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The Recycling of Mealworm (*Tenebrio molitor* L.) Frass as Biofertilizer—Effects on Soil Fertility, Biomass Growth and Nitrogen Uptake of Spring Wheat

Mario Fontana | Said Elfouki | Noelia Garcia-Franco | Thomas Guillaume | Samuel Steiner | Luca Bragazza

Agroscope, Field-Crop Systems and Plant Nutrition Group, Nyon, Switzerland

Correspondence: Luca Bragazza (luca.bragazza@agroscope.admin.ch)

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ABSTRACT

An important by-product of insect rearing is the frass, a mixture of excrements, shed exoskeletons, and undigested feed. The recycling of frass as biofertilizer in agriculture is gaining attention as potential alternative to mineral fertilizers. A better understanding of how frass affects soil fertility and crop growth is crucial to provide recommendations about benefits and limits of insect frass as biofertilizer. To reach this goal, in this study we aim at comparing how different mealworm frass amounts, that is, alone or in combination with mineral fertilizer, can affect soil fertility and spring wheat nitrogen nutrition. After a 8-week long greenhouse experiment, we observed lower soil mineral nitrogen concentration in the treatments receiving the frass either alone or in combination with the mineral fertilizer, so suggesting a potential reduction of nitrogen loss with the biofertilizer. Mealworm frass addition significantly increased the aboveground biomass compared to both the mineral fertilizer alone and the control, particularly by stimulating the growth during the initial phases of crop development. Similarly, greater belowground biomass was also associated with frass treatments where plants invested more biomass in roots with wider diameters. Based on nitrogen nutrition index, both the mineral fertilization and the frass in combination with mineral fertilizer provided the best nitrogen nutrition. Instead, the frass alone treatment, by promoting biomass growth and a dilution of nitrogen concentration, showed a nitrogen deficient status like the control even if the nitrogen export of the frass alone was like that of the frass in combination with the mineral fertilizer. We observed that frass alone stimulated biomass growth to such an extent to reduce the recovery efficiency but, on the other hand, it increased the nitrogen physiological efficiency. Overall, our study shows that the combination of mealworm frass and mineral fertilizer provides promising synergic effects at both soil and crop level.

1 | Introduction

The predicted growth of global world population from 8 to 10 billion by 2050 (United Nations 2024) will require an increase of the current agricultural productivity to meet the associated food demand (Van Dijk et al. 2021; Falcon et al. 2022). This ambitious goal clearly entails implementing and prioritizing sustainable agricultural practices that reduce the negative externalities associated with plant and animal production (Hunter et al. 2017). Among the paths to make agriculture more sustainable there is, for example, the identification of alternative sources of proteins, considering that cattle farming is a major contributor to greenhouse gas emissions (Tubiello et al. 2021). In this sense, the farming of edible insects has been proposed as a more sustainable practice to produce food and feed proteins (Van Huis and Oonincx 2017; Madau et al. 2020), even if some challenges need further attention (Biteau et al. 2025). An important by-product (= waste) of insect rearing is the frass, i.e., a mixture of insect excrement, shed exoskeletons, and undigested feed (Verardi et al. 2025). The recycling of frass as biofertilizer in agriculture, in line with the principles of circular economy, has recently gained attention as a potential alternative to chemical fertilizers for improving soil health and crop production (Watson et al. 2021; Lopes et al. 2022).

The large use of chemical fertilizers to boost crop productivity has led to serious environmental hazards (Anas et al. 2020). In this context, biofertilizers have been developed to avoid or reduce environmental pollution while, at the same time, providing nutrients to the plants (European Commission Regulation EC 2021; Puglia et al. 2021). Biofertilizers can be defined as bio-based organic fertilizers that are produced from plant or animal sources and that contain microbial strain(s) with the potential to improve the bioavailability and bio-accessibility of nutrients for the plants (Puglia et al. 2021). In this context, the recycling of insect frass as biofertilizer has been demonstrated to have very promising environmental and agronomic perspectives (Paris et al. 2024; Bouhzam et al. 2025) while, at the same time, reducing the reliance on mineral fertilizers.

In some recent reviews (e.g., Poveda 2021 a; Abd Manan et al. 2024; Hénault-Ethier et al. 2024; Verardi et al. 2025), it has been highlighted that frass can indeed provide nutrients, microorganisms and biomolecules when used as biofertilizers. Different studies have shown that frass can be an important source of macronutrients, in particular nitrogen, potassium and phosphorus, with a specific chemical composition that is, nonetheless, affected by insect species and insect diet (Poveda et al. 2019; Watson et al. 2021; Lopes et al. 2022; Zunzunegui et al. 2024). In terms of nutrient release kinetics, some studies have reported a rapid mineralization of nitrogen (N) from the frass (Watson et al. 2021), suggesting the necessity to further investigate the temporal dynamics of N requirement by the crop and the N release from the frass in order to optimize the synchronicity of fertilizer supply and plant demand. The beneficial microbes contained in the frass are expected not only to stimulate plant growth but also to increase the resilience of plants to abiotic and biotic stress (Barragán-Fonseca et al. 2022; Verardi et al. 2025). For what concerns the biomolecules, the frass contains different functional molecules that are expected to enhance plant growth, promote soil health and trigger plant defenses (Verardi et al. 2025).

