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# Assessment of the Short-Term Fertilizer Potential of Mealworm Frass Using a Pot Experiment

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The forecasted growth of insect production in the next few years will generate high quantities of frass (insect excreta). Although frass is increasingly considered a potential fertilizer, the dynamics of nutrient supply by frass is still poorly understood. Here, we aimed at gaining insight into the short-term fertilizer value of frass from mealworm (*Tenebrio molitor* L.) in order to optimize its sustainable use in agroecosystems. Using a short-term pot experiment, we showed that, even though frass has a great potential to be used as a substitute of mineral NPK fertilizer, its N fertilizer potential is mediated by its rate of application. At 10 t ha<sup>-1</sup>, due to its fast mineralization coupled with improvement in microbial activity (assessed using Biolog EcoPlate), frass was as effective as mineral fertilizer to supply N to plant. By contrast, at 5 t ha<sup>-1</sup>, the lower frass mineralization induced a reduced N uptake compared to its mineral control. Unlike N, frass was as effective as mineral fertilizer to supply P and K to plants irrespective of its application rate. This was attributed to the presence of P and K in a readily available form in frass. Taken together, our results indicate that mealworm frass supplies very rapidly N, P and K to plants but its effects on N dynamics should be better investigated to warrant its sustainable use as an alternative fertilizer for managing NPK nutrition in cropping systems.

**Keywords:** fertilizer, frass, insect excreta, mealworm, nitrogen mineralization, organic amendment, NPK, *Tenebrio molitor*

## INTRODUCTION

Fertilization of soils is essential to achieve the high yields required to feed an ever-increasing human population. However, the extensive use of chemical fertilizers leads to an increased consumption of energy and non-renewable resources (Chojnacka et al., 2020) while causing air and water pollution (Savci, 2012). In this regard, recent efforts have been channeled toward more sustainable resources for managing plant nutrition in cropping systems (Faucon et al., 2015). This has sparked a growing interest in the use of renewable feedstock from waste material to replace conventional fertilizers (Chew et al., 2019).

Recently, frass (insect excreta) has been considered a promising resource for managing plant nutrition in cropping system (Houben et al., 2020; Chavez and Uchanski, inpress). The growth of massive insect production in the near future to meet the need of finding alternative source of proteins (Derrien and Bocconi, 2018) is expected to produce frass in large quantities (Poveda, 2021). Due to its high content in nitrogen (N), phosphorus (P) and potassium (K) as well as the potential presence of beneficial microorganisms (Poveda et al., 2019), the use of frass as a fertilizer could help in reducing the use of agrochemicals. For instance, frass from black soldier

fly (*Hermetia illucens* L.) was successfully used as an organic fertilizer to promote the growth of maize (Beesigamukama et al., 2020a; Gärttling et al., 2020) and ryegrass (Menino et al., 2021). In addition, frass from mealworm (*Tenebrio molitor* L.) showed great potential to be used as a partial or a complete substitute for mineral NPK fertilizer for the growth of barley (Houben et al., 2020) while stimulating soil microbial (Poveda et al., 2019) and earthworm activity (Dulaurent et al., 2020). However, as highlighted by Beesigamukama et al. (2020b), the fertilizer potential of frass may be affected by its rate of application in soil. Although the review by Chavez and Uchanski (inpress) shows promising results with the use of frass as a fertilizer, it also highlights that the optimal rate of frass application should be clarified because it may strongly differ between the couple of existing studies so far.

Moreover, the dynamics of nutrient supply by frass is still poorly understood. Knowledge on short-term availability of N and, to a lesser extent, P and K, after application of organic fertilizers is pivotal to optimize fertilizer use with benefits for the farmer and the environment (Gutser et al., 2005). Application of organic fertilizer does not always involve a short-term increase in availability of plant nutrients and crop productivity due to, among others, microbial immobilization which subsequently reduces short-term nutrient availability to the plants (Geisseler et al., 2010). As a result, several studies have pointed out that the use of organic fertilizers may compromise crop yield as compared to inorganic fertilizers because of the reduced input of readily available plant nutrients and the absence of rapid and short-term beneficial effects on microbial properties (Pimentel et al., 2005).

Because the upscaling of the insect industry which currently takes place calls for more research on the fertilizer potential of frass (Berggren et al., 2019), the present study aimed therefore at gaining insight into the short-term fertilizer value of frass from mealworm in order to optimize the sustainable use of frass as an alternative to mineral fertilizers.

