Black Soldier Fly (*Hermetia illucens*, Diptera: Stratiomyidae) frass as plant fertilizer

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Yellow biotechnology, encompassing the biotechnological use of insects in food production, plant protection and drug development is on the rise in Europe, as its potential has been increasingly recognized over the past decade (Vilcinskas, 2014). Companies are establishing insect mass rearing and processing facilities (e.g. Bühler Insect Technology Solutions AG, CH; Protix Biosystems BV, NL; AgriProtein GmbH, D) and are mostly specializing on the breeding of the Black Soldier Fly (*Hermetia illucens*, BSF). Therefore, rearing conditions of BSF and options for post-processing must be optimized to make the production feasible and guarantee stable and safe product outputs.

Fast development, low-maintenance, and favorable nutritional values combined with the ability to degrade a broad spectrum of biogenic wastes make BSF larvae a promising candidate to tackle socio-ecological problems and contribute to circular economy (Cickova *et al.*, 2015; Clariza Samayoa *et al.*, 2016; Pastor *et al.*, 2015). During the degradation process, wastes are turned into larval protein, fat and chitin that can further be separated and transformed into secondary products (Oonincx *et al.*, 2015; Spranghers *et al.*, 2017). Protein has high demand in the feed production industry, the fat can be used to produce biodiesel and chitin can find use in agricultural and pharmaceutical applications (St-Hilaire *et al.*, 2007; Surendra *et al.*, 2016; Tharanathan and Kittur, 2003). The residues consisting of undigested waste material, larval shavings and feces can be applied as fertilizer (Xiao *et al.*, 2018). We subjected BSF larvae to three different diets, collected and characterized the resulting residues (known as frass) and applied them as soil fertilizer for ryegrass (*Lolium perenne*) in greenhouse experiments.

In feeding experiments, 300 BSF larvae were collectively nursed on ground chickenfeed (40:60 mixed with water; CF) until six days post-hatching. Subsequently, two groups of 100 larvae each were introduced to two new diets (grass-cuttings=GC and fruit/vegetable mix=FV) while a same sized control group was further fed with chickenfeed. All diets were equalized in their water and organic content. Environmental conditions were kept stable at 27 °C and 60% relative humidity. Samples (n = 4 larvae) were taken in a three-day rhythm starting with day six to determine fresh, dry and organic dry weight.

To estimate bacterial load on larval surfaces, sampled larvae were washed in sterile a. dest. and the washing solutions were plated on standard nutrient agar (0.5% peptone from meat, 0.25% yeast extract, 0.1% glucose, 1.5% agar; pH=7), ChromoCult® coliform agar (Merck KGaA, Darmstadt, Germany) and XLT-4 *Salmonella* agar (Merck KGaA, Darmstadt, Germany). Samples from frass and soil were diluted in sterile 0.95% NaCl solution, shaken and plated on ChromoCult® coliform agar.

The greenhouse experiments were conducted in four 500 mL pots per treatment (NH_4NO_3 control, CF frass, GC frass, FV frass). The soil was sieved (< 4 mm) and mixed with a vermiculte/sand blend at a ratio of 2:1 w:w (soil:blend) and the four beforehand characterized amendments were added in amounts of 40 mg N kg^{-1}. Each pot was seeded with 0.15 g of *Lolium perenne* seeds and incubated for 28 days at 20 °C. After incubation, plants were removed, and soil was sieved (< 2mm). Soil physicochemical parameters, available nutrients, microbial respiration and plant biomass were determined.

The type of diet (CF, GC, FV) defined the developmental progress of the BSF larvae and led to significant differences in the metabolization of substrates. In FV treatments, the highest fraction of substrate was degraded without being invested in larval biomass while CF treatments resulted in highest amounts of residues. All larvae subjected to a GC diet reached prepupal stage within 19 days after treatment start, while at this point only 22% FV-fed and 55% of CF-fed larvae transitioned to prepupal stage. After day six, *E. coli* were present in all samples from CF treatments with abundances in the range of 10^5 to 10^6 CFUs mL^{-1}. In the GC and FV treatments *E. coli* were detected only in lower numbers at two sampling time points. No *Salmonella* sp. were detected during the trial.

After termination of the feeding experiment, residual frass was collected and characterized. Frass was then amended to the experimental soils (Table 1). Although administered diets were different, the frass fractions of the treatments showed comparable physicochemical properties.

Table 1. Physicochemical characterization of Black Soldier Fly frass derived from different diets.

	GC-Frass	FV-Frass	CF-Frass
pH	5.40 ± 0.03	5.58 ± 0.01	6.22 ± 0.14
EC [mS cm^{-1}]	3.06 ± 0.03	2.36 ± 0.11	5.67 ± 0.27
C_{tot} [g kg^{-1}]	443 ± 6	488 ± 4	479 ± 8
N_{tot} [g kg^{-1}]	24.4 ± 0.2	18.3 ± 1.2	25.9 ± 0.9
C:N ratio	18.2 ± 0.4	26.6 ± 1.7	18.5 ± 0.3
VS [g kg^{-1}]	825 ± 9	873 ± 4	910 ± 7

A more detailed characterization of the BSF frass amended soils and a comparison with a NH_4NO_3 amended control was done after 28 days when the greenhouse experiments were terminated. There was no significant difference of plant biomass, soil total carbon, as well as soil total nitrogen content between treatments and control. Nitrate and dissolved nitrogen content were higher in the control group than in the frass treated soils (45.2 ± 0.13 vs 14.8 ± 4.01 mg kg^{-1} for NO_3^-; 35.6 ± 3.9 vs 15.77 ± 1.55 mg kg^{-1} for dissolved N); On the other hand, frass amended soils showed higher phosphorus bioavailability (0.77 ± 0.5%) compared to the control (0.67 ± 0.1%).

The mass rearing of insects such as the BSF, where each component of the organism can be industrially processed, can contribute to the increasing demand of animal feed for fisheries and poultry farming but can also offer an alternative to organic waste management. In this study we tried to further close the loop by making use of BSF frass that accumulates during the degradation of various diets and investigated its performance as fertilizer. The characteristics of frass amended soil were similar to NH_4NO_3 amended soil and resulted in comparable plant biomass output. Moreover, the amendment of *E. coli* contaminated frass led to a complete reduction of these pathogens when used as soil amendment, suggesting that the use of frass as fertilizer could contribute to a circular economy.

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