Most of the studies dealing with frass as biofertilizer are mainly focused on the frass from the black soldier flies (*Hermetia illucens* L.) (Abd Manan et al. 2024; Lomonaco et al. 2024), whereas less studies are available for the frass from mealworm (*Tenebrio molitor* L.) rearing (Verardi et al. 2025). By considering the importance of insect species in affecting frass quality (Praeg and Klammsteiner 2024), here we want to contribute to better understanding the effects of mealworm frass when applied alone or in combination with a mineral fertilizer by specifically looking at the effects on: 1) soil biochemical properties, in particular for what concerns the soil N cycling; 2) above- and belowground crop productivity of spring wheat (*Triticum aestivum* L.) with a focus on temporal development of aboveground plant biomass and root morphology; 3) crop N nutrition, in particular for what concerns the N status.

2 | Material and Methods

2.1 | Experimental Design

A greenhouse experiment was carried out at Agroscope (Nyon, Switzerland) using spring wheat (*T. aestivum* L., variety Fiorina) as crop model. Plants were grown in pots (diameter 27 cm and height 24 cm) during the period October-November 2023. All the pots contained about 6 kg of soil a loamy (338 g kg^{-1} sand, 425 g kg^{-1} silt and 237 g kg^{-1} clay) soil that was manually collected from the surface layer (0–15 cm) of an agricultural soil classified as Calcaric Cambisol and situated close to the greenhouse. Before potting, the soil was air-dried, ground, sieved through a 1-cm mesh and mixed thoroughly. The soil physico-chemical properties were: pH (1:2.5, H_2O) of 7.9, total N content of 1.21 g kg^{-1}, total organic C content of 13.3 g kg^{-1}, Olsen-P_2O_5 content of 0.14 g kg^{-1}, and exchangeable K_2O content 0.29 g kg^{-1}. Three fertilization treatments were selected so to provide the equivalent recommended amount of 120 kgN ha^{-1} (Sinaj et al. 2017), that is, as pure frass (frass alone), as mineral N fertilizer (= ammonium nitrate, 27% N), and as a mixture of 50% frass and 50% mineral fertilizer (50/50 treatment) to reach the recommended N dose. In addition, a control treatment receiving no N addition was also included.

The mealworm frass here used was produced by Swissinsect GmbH (Hergiswil, Nidwalden, Switzerland). The diet of mealworms consists of approximately 95% Swiss-produced wheat bran and approximately 5% organic-certified carrots. Based on the chemical analyses of the mealworm frass (Table S1), each pot with six growing spring wheat plants was supplied with 5.63 g of pure frass for the frass alone treatment, with 0.67 g of ammonium nitrate for the mineral fertilizer alone, or half of each dose for the 50/50 treatment. All the pots received an equal amount of mineral phosphorus and potassium corresponding to the requested Swiss recommendations (Sinaj et al. 2017). Overall, twenty-four pots were cropped with spring wheat resulting from a combination of four fertilization treatments and six replicates.

In each pot, 10 spring wheat seeds were sown. After 2 weeks, the six most robust plants were kept, so to resemble the field density, whereas the rest of the seedlings were pulled out and left on the surface. Pot watering was done manually to keep a soil moisture content of around 70%–100% of the field capacity. Pots were regularly moved (i.e., each 1–2 weeks) to avoid any bias due to potential greenhouse heterogeneity. To warrant optimal photosynthetic conditions, daily temperature was kept between 18°C and 25°C. In addition to natural daylight, high-pressure sodium lamps (400 W m^2) were used from 6 a.m. to 8p.m. to maintain the light intensity over 250 W m^{-2}. In order to isolate the effect of spring wheat plants on soil biochemical properties, three replicates of bare soil without crops were incubated in the same greenhouse conditions for each fertilization treatment totaling 12 pots, that is, four fertilization treatments ×3 replicates. The top of the bare soil pots was regularly and gently scratched with a fork (≈ each 2 weeks) to avoid any compaction and anoxic conditions. Overall, the experiment totalized 24 pots with spring wheat and 12 pots with bare soil for a total of 36 pots.

2.2 | Aboveground Biomass Measurements

The collar diameters and plant heights were measured at 37, 45 and 52 days after sowing (DAS), whereas the relative leaf chlorophyll content (RLCC) was measured at 31, 37, 45 and 52 DAS in the uppermost mature leaf using a portable chlorophyll meter (CL-01 Hansatech). After 8 weeks, the developmental stage (according to Meier 2018) of each of the six plants was recorded and averaged for each pot just before the aboveground biomass harvest. Fresh aboveground biomass was directly weighted whereas humidity was determined by weighing aboveground biomass after oven drying (48 h at 40°C).