## MATERIALS AND METHODS

### Frass

Frass (YnFrass) from mealworm (*T. molitor* L.) was provided in the form of powder by Ynsect (Paris, France), an industrial company farming this insect at the large-scale. The mealworms were fed exclusively on raw materials authorized by French and European regulations for farm animal feeds. The frass was provided and used as such, that is with no chemical input, making it a fertilizer compatible with organic farming and not subject to any specific restrictions. Table 1 shows the chemical characteristics of the mealworm frass used in this study.

### Soil

The studied soil was sampled in Beauvais (Northern France) and was classified as a Haplic Luvisol. Soil characterization was carried out by Houben et al. (2020) and revealed that the soil was a silt loam (USDA classification) with 16% sand, 67% silt, and 17% clay. Organic C was 1.54% and total N was 0.18%. Available concentrations as assessed using the acetate ammonium-EDTA extraction (Houben et al., 2011) were Ca 3869 mg kg<sup>-1</sup>, Mg 101 mg kg<sup>-1</sup>, K 292 mg kg<sup>-1</sup>, P 72 mg kg<sup>-1</sup>. The cation exchange capacity (CEC) was 12.5 cmol<sub>c</sub> kg<sup>-1</sup> and pH was 7.8.

### Treatments

The frass was applied to the soil at a rate of 5 and 10 t ha<sup>-1</sup> (hereafter called “Frass-5” and “Frass-10,” respectively). Two mineral treatments adding the same quantity of N, P and K as in the Frass-5 and Frass-10 treatments were achieved by mixing the soil with appropriate amount of inorganic nutrients (NH<sub>4</sub>NO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and KCl) in solution (hereafter called “NPK-5” and “NPK-10” treatments, respectively). Untreated (hereafter called “Control”) soil was also part of the experimental design.

### Pot Experiment

A pot experiment was conducted to assess the effect of frass on the nutrient availability for plants. Plastic plant pots (11.5-cm diameter, 10.5-cm height) were filled with 450 g of each mixture in four replicates. Before sowing, the pots were placed in a controlled dark room and the mixtures were equilibrated during one week at 80% WHC. After the equilibration period, the pots were transferred to a greenhouse glass and were arranged according to a randomized design. In each pot, 1.5 g seeds of Italian ryegrass (*Lolium multiflorum* Lam.) were sown. The trials were conducted under controlled greenhouse conditions (temperature 18–25°C, 16-h photoperiod) with daily sprinkler watering. After 4 weeks, shoots were harvested by cutting 1 cm above the soil with ceramic scissors, dried (60°C; 72 h), weighed and crushed. The nutrient concentration of the aerial parts was then analyzed by ICP-AES after *aqua regia* digestion.

### Incubation Experiment: C and N Mineralization

The kinetics of frass mineralization were followed during laboratory incubations of control and frass treatments, based on the French normalization (AFNOR, 2016). An amount equivalent to 100 g of dry soil mixture was incubated in 1.2 L hermetic jars kept in a dark room at 22°C. The experiment was conducted in four replicates and lasted 32 days. The water content of the mixtures was adjusted at field capacity with demineralized water and controlled during the incubation period. In each glass jar, C mineralized as CO<sub>2</sub> was trapped

**TABLE 1** | Chemical characteristics of frass (data from Houben et al., 2020).

Organic C g kg <sup>-1</sup>	Total N g kg <sup>-1</sup>	Total K g kg <sup>-1</sup>	Total P g kg <sup>-1</sup>	Soluble fraction %Corg	Hemicellulose- like fraction %Corg	Cellulose-like fraction %Corg	Lignin-like fraction %Corg
393	50	17	20	49.3	31	15.2	4.4

in 30 mL of 1 mol L<sup>-1</sup> NaOH. The C-CO<sub>2</sub> trapped in NaOH was determined using the alkali absorption/conductivity method (Rodella and Saboya, 1999). As recommended by Doublet et al. (2011), the dynamics of C mineralization in soil was calculated by subtracting C-CO<sub>2</sub> mineralized in the control treatment to C-CO<sub>2</sub> mineralized from the frass treatment and the results were expressed as a percentage of the total organic C (TOC) in frass. Similar to the recommendations by Flavel and Murphy (2006) for other organic amendments, the release of mineral N from frass was predicted on the basis of the strong relationship between C-CO<sub>2</sub> mineralized from frass and N mineralized from frass found in our previous study (Houben et al., 2020) (see details of the regression equation in **Supplementary Figure S1**).

