha.

removed. Four seeds per pot were sown at 5-cm depth, and plants were reduced to three after 1 wk.

The pots were watered with demineralized water to their water-holding capacity and readjusted gravimetrically. Target weights were elevated during plant growth to ensure the complete moistening of the pot volume. Those minimum and maximum pot weights were elevated during plant growth, so that the amount of water was enough to moisten the pots to the ground. Weeds were removed. In regular intervals, the pots were randomized using the randomization software Research Randomizer (Urbaniak and Plous 2013).

The average temperature in the greenhouse chamber was 20.9°C [coefficient of variation (CV) = 0.104], with 22.2°C (CV = 0.094) daytime and 19.3°C (CV = 0.048) nighttime temperatures (12: 12 h) (Fig. 2). Average relative humidity (RH) was 67.8 % RH (CV = 0.103), 68.0 % RH (CV = 0.061) at day, and 67.5 % RH (CV = 0.116) at night (Fig. 3). Sodium-vapor lamp lighting was set to 16 h a day.

### Data Processing and Statistical Analysis

Individual datasets were created for the time-related measures during growth, for the final analyses and for the final Yara-N and N\textsubscript{t} results used for the regression function. A one-way analysis of variance (ANOVA) was carried out for all parameters measured at harvest date, followed by the appropriate post-hoc-test if the P-value indicated significant differences between the factor levels. Before applying the ANOVA, all parameters were tested on the assumptions of normality and homoscedasticity. To avoid distortion through extreme values, these were identified in a boxplot of the residues (>3 × height of the box) and excluded if they also showed a Cook’s Distance >1. Outliers were not excluded in order not to unbalance the dataset. The assumption of independency of the samples was assured by the experimental design. For normality, the Shapiro–Wilk test was carried out on the residues and for homoscedasticity, a Levene test was performed. When the assumptions were not met, a square-root- or ln-transformation was performed to fulfill the ANOVA assumptions. If this was successful, the means and the CIs were backtransformed to be displayed in the results after the ANOVA and Tukey’s post-hoc test had been carried out, if not, a Kruskal–Wallis test including the results of the pairwise comparisons was applied on the untransformed data. All statistical analysis was carried out with the SPSS ver. 24.0 (IBM Corp. 2016).
Results

Byproduct Analysis

In Table 3, the results of the analysis of the different insect fertilizers are displayed. Salmonella was not detected in any of the samples (data not shown), while Enterobacteriaceae was present in all of them (>150,000 CFU/g). Drying led to a reduction, whereas freezing did not. The pH value was relatively high in all insect-derived products (mean 7.75). The adult flies (AD) showed a higher water content (41.7 %), in consequence less FM and OM (55.7 %) contents and residue on ignition (2.6 %) than the frass (FR) and the larval skins (LS).

Although the N\textsubscript{i} content in FM and DM were the highest in AD, the most ammonium (NH\textsubscript{4}+\textsubscript{-N}) was found in FR (Table 3). N:P ratios were 2.22 for FR, 3.93 for LS, and 10.79 for AD. The K\textsubscript{2}O contents were lower than N and P\textsubscript{2}O\textsubscript{5} in all insect-related substrates. However, when considering the element forms, potassium concentrations were lowest in AD, whereas phosphorus concentrations were lowest in FR and LS.

The analytical methods used are displayed in Supp Table 1 (online only).

Table 3. Nutrient contents of the BSF by-products

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Frass</th>
<th>Larval skins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical and technical parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>9.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Water content % DM</td>
<td></td>
<td>18.3</td>
<td>15.5</td>
</tr>
<tr>
<td>Organic matter % DM</td>
<td></td>
<td>90.4</td>
<td>91.2</td>
</tr>
<tr>
<td>Residue on ignition (550°C) % DM</td>
<td></td>
<td>9.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Value-determining components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen (N\textsubscript{i}) % DM</td>
<td></td>
<td>3.30</td>
<td>4.80</td>
</tr>
<tr>
<td>Ammonium nitrogen (NH\textsubscript{4}+\textsubscript{-N}) % DM</td>
<td></td>
<td>1.00</td>
<td>0.23</td>
</tr>
<tr>
<td>Phosphate (P\textsubscript{2}O\textsubscript{5}) % DM</td>
<td></td>
<td>3.40</td>
<td>2.80</td>
</tr>
<tr>
<td>Calcium (CaO) % DM</td>
<td></td>
<td>0.40</td>
<td>0.70</td>
</tr>
<tr>
<td>Magnesium (MgO) % DM</td>
<td></td>
<td>1.00</td>
<td>0.68</td>
</tr>
<tr>
<td>Total sulfur (S) % DM</td>
<td></td>
<td>0.53</td>
<td>0.48</td>
</tr>
<tr>
<td>Sodium (Na) % DM</td>
<td></td>
<td>0.26</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Plant Analyses of Maize