2.3 | Soil Sampling and Analyses

Around 200 g of fresh soil were collected using a cylindrical auger with a diameter of 2 cm through 2 transverse punctures from the top to the bottom of the pots. Half of this fresh soil was put in a closed plastic bag and stored in a cold chamber before analyses of enzymatic activities, mineral N concentration and soil moisture. Soil moisture was measured by weight loss (24 h at 105°C). Soil mineral N (i.e., NO_3^- and NH_4^+) was extracted by KCl (1 M) and quantified by colorimetry using a microplate reader (BioTek, Instruments, US). Briefly, the activities of acidic phosphatase, β-N-acetylglucosaminidase, β-Glucosidase, cellobiohydrolase were measured using synthetic fluorogenic substrates according to a modified procedure by Marx et al. (2001) and German et al. (2011). Briefly, 5 g of fresh soil was shaken in 20 mL of distilled water for 1 h and then centrifugated (10000 rpm for 5 min) to analyze the supernatant. Fluorogenic 4-methylumbelliferone (MUF)-based substrate was used to determine the activities of the selected enzymes. The activities of enzymes were measured on a microplate reader (BioTek, Instruments, US) at 450 nm emission and 330 nm excitation wavelength after 30 min, 2 h, 2 h 30 min, and 2 h 30 min of incubation for, respectively, acidic phosphatase, cellobiohydrolase, β-N-acetylglucosaminidase, and β-Glucosidase. Enzyme activities were calculated from the regression slopes of the standard measurements along with the fluorescence average values of the triplicates for each sample and they were reported as mmol substrate (MUF or MUC) g^{-1}dry soil h^{-1}.

The remaining soil was air dried and sieved (2 mm mesh size) to be analyzed for soil organic carbon (SOC) using the sulfochromic oxidation (NF ISO 14235). An elemental analyzer (Thermo, flash 2000) was used to measure the total soil N (NF ISO 13878).

2.4 | Root Sampling and Analyses

The remaining soil pot containing the root systems were put in a water bucket before washing root system. Spring wheat root systems were carefully separated. Root systems were then stored in a cold (+ 5°C) chamber. Further, root systems were put in plastic water pan with a concern of avoiding root overlapping as much as possible. One image was obtained for each root system part. The scan images were analyzed using winRHIZO software (Regent Instruments Inc., Ottawa, Canada), allowing to quantify root length, root area and root volume for various root class diameters. Then, the root systems contained in each pot were oven-dried (48 h at 45°C) and weighed.

2.5 | Determination of Crop N Nutrition Status

The N physiological efficiency identifies plants that have a higher ability to produce aboveground biomass per unit of available nitrogen (Congreves et al. 2021):

$$\text{NPHYE} = (\text{Biomass} - \text{BiomassC})/(\text{PlantN} - \text{PlantNc})$$

where Biomass is the total aboveground biomass in each pot and BiomassC is the average total biomass of the control treatment (= unfertilized pots), whereas the PlantN and PlantNc are the total N content (= stock) in aboveground biomass in, respectively, the fertilized and control pots.

The N recovery efficiency (NRE) indicates the apparent amount of applied N that is assimilated into the aboveground plant biomass (Congreves et al. 2021):

$$\text{NRE} = (\text{PlantN} - \text{PlantNc})/\text{FertilizerN}$$

where PlantN and PlantNc arethe total N content (= stock) in aboveground biomass in, respectively, the fertilized and control pots, whereas FertilizerN is the amount of added N fertilizer.

The N nutrition index (NNI) is the ratio between the actual N concentration (%N) in aboveground biomass and the critical N concentration (%Nc), the later here calculated using the aboveground biomass and the critical N dilution curve equation according to Ziadi et al. (2010).

Nitrogen concentration in plant biomass was determined using the Dumas method (NF ISO 13 878).

2.6 | Data Analysis

Statistical analyses were performed in the R environment, versions 4.0.2 (R Core Team 2020), and with TIBCO Statistica. The treatment effect was tested using one-way ANOVA with post-hoc Tukey test. The *p*-values were determined with least significant difference.

3 | Results

3.1 | Bio-Chemical Properties of Bare Soils

In bare soils, after 8 weeks of incubation, the total N concentration for all three treatments receiving a N fertilization was higher compared to the control but not different among fertilization treatments (Table 1). The soil nitrate (NO_3) concentration was lower in the mineral fertilization and in the 50/50 treatments compared to the control and the frass alone treatments. No differences were observed among treatments for the pH and ammonium (NH_4) concentration, whereas the total organic carbon (C_{org}) was slightly higher in the 50/50 treatment (Table 1).