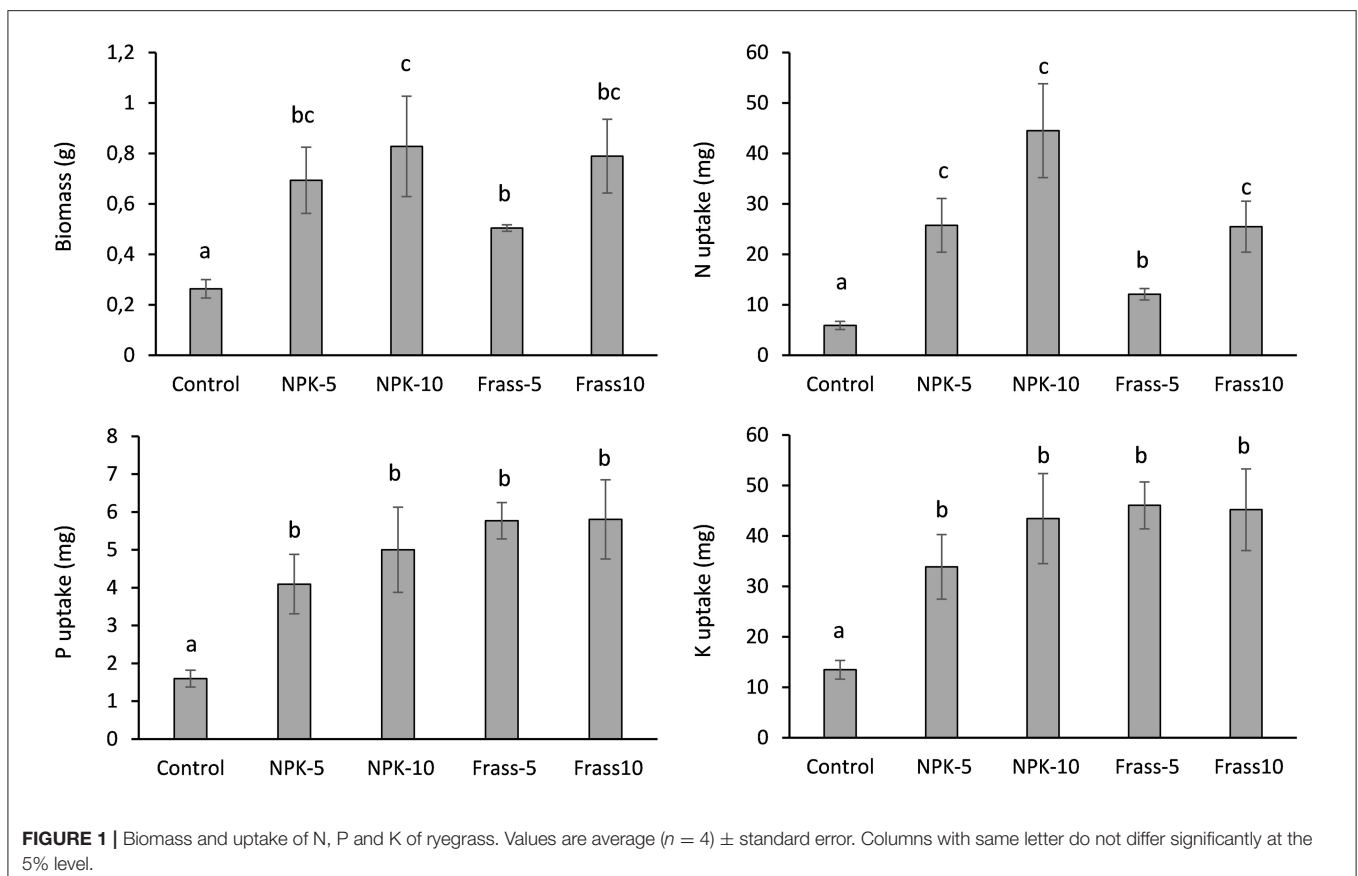
## Community-Level Physiological Profiles

The effect of frass on metabolic functions, including those involved in nutrient cycling, in soil was assessed by comparing the patterns of potential C source utilization by soil microbial communities under the Control, the NPK-10 and the Frass-10 treatments using Biolog Ecoplate. Briefly, each 96-well plate consisted of three replicates, each one comprising 31 sole C sources and a water blank. Five grams of soil were shaken with 45 ml of sterile 0.85% NaCl for 30 min at 200 rpm and then diluted to 1:1000. Each plate well was inoculated with 150  $\mu$ L of the dilution and the plates were incubated at 25°C. Color development for each well was obtained in terms of optical density (OD) at 590 nm using an automated plate reader.