Maize nutrient and plant growth parameters of control treatments (ORG, NPK) were comparable to each other (Table 4; P > 0.05). Generally, DM contents decreased with increasing N supply, but significant differences were only found between the highest-yielding (NPK control, 10.9 %) and the lowest-yielding (FR P\textsubscript{opt}, 14.3 % and LS P\textsubscript{opt}, 15.0 %) treatments. The N\textsubscript{i} content tended to increase with higher N fertilization. P\textsubscript{2}O\textsubscript{5} was high, where N was low and vice versa. Since C\textsubscript{i} did not show notable differences, the plant C:N ratio was mainly influenced by N\textsubscript{i}. The N:P ratio increased in the order FR < LS < controls < AD and, within one fertilizer, in the order P\textsubscript{opt} < N\textsubscript{uc} < N\textsubscript{max}. At the N\textsubscript{max} application level, AD fertilization led to higher N and lower P\textsubscript{2}O\textsubscript{5} contents than FR and LS treatments, leading to a wider plant N:P ratio. Due to differing N\textsubscript{i} contents, the plant C:N ratio was also lower for AD N\textsubscript{max} but not significantly.

A similar pattern appeared when the plants were fertilized at N\textsubscript{opt} level, resulting in a grouping of FR/LS versus AD/controls: N\textsubscript{i} contents of FR and LS were comparable inter alia and lower than AD, which was comparable to the NPK control. ORG was in between and not significantly different from any other treatment. P\textsubscript{2}O\textsubscript{5} contents were low for AD and the ORG control and high for LS and FR, leaving the NPK control in between. This ranking followed the P\textsubscript{2}O\textsubscript{5} application rates in this treatment. The C:N ratio of AD was lower than that of FR and LS, which were comparable to each other. The plants’ N:P ratio decreased in the order AD > ORG > NPK > LS > FR, analogous to the fertilizers’ N:P ratios.

In the P\textsubscript{opt} treatment, the N\textsubscript{i} contents followed N application rates. Despite an equal P\textsubscript{2}O\textsubscript{5} application rate, P\textsubscript{2}O\textsubscript{5} contents were high in lower-yielding FR and LS and low in higher-yielding AD and ORG.

Nitrogen Nutrition of Treatments

Concerning the NUE, N output and NNI in the moderately fertilized N\textsubscript{uc} treatments, FR and LS grouped together, as well as AD and the controls (Table 5). This difference between FR/LS and AD was also visible in the N\textsubscript{max} application rate. Within all fertilizers, NUE increased with higher N application rates except for the highly N fertilized AD P\textsubscript{opt} treatment. In this treatment, the highest NNI (1.20) and N surplus (270.32 kg/ha) was observed.
**Table 4.** Nutrients, fractions, and nutrient ratios of the aerial maize biomass

<table>
<thead>
<tr>
<th>Fert.</th>
<th>Appl. level</th>
<th>DM (% FM)</th>
<th>N&lt;sub&gt;i&lt;/sub&gt; (% DM)</th>
<th>P&lt;sub&gt;O&lt;sub&gt;i&lt;/sub&gt;&lt;/sub&gt; (% DM)</th>
<th>C:N ratio</th>
<th>N:P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>FR N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>12.705 (±0.518)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.934, [0.762, 1.319]&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.149, [1.162, 3.974]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.04, [1.801, 3.66]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.047 (±4.027)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.722, [0.498, 1.047]&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>FR N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>13.966 (±0.661)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.089, [0.753, 0.869]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.568, [1.801, 3.66]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.047 (±0.027)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.722, [0.498, 1.047]&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>LS N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>12.879 (±0.961)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.147, [0.661, 1.99]&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1.624, [0.851, 3.101]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.051 (±4.228)&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.997, [0.669, 1.485]&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>LS N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>14.645 (±0.767)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.869, [0.623, 1.214]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.188, [0.942, 1.761]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50.496 (±4.992)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.545, [1.037, 2.301]&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AD N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>12.887 (±0.758)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.943, [0.104, 3.48]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.377, [0.228, 0.624]&lt;sup&gt;j&lt;/sup&gt;</td>
<td>21.159 (±2.07)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47, [0.358, 0.617]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AD N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>13.185 (±0.877)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.089, [0.753, 0.869]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.568, [1.801, 3.66]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.047 (±4.027)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.722, [0.498, 1.047]&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ORG</td>
<td>13.417 (±0.748)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.537, [0.999, 2.367]&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.377, [0.228, 0.367]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.159 (±2.07)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47, [0.358, 0.617]&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>NPK N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>10.902 (±0.572)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.052, [1.356, 3.107]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.377, [0.228, 0.624]&lt;sup&gt;j&lt;/sup&gt;</td>
<td>21.159 (±2.07)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47, [0.358, 0.617]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NPK N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>12.879 (±0.961)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.147, [0.661, 1.99]&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1.624, [0.851, 3.101]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.051 (±4.228)&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.997, [0.669, 1.485]&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NPK</td>
<td>14.645 (±0.767)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.869, [0.623, 1.214]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.188, [0.942, 1.761]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50.496 (±4.992)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.545, [1.037, 2.301]&lt;sup&gt;j&lt;/sup&gt;</td>
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</tbody>
</table>