The two treatments receiving the frass, particularly the treatment with frass alone, were characterized by higher activity, although statistically not significant, of the enzymes β-N-acetylglucosaminidase, β-Glucosidase and cellobiohydrolase, indicating an enhanced cycling of N and C compounds (Table 1). No effect was observed on acidic phosphatase activity.

3.2 | Bio-Chemical Properties of Cropped Soils

In cropped soils, after 8 weeks of crop growth, total N concentration was similar in all the three fertilization treatments (Table 1). Soil nitrate and ammonium concentration was lower in the two treatments receiving the frass as biofertilizer compared to the mineral fertilization treatment (Table 1). No differences were observed in the activity of the C- and N-degrading enzymes, whereas acid phosphatase was lower in the frass-receiving treatments (Table 1).

TABLE 1 | Selected properties of bare (= without plants) soils and cropped (= with growing plants) soils after 8 weeks of incubation for each fertilization treatment.

	Control	Min	Frass	50/50
Bare soil properties				
C_{org} (g kg^{-1})	14.6^{b}	14.6^{b}	14.8^{a,b}	15.1^{a}
N_{tot} (g kg^{-1})	1.78^{b}	1.85^{a}	1.87^{a}	1.85^{a}
NO_3 (mg kg^{-1})	25.2^{a}	20.1^{b}	26.2^{a}	22.3^{b}
NH_4 (mg kg^{-1})	0.08^{a}	0.05^{a}	0.09^{a}	0.06^{a}
pH	7.97^{a}	7.83^{a}	7.90^{a}	7.87^{a}
Acidic phosphatase (μmol MUF g^{-1} h^{-1})	2.53^{a}	2.68^{a}	2.63^{a}	2.54^{a}
β-N-acetylglucosaminidase (μmol MUF g^{-1} h^{-1})	0.13^{b}	0.06^{c}	0.43^{a}	0.20^{a,b}
β-Glucosidase (μmol MUF g^{-1} h^{-1})	0.21^{b,c}	0.10^{c}	0.38^{a}	0.34^{a,b}
Cellobiohydrolase (μmol MUF g^{-1} h^{-1})	0.070^{a}	0.067^{a}	0.093^{a}	0.090^{a}
Cropped soil properties				
C_{org} (g kg^{-1})	15.1^{a}	15.5^{a}	15.4^{a}	15.7^{a}
N_{tot} (g kg^{-1})	1.81^{a}	1.75^{a}	1.77^{a}	1.74^{a}
NO_3 (mg kg^{-1})	0.92^{b}	2.61^{a}	0.68^{b}	0.74^{b}
NH_4 (mg kg^{-1})	0.11^{b}	0.23^{a}	0.12^{b}	0.16^{a,b}
pH	8.05^{a}	8.12^{a}	8.10^{a}	8.15^{a}
Acidic phosphatase (μmol MUF g^{-1} h^{-1})	3.48^{a}	2.73^{b}	2.31^{c}	2.37^{c}
β-N-acetylglucosaminidase (μmol MUF g^{-1} h^{-1})	0.38^{a}	0.36^{a}	0.35^{a}	0.35^{a}
β-Glucosidase (μmol MUF g^{-1} h^{-1})	0.94^{a}	0.94^{a}	0.78^{a}	1.27^{a}
Cellobiohydrolase (μmol MUF g^{-1} h^{-1})	0.10^{a}	0.10^{a}	0.11^{a}	0.16^{a}

Note: Control is the soil without any fertilizer addition; Min refers to the mineral fertilization treatment with ammonium nitrate; Frass is the treatment receiving frass alone; 50/50 refers to the treatment receiving half frass and half ammonium nitrate. For all treatments the amount of N input was equivalent to the fertilization recommendation for spring wheat, that is, 120 kg N ha^{-1}. Values are the mean of three replicates for the bare soils and six replicates for the cropped soils. Significant differences ($p < 0.05$) between fertilization treatments are indicated, within the same row, by different letters.