Kinetic curves suggested that after 72 h incubation time, the wells with the most active microbial communities reached the asymptote of color development. Therefore, this point was considered as the optimal incubation time for further statistical analyses, as suggested by Doan et al. (2013). The C sources were grouped into six categories representing different substrate guilds according to Sala et al. (2010): amino acids (L-arginine, L-asparagine, L-phenylalanine, L-serine, glycyl-L-glutamic acid, L-threonine), amines (phenylethylamine, putrescine), carbohydrates (D-mannitol, glucose-1-phosphate, D,L-alpha-glycerol phosphate, beta-methyl-D-glucoside, D-galactonic acid-gamma-lactone, erythritol, D-xylose, N-acetyl-D-glucosamine, D-cellobiose, alpha-D-lactose), carboxylic acids (D-glucosaminic acid, D-malic acid, itaconic acid, pyruvic acid methyl ester, Dgalactouronic acid, alpha-ketobutyric acid, gamma-hydroxybutyric acid), phenolic compound (2-hydroxy benzoic acid, 4-hydroxy benzoic acid) and polymers (Tween 40, Tween 80, alphacyclodextrine, glycogen). Substrate average well color development (SAWCD) values for each substrate categories were calculated with the same equation:  $AWCD = \sum ODi/N$ , where ODi is the corrected OD value of the substrates within the substrate category and N is the number of substrates in the category.

## Statistical Analyses

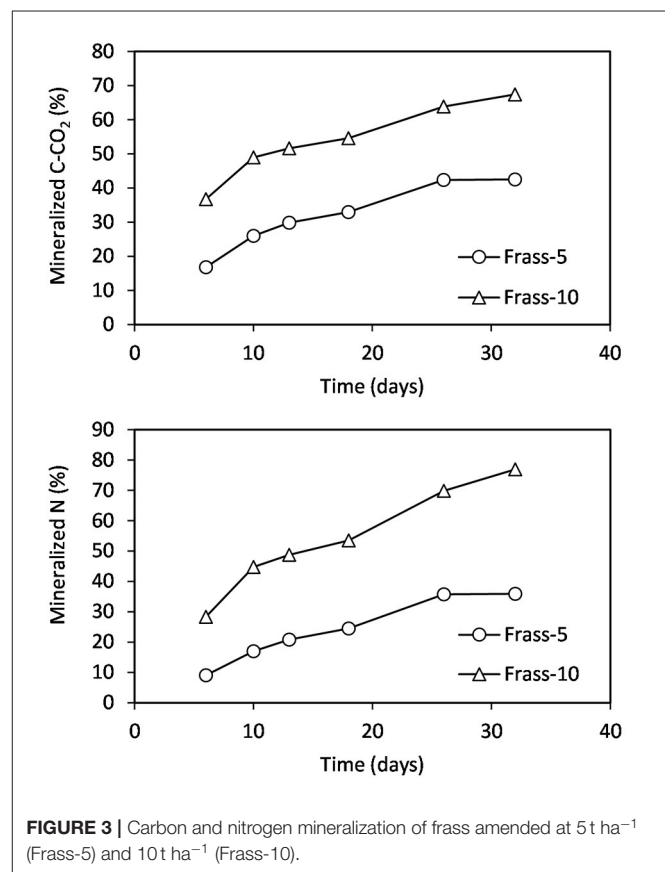
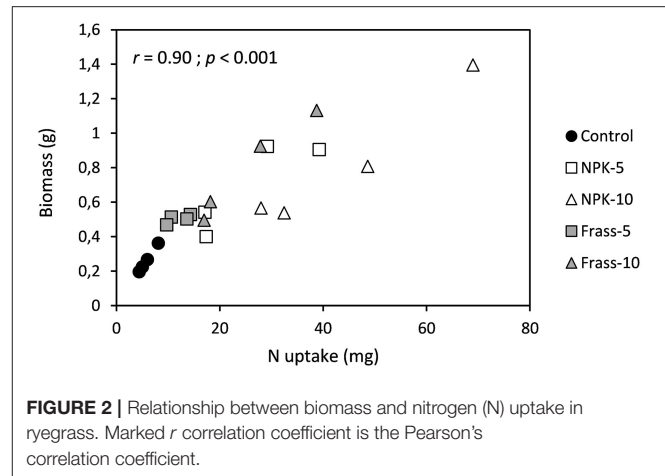
All recorded data were analyzed using descriptive statistics (mean  $\pm$  standard error) and normality was determined using



the Shapiro-Wilk test. One-way ANOVAs and Tukey's multiple comparison tests or Kruskal-Wallis and Mann-Whitney tests were used to compare biomass and nutrient concentrations in the shoot according to whether the distribution was normal or not, respectively. Pearson's correlation coefficient was used to analyze the relationship between biomass and N content. All statistical analyses were performed using R software version 3.5.0 and the package Rcmdr (Fox, 2005).