**Table 5.** Nitrogen nutrition key figures of the fertilizing experiment

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>Appl. level</th>
<th>NUE (%)</th>
<th>N output (kg/ha)</th>
<th>N surplus (kg/ha)</th>
<th>NNI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frass N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>14.96, [12.15, 18.42]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>32.169, [26.122, 39.616]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>182.629 (± 2.07)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.47, [0.358, 0.617]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Frass N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>15.26, [11.55, 20.16]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>27.467, [20.787, 36.294]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.231 (± 2.247)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.409, [0.322, 0.52]&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Larval skins P&lt;sub&gt;m&lt;/sub&gt;</td>
<td>17.29, [15.34, 19.24]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>37.151, [33.38, 41.349]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>177.773 (± 1.286)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.321, [0.354, 0.766]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Larval skins P&lt;sub&gt;n&lt;/sub&gt;</td>
<td>14.54, [11.12, 19.02]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>26.18, [20.015, 34.243]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>133.54 (± 1.125)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.384, [0.302, 0.49]&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Adult flies P&lt;sub&gt;m&lt;/sub&gt;</td>
<td>17.29, [15.34, 19.24]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>37.151, [33.38, 41.349]&lt;sup&gt;d&lt;/sup&gt;</td>
<td>177.773 (± 1.286)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.321, [0.354, 0.766]&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Adult flies P&lt;sub&gt;n&lt;/sub&gt;</td>
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<td>26.18, [20.015, 34.243]&lt;sup&gt;c&lt;/sup&gt;</td>
<td>133.54 (± 1.125)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.384, [0.302, 0.49]&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>P&lt;sub&gt;n&lt;/sub&gt;</td>
<td>11.18, [10.08, 15.02]&lt;sup&gt;i&lt;/sup&gt;</td>
<td>15.26, [10.015, 17.81]&lt;sup&gt;i&lt;/sup&gt;</td>
<td>113.724 (± 0.871)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.304, [0.269, 0.344]&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Organic fertilizer N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>34.3, [28.72, 40.96]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>73.774, [61.78, 88.09]&lt;sup&gt;f&lt;/sup&gt;</td>
<td>140.905 (± 4.078)&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.999, [0.738, 1.351]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Organic fertilizer N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>32.53, [27.91, 37.91]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>58.557, [50.245, 68.244]&lt;sup&gt;f&lt;/sup&gt;</td>
<td>121.219 (± 2.874)&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.859, [0.624, 1.183]&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NPK N&lt;sub&gt;m&lt;/sub&gt;</td>
<td>30.94, [21.51, 44.51]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>55.701, [38.724, 80.121]&lt;sup&gt;e&lt;/sup&gt;</td>
<td>123.218 (± 9.509)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.726, [0.502, 1.054]&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>NPK N&lt;sub&gt;n&lt;/sub&gt;</td>
<td>46.95, [42.33, 52.09]&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>84.521, [76.192, 93.761]&lt;sup&gt;e&lt;/sup&gt;</td>
<td>270.321 (± 14.873)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.204, [1.112, 1.304]&lt;sup&gt;f&lt;/sup&gt;</td>
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**F-value**<br>P-value<br>P-value | <br>P = 0.000 | <br>P = 0.000 | <br>P = 0.000 | <br>P = 0.000 | <br>P = 0.000 |