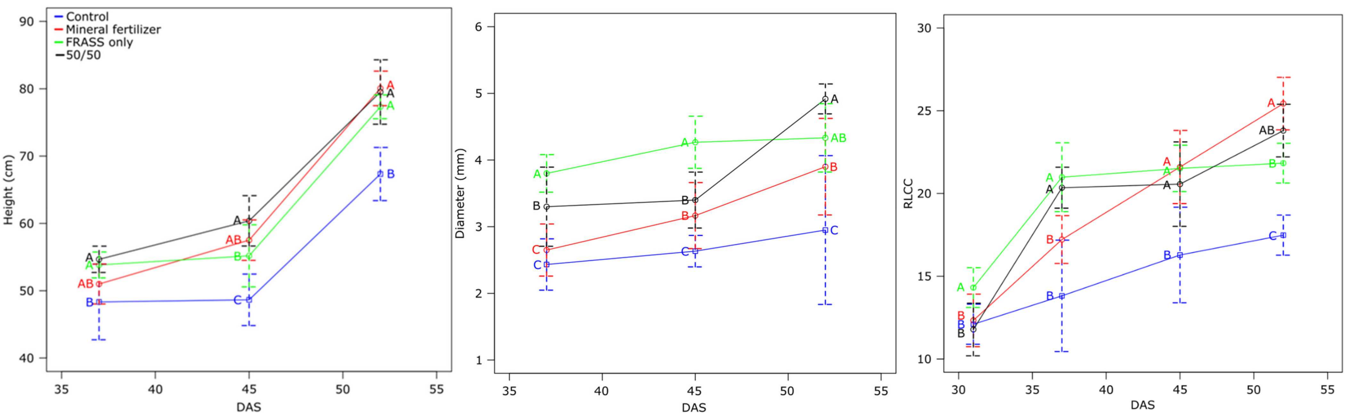

FIGURE 1 | Temporal trend of stem height (cm), stem collar diameter (mm) and relative leaf chlorophyll content (RLCC) of spring wheat at different days after sowing (DAS). Different letters indicate significant differences ($p < 0.05$) among treatments for the same day. Green line = treatment receiving insect frass only; black line = treatment receiving half frass and half ammonium nitrate (50/50); red line = mineral fertilizer; blue line = control. Each value is the mean (± s.d.) of six replicates.

Overall, there was an increase of enzymatic activity in all the treatments in presence of growing plants compared to bare soil (Table 1).

3.3 | Effect of N Fertilization on Growth Dynamic and Leaf Chlorophyll Content of Spring Wheat

After about 1-month, higher plant height was observed in the frass-receiving treatments compared to the control, whereas no significant differences were observed between the frass and the mineral fertilization treatment (Figure 1). After 45 days of growth, the frass-only treatment was characterized by smaller plants, particularly if compared to the 50/50 treatment, whereas the control showed the plants with the lowest height. At the end of the experiment, that is, after 52 days, the height of the plants in the three fertilization treatments did not differ, whereas the control still showed the smallest plants (Figure 1).

The stem collar diameter was also greater, after about 1 month, in both the treatments receiving the frass compared to both the control and the mineral fertilizer (Figure 1). However, at the end of the experiment, the treatments receiving the frass showed greater stem diameters (Figure 1).

The chlorophyll content was higher in the frass alone treatment compared to all the control and the 50/50 treatments after 1 month of plant growth (Figure 1). At the end of the experiment the mineral fertilizer showed a higher, but not significantly different chlorophyll content if compared to the two frass treatments (Figure 1). The control treatment was characterized by lower values of chlorophyll compared to the other three treatments during most of the growth period (Figure 1).

3.4 | Fertilization Effect on Aboveground and Belowground Productivity of Spring Wheat

The mean phenological stage of the plants in each of the four treatments corresponded to 47 for the control and to 49 for the other three fertilization treatments with no significant differences among treatments. All the fertilization treatments had a significantly greater aboveground biomass compared to the control at the end of the experiment (Table 2). In addition, the aboveground biomass in the frass alone treatment was significantly greater than mineral fertilizer treatment. A similar pattern was observed also for the belowground productivity with both the frass-receiving treatments showing a higher root biomass (Table 2). The total productivity, i.e. the aboveground and belowground biomass, was significantly higher in the two frass-receiving treatments compared to the control and mineral fertilizer treatments (Table 2).

The root-to-shoot ratio did not differ significantly between the treatments even if the 50/50 treatment showed a higher ratio (Table 2).

3.5 | Fertilization Effect on Biomass Allocation in Different Root-Diameter Classes

The plants in the control treatment had a higher proportion of fine roots (diameter class: 0–0.5 mm) compared to the other three fertilization treatments (Table 3). Both the two frass-receiving treatments were characterized by plants with a lower allocation of biomass in the 0.5–1 cm diameter class, but with significantly higher biomass allocation in large diameter roots (>1.5–3 mm) compared to the control and mineral fertilizer treatments. Overall, the mean root diameters of spring wheat did not differ among treatments (Table 3).

3.6 | Fertilization Effect on Spring Wheat Nitrogen Status, Nitrogen Recovery Efficiency and Nitrogen Physiological Efficiency

The N nutritional index (NNI) of spring wheat plants indicated luxury N consumption in the mineral fertilizer treatment (mean NNI = 115), an optimal N status in the 50/50 treatment (mean NNI = 102), and N deficient status for the frass-only (mean NNI = 82) and the control treatments (mean NNI = 76) (Figure 2). The N recovery efficiency was significantly higher for the mineral treatment compared to the frass alone treatment. In contrast, the N physiological efficiency was higher for the treatments receiving the frass, in particular for the frass alone treatment (Figure 2).