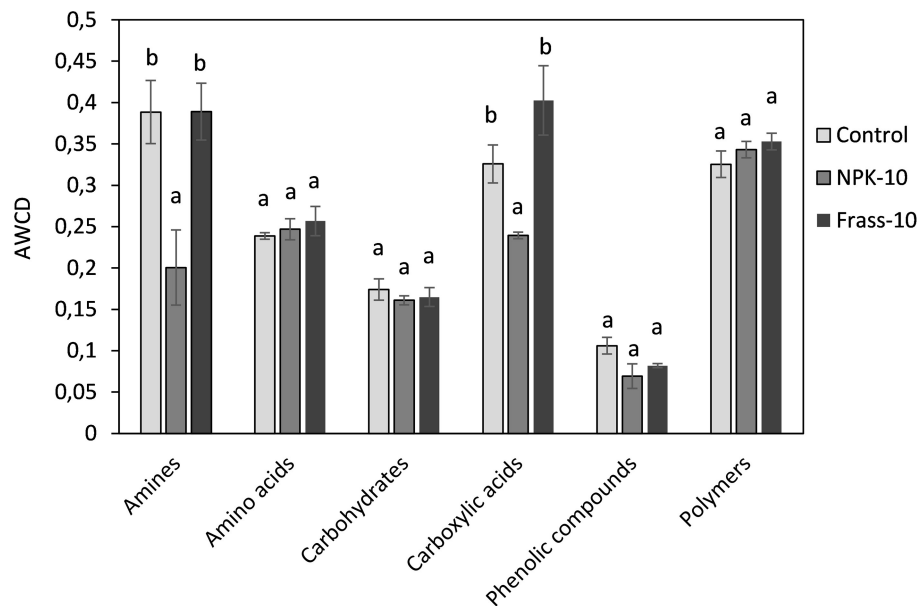
## RESULTS AND DISCUSSION

Recent studies have reported that frass application to soil might sustain plant biomass production (Beesigamukama et al., 2020a; Dulaurent et al., 2020; Houben et al., 2020; Schmitt and de Vries, 2020; Poveda, 2021). Here, we showed that, irrespective of the application rate, the biomass of ryegrass was significantly higher in the presence of frass than in the control (Figure 1). Using also ryegrass as the study plant, Kebli and Sina (2017) and Menino et al. (2021) found similar results with frass from black soldier fly. The authors concluded that higher ryegrass biomass after frass application was due to an improvement of plant N nutrition. In the present study, the strong relationship between biomass and plant N content corroborates these findings (Figure 2). Nitrogen is usually considered the key nutrient for plant growth and plant yield is closely related to the N supply (Marschner, 1995). Therefore, to be used as an alternative to inorganic fertilizers, organic resources are expected to provide a rate of available N similar to that provided by inorganic fertilization (Hernández et al., 2016). Compared to the Control, N uptake was significantly increased by the application of frass. This high short-term ability to supply N contrasts with other organic amendments which usually supply N more slowly (Delin et al., 2012; Cassity-Duffey et al., 2020). The amount of N released to plants by organic fertilizers depends on their chemical composition, including N content, C:N ratio, and contents of labile C, hemicellulose, cellulose, and lignin (Gutser et al., 2005; Mohanty et al., 2011). As shown in Figure 3, frass is rapidly mineralized after its incorporation into the soil which is related to its high labile C and its low recalcitrant C contents (Table 1), as previously suggested (Houben et al., 2020). The subsequent high N mineralization (Figure 3) can thus explain the rapid N supply by frass to plants. Higher N mineralization in the presence of frass was also reflected by BIOLOG Ecoplate results (Figure 4). In agreement with Chakraborty et al. (2011), the application of mineral N had compromised the ability of the soil microbial communities to catabolize amines as the AWCD values for these compounds was significantly lower in NPK-10. By contrast, the application of frass at 10 t ha<sup>-1</sup> restored the ability of microbes to catabolize amines compared with the control. It is noteworthy to mention that frass also stimulated significantly the substrate utilization of carboxylic acids compared with the NPK control. As observed for other organic amendments (Trabue et al., 2016), this likely reflects the fast decomposition of easily degradable organic compounds added by frass but also of more complex compounds, possibly originated from the soil organic matter (Kolton et al., 2017). According to Lazcano et al. (2013) who



reported similar findings for rabbit manure and vermicompost, frass might thus increase N availability not only by supplying N but also by promoting N turnover from organic matter through increased microbial activity.