Intransformed and backtransformed data are means with confidence intervals (CI95 = []), unprocessed data are means (±SE), n = 4; α = 0.05.
Aboveground DM yields of maize and their corresponding nitrogen concentration (%N) are shown in Fig. 4. They are put in context to the critical N concentration curve for maize and its minimal and maximal envelope curves published by Lemaire et al. (1997). Since Lemaire’s equations for Nc% are only valid for yields >1 t DM/ha, a constant of 3.40% was used for %Nc for 3 of the 44 pots, thereby following his proposal. The observed minimum plant %N was at 0.698% DM. There is a greater part of the values below the %Nc curve than above. Even about half of the pots (n = 20) were below Lemaire’s minimal envelope curve and allocated near the minimum plant %N constant. When reversed, the displayed values in Fig. 4 build up an optimum curve of the DM yields against %N, having the highest scatter in the optimum range. A very similar graph can be observed when %N is replaced by the NNI.

Yield-Related Plant Parameters
At the same N nutrition level, the applied fertilizers were not distinguishable from each other in these parameters (Table 6). In leaf area, the NPK treatment was comparable to AD and ORG. The increase in N supply from Nuc to Nmax was not reflected in growth parameters, but N supply below Nuc revealed negative effects. Control and AD treatments were not distinguishable from each other. Overall, growth parameters tended to increase with higher N application and showed better performance of the controls, especially NPK, over the insect-derived treatments. N application levels affected plant growth parameters more strongly than different N availability caused by the fertilizer’s characteristics; in that aspect, only trends were visible.

Discussion
Byproduct Characteristics
High DM contents of BSF frass improved its conservation properties and led to high nitrogen concentrations in FM (Table 3) compared with other farm manures and composts which (apart from chicken
manure) are below 1% in FM (LfL Bayern 2018). Thus, lower application rates are needed for the nutrient application. An even N:\P2O5 ratio with slightly lower K2O contents (Table 3) is not common among farm manures. In most manures, N dominates P2O5, as is the case for the nitrogen removal of most agricultural crops (LfL Bayern 2018). Although the NH4+\-N fraction of N was very high for frass from terrestrial insects (Kagata and Ohgushi 2011), it was low compared with other farm manures (Gutser et al. 2005), and ammonia volatilization with the increasing humidity after application was possible due to the high pH observed (Table 3).

Both LS and AD fertilizers showed higher N, but lower NH4+ concentrations than FR, combined with moderate pH values (Table 3). The fertilizer gained from adult flies has by far higher N concentrations in DM but should be dried to allow grinding and enhance conservation properties. Based on its N:P ratio, AD was the only byproduct fertilizer that was nitrogen dominated. AD had N concentrations comparable to usual commercial organic fertilizers.

Although growth depression after frass application occurred in other experiments, this was not the case for this experiment. Alattar et al (2016) observed it for sweet corn, which was associated with ammonium toxicity. Although corn was comparably insensitive to ammonium toxicity (Moritsugu et al. 1983, Spiegel et al. 1986), it affected sweet corn to a greater extent than silage maize (Hauck et al. 1984).

As Berggren et al. (2019) summarized, the current data situation regarding the effects of frass fertilization on plant growth is small and unclear in its outcomes. Different frass qualities critically influenced its effects on plant growth (Kagata and Ohgushi 2012). The authors stating negative effects of frass application on plant growth (Newton et al. 2005, Temple et al. 2013, Alattar 2016) compared it with compost or similar materials at fresh weight or volume equivalents. However, the utilized frass showed threefold higher %N in FM compared with common biowaste composts (LfL Bayern 2018), in addition to higher NH4+ concentrations. When lower rates, e.g., comparable to manure application, were applied, like in Zahn (2017), an optimum effect in plant growth was observed. Based on the reviewed literature, the characteristics of frass that lead to plant growth inhibition could not be identified definitively. The choice of silage maize as experimental crop and comparably low application rates may have helped to avoid negative effects on plant growth.

The finding that the concentration of Salmonella spp. was below the detection limit was concordant with the literature (Lalander et al. 2013, 2015), where an effective reduction of Salmonella spp. through BSF larvae was reported. Escherichia coli as a member of Enterobacteriaceae was described to be reduced in substrates through BSF treatment (Erickson et al. 2004, Liu et al. 2008). However, those suppressive effects were partly attributed to temperature (Liu et al. 2008) and uncharged ammonia concentrations (Lalander et al. 2015). The fact that high Enterobacteriaceae infestation was present in all BSF fertilizers could either have resulted from differences in

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**Fig. 3.** Time course of the mean relative humidity in the climate chamber during the day. Measurements took place every 12 min during the 10-wk experiment. Of each measurement time, mean and variation range (maximum – minimum) are depicted.