4 | Discussion

4.1 | Effect of Mealworm Frass Addition on Soil Fertility

At the end of 8 weeks of incubation of the bare soils (i.e., the soil samples from pots without any plant), we observed that soil NO_3^- concentration and soil enzymatic activities were higher in the treatments receiving the frass alone and the frass in combination with the mineral fertilizer (i.e., the 50/50 treatment) compared to the mineral fertilizer treatment (Table 1). A higher enzymatic activity, in particular of C- and N-degrading enzymes, suggests an enhanced microbial metabolism in response to frass addition as previously reported by other studies (Poveda 2021; van de Zande et al. 2023). The increase of chitinase (β-N-acetylglucosaminidase) activity, for example, was already observed in soils incubated with frass from black soldier fly (Gebremikael et al. 2022) and can be explained by the fact that frass contains chitin of insect origin, a substrate that can promote, in particular, the fungal activity (Barragán-Fonseca et al. 2022; Watson et al. 2025). Overall, the increase of β-Glucosidase and, to a lower extent, of cellobiohydrolase activity (Table 1) seems to reflect a faster C-cycling as consequence of the enhanced microbial activity (Lovett and Ruesink 1995).

It is interesting to underline that in bare soil the NO_3^- concentration in the mineral fertilizer treatment was lower not only compared to the frass-receiving treatments, but also compared to the control, a result that may be explained by the inhibition of microbial N mineralization due to mineral N addition (Carpenter-Boggs et al. 2000). The fact that the frass-receiving treatments have a soil NO_3^- content similar to the control, but higher than the mineral fertilization treatment, suggests that

TABLE 2 | Aboveground and root biomass of spring wheat in the four fertilization treatments after about 8 weeks of growth.

	Control	Min	Frass	50/50
Aboveground biomass (g pot^{-1})	6.5^{c}	10.0^{b}	12.8^{a}	11.3^{a,b}
Root biomass (g pot^{-1})	2.1^{c}	3.0^{b,c}	4.0^{a,b}	4.8^{a}
Total biomass (g pot^{-1})	8.6^{c}	13.0^{b}	16.7^{a}	16.0^{a}
Root-to-shoot ratio (g g^{-1})	0.32^{a}	0.30^{a}	0.31^{a}	0.42^{a}

Note: Control is the soil without any fertilizer addition; Min refers to the mineral fertilization treatment with ammonium nitrate; Frass is the treatment receiving insect frass only; 50/50 refers to the treatment receiving half frass and half ammonium nitrate. For each fertilization treatment, an equivalent amount of nitrogen was given so to reach the correspondent nitrogen fertilization norm for spring wheat, that is, 120 kgN ha^{-1}. Values are the mean of six replicates. Significant differences ($p < 0.05$) between fertilization treatments are indicated, within the same row, by different letters.

TABLE 3 | Mean relative root biomass (% of the total root biomass) of in the different diameter classes and mean root diameter (mm) in response to the four fertilization treatments.

Root-diameter class (mm)	Volume of roots per class of diameter (%)			
	Control	Min	Frass	50/50
> 0–0.5	33.7[a]	26.2[a,b]	22.8[b]	22.7[b]
> 0.5–1	30.6[a]	28.5[a,b]	20.6[c]	24.3[b,c]
> 1–1.5	16.5[a]	19.2[a]	17.8[a]	17.1[a]
> 1.5–2	7.3[c]	9.5[b,c]	15.8[a]	13.3[a,b]
> 2–2.5	3.3[b]	3.9[b]	7.9[a]	7.9[a]
> 2.5–3	1.1[b]	1.2[b]	3.3[a]	3.0[a]
> 3–3.5	1.3[a]	0.9[a]	1.4[a]	1.3[a]
> 3.5–4	0.1[b]	0.2[ab]	0.7[a]	0.4[a,b]
> 4–4.5	1.1[a]	0.1[a]	0.2[a]	0.1[a]
> 4.5	3.8[a]	9.4[a]	9.8[a]	9.0[a]
Mean root diameter (mm)	0.26[a]	0.29[a]	0.29[a]	0.29[a]

Note: Control = no fertilization; Min = mineral fertilization with ammonium nitrate; Frass = insect frass only, whereas 50/50 = half frass and half ammonium nitrate. Values are the average of 6 replicates. Significant differences ($p < 0.05$) between fertilization treatments are indicated, within the same row, by different letters.