Although frass increased N uptake by plants, its N fertilizer potential was interestingly mediated by its application rate. As shown in Figure 1, while N uptake was significantly lower in Frass-5 than in NPK-5, the application of frass at 10 t ha<sup>-1</sup> (Frass-10) induced a similar N uptake compared to its mineral control



**FIGURE 4** | Average well-color development (AWCD) for each group of substrates metabolized in BIOLOG EcoPlate. Values are average ( $n = 3$ )  $\pm$  standard error. Columns with same letter do not differ significantly at the 5% level.

(NPK-10). The lower efficacy of frass to supply N when applied at  $5 \text{ t ha}^{-1}$  might be explained by its lower mineralization rate. As shown in **Figure 3**, the proportion of frass-derived C and N that was mineralized during the incubation experiment was lower when frass was applied at  $5 \text{ t ha}^{-1}$  than at  $10 \text{ t ha}^{-1}$ . This likely results from the higher input of easily-degradable C in the Frass-10 treatment, which in turn stimulates further microbial activity (Mohanty et al., 2011). Overall, the high mineralization of frass, especially at  $10 \text{ t ha}^{-1}$ , suggests that frass application might be efficient to increase short-term soil N supply to plants. On the other hand, application of frass long before sowing or in excess with respect to the crop needs might cause a substantial loss of N mineralized, as reported for other organic fertilizers (Abbasi et al., 2007). Although in field studies in a longer run should be performed, these preliminary results indicate that frass might be well-suited for a synchronized application of N according to the plant demand. More specifically, application of frass with sowing could be recommended so that the quickly mineralized N may be readily utilized by crops.

Compared with the control, all treatments increased P and K uptake (**Figure 1**). More interestingly, the uptakes of P and K in the presence of frass or mineral fertilizer were similar, indicating that frass was as efficient as mineral fertilizer to supply P and K to plants. Investigating P and K amounts in barley shoot after the application of mealworm frass applied at  $10 \text{ t ha}^{-1}$ , previous studies also concluded that frass was as efficient as mineral fertilizer to quickly provide nutrients to plants (Dulaurent et al., 2020; Houben et al., 2020), which was due to the rapid mineralization of frass and the presence of P and K in a readily available form. Our results confirm thus the potential of frass to be used as a fertilizer.

By contrast to N, high application rate had no significant effect on P and K uptakes compared to low application rate. These findings agree with Brod et al. (2012) who investigated the fertilizer potential of various organic by-products using a similar pot experiment with ryegrass. According to the authors, P and K uptake by ryegrass did not respond to high application because nutrient supply was higher than the plant requirements.

## CONCLUSION

Insect production is forecasted to grow in the next few years in response to the increasing need of finding alternative sources of protein and this should generate high quantities of frass. Using a short-term pot experiment, this study indicates that frass has a great potential to be used as a substitute for mineral NPK fertilizer even though its N fertilizer potential is mediated by its rate of application. Indeed, at  $10 \text{ t ha}^{-1}$ , the fast frass mineralization coupled with improvement in microbial activity seemed to be enough to maintain the uptake of N by ryegrass as compared to mineral fertilization. By contrast, at  $5 \text{ t ha}^{-1}$ , the lower frass mineralization induces a reduced N uptake compared to its mineral control. Unlike N, frass was as effective as mineral fertilizer to supply P and K to plants, likely due to their presence in a readily available form in frass. Although this work must be considered a preliminary step which needs to be completed by studies on a longer term and with other crops, our findings also indicate that plant biomass responds mainly to the supply of N by frass. As a result, increasing N supply by frass by using higher application rate will result in better yield. On the other hand, due to its fast mineralization, higher application

rate of frass might cause substantial loss of N to groundwater. The next challenge will be, therefore, to optimize the use of frass as a sustainable resource for managing NPK nutrition in cropping system.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

DH, GD, and A-MD: Conceptualization. DH and A-MD: methodology. DH and A-MD: investigation. DH: writing—original draft preparation. A-MD and GD: writing—review and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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## SUPPLEMENTARY MATERIAL

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