**Fig. 4.** Relationship between %N and aerial biomass (W). %Nc function with minimum (%Nmin) and maximum (%Nmax) envelope curves from Lemaire (1997). Constant minimum plant N based on the least %N of the dataset.
the above-mentioned parameters or from a different proportion of 
E. coli in Enterobacteriaceae (Lalander et al. 2015). Drying (80°C, 
1 h) was the better way for the reduction of Enterobacteriaceae in 
comparison to freezing but did not lead to an eradication. Both 
increased DM contents (Himathongkham and Riemann 1999) and 
high drying temperatures (Ghaly and Alhattab 2013) could have 
had reduced the microbial load. However, drying manures of high pH 
at high temperatures can lead to a substantial volatilization of am- 
monia (Ghaly and Alhattab 2013, Pantelopoulos et al. 2016). The ef-
fects of different drying temperatures and durations were not tested.

Nitrogen Fertilizing Effect Through Insect-Derived 
Fertilizers
High N supply led to low DM, which was most clearly visible for AD 
and NPK treatments (Table 4). Also treatments with high DM yields 
exhibited low DM contents. A well-nourished and therefore high 
yielding plant matures as fast as a plant in N deficiency (Marschner 
2012) and, as a result, generative growth which is associated with 
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facts of different drying temperatures and durations were not tested.
seemed to exhibit the highest N supply (Ciampitti et al. 2013). At very high N application rates (AD $P_{\text{o}}$), the ratio decreased because P assimilation increased faster than N assimilation. Lowered yields (Table 6) and NUE (Table 5) indicated N oversupply when fertilizing on phosphorus demand, so the fertilizer’s N:P ratio did not meet the optimum assimilation ratio of maize.

The close fertilizer N:P ratio in FR and LS led to an even lower plant N:P ratio caused by high plant P concentrations (Table 4). Plants when experiencing N deficiency respond through the enhancement of root growth which results in high root DM weights and a lower SRR (Marshner 2012). As P is not as soluble as N, the enhanced root system improves more P than N nutrition (Blume et al. 2010), resulting in higher P concentrations in DM. Thus, the increased P concentrations of FR and LS treatments must be rather associated with N deficiency than with pure P fertilizing effects.

However, high NH$_4^+$ fertilization stimulates P assimilation (Marshner 2012) and could thereby have contributed to the higher P concentrations. Another reason for high P contents could be an increased mycorrhization (Grant et al. 2005), as reported by Zahn (2017) and Lovett and Ruesink (1995) in frass. However, no parameters were investigated in this experiment that could support this hypothesis.

Discussion of Methods

The choice of maize as experimental crop was appropriate, as it demands the main part of N and P within the 10-wk growing period (Ciampitti et al. 2013) and is not affected severely by NH$_4^+$ toxicity (Moritsugu et al. 1983), which was an obstacle reported in former frass experiments (Temple et al. 2013, Zahn 2017).

By drying the insect-derived fertilizers before application, ammonia volatilization was expected and could have occurred. The proportions of NH$_3$-N in total nitrogen (Table 3) were high compared with other BSF frass analyses (Garrtling and Schulz 2019) but still lower than poultry manure and various slurries (Gutser et al. 2005). Therefore, a tolerably low volatilization was expected, as drying was necessary to enhance conservation properties and as freeze-drying was not available. However, ammonia losses were not investigated and should be quantified in future to assess the volatilization risk of this process. Ammonia losses during postprocessing could partially explain the bad performance of frass as well as the absence of ammonium toxicity symptoms in the plants. As dry frass was associated with ammonia volatilization during rearing and storage (Halloran et al. 2016), the impact of the rearing system on the nutrient contents of frass should be addressed in future research.

The utilized experimental design with two nitrogen-balanced and one phosphorus-balanced application level without an additional N-leveling was not suitable to assess the phosphorus fertilizing effect of the fertilizers, as it was distorted by alternating N nutrition. A better but more complex design would have been a solely N-based increase series, maybe in combination with a separate P-increase series. However, N-leveling with a mineral fertilizer in this series is not appropriate to offset distortion by different N nutrition because of the fertilizers’ variable N mineralization characteristics.