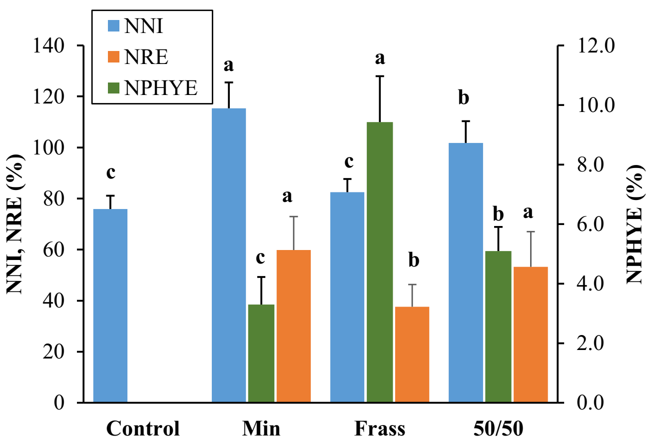

FIGURE 2 | Mean values (± s.d., $n = 6$) of the nitrogen nutrition index (NNI), the nitrogen recovery efficiency (NRE), and the nitrogen physiological efficiency (NPHYE) at the end of the experiment for the control, the mineral fertilizer (Min), the frass alone (Frass), and the half frass and half ammonium nitrate (50/50) treatments. Different superscripts indicate significant differences between treatments for the same parameter ($p < 0.05$).

the addition of nitrogen as frass does not inhibit microbial nitrification (Beesigamukama et al. 2020). Overall, the results from the bare soils clearly point to the important role of frass microbiome in driving nutrient cycling (Gómez-Brandón et al. 2025).

At the end of the incubation period of the cropped soils (i.e., the soil samples from pots with plants), we observed an overall decrease of about 13-times of the soil NO_3^- content, an increase of about 2-times of the soil NH_4^+ content, and an increase between 1.5 and 4 times of the enzymatic activity compared to bare soils (Table 1). These changes are clearly due to the presence of growing plants and their role in affecting soil microbial activity and nutrient cycling (Coskun et al. 2017). Noteworthily, the frass-receiving treatments were both characterized by significantly lower soil NO_3^- content compared to the mineral fertilization treatments (Table 1). Such a result suggests that most of the easily available N provided by the frass, which has been estimated to be between 4% and 22% for black soldier frass and up to 40% for mealworm frass (Houben et al. 2020; Gebremikael et al. 2022), was readily absorbed either by the crop or by the soil microbes (Gebremikael et al. 2022; Gurung et al. 2024). From an environmental point of view this result is of interest because it indicates that providing N in the form of frass may reduce the mineral N loss risk.

4.2 | Effect of Mealworm Frass Addition on Aboveground and Belowground Plant Productivity

The fertilization with mealworm frass increased the aboveground biomass of spring wheat compared to both the control and the mineral fertilizer treatment (Table 2). Previous studies have reported that frass addition can increase biomass productivity compared to the control, but not necessarily compared to mineral or organic fertilizers (see Houben et al. 2020, 2021; Menino et al. 2021; Gebremikael et al. 2022; Cardarelli et al. 2023). Furthermore, a decrease in biomass production was also observed in response to a fertilization with a black soldier frass (see Alattar et al. 2016; Gebremikael et al. 2022). In our case, the quality of the applied mealworm frass seems to be particularly effective in promoting the aboveground biomass growth, a result that highlights the importance of taking into account the frass origin in terms, for example, of insect species and feed source for future use of this by-product as biofertilizer (Praeg and Klammsteiner 2024).

The temporal pattern of aboveground biomass development showed that the frass alone stimulated plant growth at the beginning of the growth cycle (Figure 1), in particular the diameter, suggesting a possible shortening of the vegetative growth as previously reported (Foscari et al. 2024). At the end of the experiment, when the phenological stage corresponded to just before the head emergence, the plants from all three fertilization treatments showed comparable height while the diameter was greater in the frass treatments compared to mineral fertilization, in particular for the 50/50 treatment (Figure 1). We may argue that if such morphological differences will be maintained under field conditions, this may increase wheat plants resistance to lodging (Zuber et al. 1999; Khobra et al. 2019). For the belowground productivity, the treatments receiving the mealworm frass alone or in combination with mineral fertilizer were characterized by a higher root biomass compared to both the control and the mineral fertilizer treatment (Table 2). In addition, a different root architecture was observed in response to frass addition so that a greater proportion of root biomass was allocated in the 1.5–3.0 mm diameter class (Table 3). Root architecture is well known to be affected by nutrient availability (Svoboda and Haberle 2006; Rasmussen et al. 2015; Yuan et al. 2016) and higher N availability is generally associated with a higher abundance of larger diameter roots (Rahman M. et al. 2000; Zhu et al. 2022). The effect of lower N availability on root architecture is clearly visible in our control treatment where there was a higher proportion of smaller diameter roots, that is, < 1 mm, that are supposed to be devoted to nutrient uptake (van de Zande et al. 2023). Roots with larger diameter are normally associated with nutrient transport (van de Zande et al. 2023) so that a greater nutrient availability reduces the necessity for the plant to invest in finer roots, as clearly observed in all the three fertilization treatments (Table 3). However, the fact that larger roots were more abundant in the frass-receiving treatments may rather reflect a growth-promoting effect of the frass due to the presence of active biomolecules (Blakstad et al. 2023) and/or the presence of growth-promoting bacteria (Poveda et al. 2019). The synergy between the frass stimulation and the mineral fertilization increased more strongly the root biomass production than the aboveground biomass for the 50/50 treatment, resulting in a slightly higher root:shoot ratio compared to other treatments (Foughar et al. 2024; Zunzunegui et al. 2024). If this pattern would be maintained under field conditions, it could be an helpful outcome for improving nutrient and water uptake in face of biotic (e.g., weed root competition) or abiotic stress (e.g., drought) (Calleja-Cabrera et al. 2020; Ober et al. 2021).