The variation in yields at the same NNI indicated that the instantaneous NNI did not reflect the N supply during growth appropriately. Higher-yielding plants suffering from N deficiency at harvest date had a better N supply during growth than lower-yielding plants of the same NNI. For an increased informative value of the NNI, it could have been integrated over the growth period, thereby demanding a preharvest of additional pots. The data of the %N$_c$ equations for maize were obtained from a series of field experiments.

The main ecophysiological reason for the decrease of %N$_c$ was suggested to be the changing ratio of structural and metabolic tissue (Lemaire 1997). Under greenhouse conditions, stem thickening was slower than it would have been expected in open field. With a slower increase of the fraction of structural tissue in plant DM, %N$_c$ was estimated to be lower, maybe resulting in an overestimation of the NNI and, thus, the plant nutrition status.

Application Purposes and Implications for Postprocessing

Although larval skins and especially adult flies exhibited better fertilizing effects than frass, a separate fertilizer processing would not be economical, as both byproducts are a quite small part of the byproduct output of BSF production. Thus, both substrates could be used to blend with the frass fertilizer, which is by far the greatest by-product output (Devic 2016), thereby improving the N:P profile of this substrate. The problems of different particle sizes of the products could be overcome by milling, sieving, and/or pelletizing. However, a further valuation by chitin extraction could also be possible (Cammack and Tomberlin 2017).

Currently, most of the frass fertilizers are marketed as garden fertilizers, but its use especially in organic agriculture and horticulture is also possible, as there is a higher demand for high-value organic fertilizers. Based on the performance in this trial, an application in basic fertilizer is recommended, so it could be further supplemented by other fertilizers, oriented toward the needs of the crop. Slow-releasing N fertilizers also provided N to the subsequent crop, which ultimately increased their long-term efficiency (Gutser et al. 2005).

As the analyses on frass pH and NH$_4^+$ concentrations revealed favorable conditions for ammonia volatilization, postprocessing of the substrate seems reasonable but is not well-explored. Drying reduces phytotoxic effects (Alattar et al. 2016) and, as also shown in this study for Enterobacteriaceae, pathogens (Himathongkham and Riemann 1999, Ghaly and Alhattab 2013), but increases the risk of ammonia volatilization (Kagata and Ohgushi 2012, Halloran et al. 2016). Acidification of manures is known to reduce ammonia volatilization in farm manures (Pantelopoulos et al. 2016) and can also be used for chitin-rich substrates (Spiegel et al. 1986). Acidified manures show low ammonia volatilization even at high drying temperatures (Pantelopoulos et al. 2016), which could also improve hygienization effects. The amplification of the C:N ratio through blending with compost is suggested to reduce volatilization (Spiegel et al. 1986, Cortes Ortiz et al. 2016) and opens new application purposes, e.g., as formulated potting substrate. Postprocessing is not only recommendable to reduce greenhouse gas emissions of the whole production system but can contribute to improve N fertilizing effect of frass to widen the application purposes in agriculture and horticulture.

Conclusions

The hypothesis of nutritional qualities of all insect derived fertilizers being comparable with other organic fertilizers was not met. BSF frass itself performed worst in most parameters of the conducted trial, especially concerning its N fertilization effect. Plants fertilized with frass showed lower performance in NNI and NUE as well as smaller leaf area and narrower SRR, compared with adult fly fertilizer and especially the controls. Thus, nutrient status and habitus of frass and AD/controls were assumed to differ. DM contents indicating physiological age as well as yields displayed minor differences but seemed more related to application levels than to the
choice of fertilizer, which may have resulted from the limited duration of the experiment and premature harvest. Due to the experimental design, the P-fertilization effects found for frass could not clearly be separated from the N-fertilization effects.

Although drying seems to be reasonable for conservation and hygienization purposes, it may have led to substantial volatilization of ammonia, thereby affecting the performance of frass in the trial. Other conservation methods or acidification prior to drying seem reasonable. Therefore, the results point to BSF frass use as a possible lower-level organic composite NPK fertilizer.

The literature on BSF frass suggests several beneficial and disadvantageous effects which have not been assessed in this study. For a valuation of frass as a fertilizer at larger scale, this seems to be a promising research area, e.g., regarding plant protection characteristics or for identifying and avoiding phytotoxic effects through frass application.

Supplementary Data

Supplementary data are available at Journal of Insect Science online.

Acknowledgments

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