4.3 | Effect of Mealworm Frass Addition on N Nutrient Status

Mineral fertilization and frass addition changed the dynamic of wheat N status during biomass accumulation. In the light of the positive linear relationship (adj$R^2 = 0.66$, $p < 0.001$, $n = 24$) between the relative chlorophyll leaf content (RLCC) at 52 days and the correspondent nitrogen nutritional index (NNI) and in accordance with previous studies showing that RLCC is relevant for NNI estimation (Debaeke et al. 2006; Ravier et al. 2017), we used the temporal pattern of RLCC values as a proxy of the temporal pattern of NNI (Figure 1). Hence, N status rapidly improved in the frass alone treatment compared to the other treatments to 37 days, likely due to a quick N mineralization of frass. Similarly, chlorophyll content also increased during the initial developmental stages of lettuce fertilized with black soldier frass (Cardarelli et al. 2023). However, the N status remains roughly stable after 37 DAS with the frass alone addition. In contrast, mineral fertilization increased slowly, but constantly, the N status so to reach a higher RLCC value and NNI value at the end of the experiment compared to the frass alone treatment (Figure 1). After 8 weeks of growth, based on the NNI, the mineral fertilization resulted in N luxury consumption (NNI = 115%) while the NNI values of the frass (83%) and the control (76%) treatments suggested a N deficient status (Figure 2). The lower biomass production of the control was likely due to a severe N limitation, whereas the stimulating effect of the frass on aboveground biomass (Barragán-Fonseca et al. 2022; Blakstad et al. 2023) caused a dilution of N concentration that decreased the NNI values. It is worthy to note that the mean total N content in the aboveground biomass (= N export at pot level) was significantly lower in the control treatment (115 mg ± 19.5) compared to all the three other treatments ($p < 0.001$), whereas no significant difference ($p = 0.77$) was observed between the 50/50 treatment (211 mg ± 24.8) and the mineral fertilization (223 mg ± 23.8) with the frass alone treatment that shows a total N content (183 mg ± 15.5) no significantly different from the 50/50 treatment ($p = 0.13$).

Our study shows that, although frass alone resulted in a low N recovery efficiency (Figure 2), the total aboveground biomass production was higher compared to the mineral fertilization treatment (Table 2). Therefore, the low N status was compensated for by a higher N physiological efficiency (Figure 2). The 50/50 treatment appeared to be a good trade-off because it certainly boosted the initial growth but also maintained an optimal N status (i.e., NNI > 100%), in line with previous studies stating that mealworm frass can be combined with a mineral N source to ensure optimal N nutrition (Gebremikael et al. 2022; Blakstad et al. 2023; Nyanzira et al. 2023).

In view of the N concentration dilution due to the enhanced biomass production and the associated low NNI, future field studies are necessary to test to what extent the frass stimulation effect could last until grain production and if the initial low N status could affect grain protein content. Indeed, we harvested the spring wheat biomass just at the beginning of the inflorescence emergence, so that an early N deficiency, as in our case, does not necessarily translate in a lower grain yield (Yao et al. 2023; Rodriguez et al. 2024).

5 | Conclusions

Our greenhouse study has shown that the addition of frass enhanced the growth of aboveground and belowground crop biomass. Compared to frass alone and mineral fertilizer alone, mixing the frass with a mineral fertilizer showed a synergic action by promoting N nutrition for the studied spring wheat while reducing the risk of N loss. The result underscores the biofertilizer value of mealworm frass and the potential to promote a circular economy in agroecosystems in response to the global backdrop of reduced availability of non-renewable fertilizers.

Author Contributions

Mario Fontana: investigation, data curation, formal analysis, writing – review and editing. **Said Elfouki:** investigation, writing – review and editing. **Noelia Garcia–Franco:** writing – review and editing. **Thomas Guillaume:** writing – review and editing. **Samuel Steiner:** writing – review and editing. **Luca Bragazza:** conceptualization, funding acquisition, project administration, supervision, formal analysis, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data available on request from the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Table S1: Chemical characterization of the mealworm frass applied in the study.