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Research article

Yellow mealworm frass: A promising organic fertilizer for common sowthistle (*Sonchus oleraceus* L.) and bristly oxtongue (*Helminthotheca echioides* (L.) Holub) cultivation

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ABSTRACT

Common sowthistle (*Sonchus oleraceus* L.) and bristly oxtongue [*Helminthotheca echioides* (L.) Holub] are winter broad-leaved weeds that have gained interest for cultivation as leafy vegetables. The aim of this study was to examine the effects of frass from the yellow mealworm (*Tenebrio molitor* L.) on nutrient content in soil, growth parameters, and nutrient content in above-ground plant tissues of common sowthistle and bristly oxtongue. Thus, two pot experiments were carried out with 5 treatments [control, calcium ammonium nitrate (CAN) applied at a dose of 100 kg N ha^{-1}, and insect frass applied at a rate of 3500 kg ha^{-1} (0.5 % w/w) 7000 kg ha^{-1} (1 % w/w), and 14,000 kg ha^{-1} (2 % w/w)]. Our results showed that the lowest values of growth parameters for both plant species were recorded in the control treatment. At the final rosette growth stage [e.g., 152 days after sowing (DAS)], the CAN treatment exhibited the highest values of rosette diameter and above-ground dry weight, followed by the highest rate of insect frass. Similarly, at 152 DAS the SPAD index values in the CAN treatment were 28.4–41.5 % higher compared to the control treatment in both species. Regarding root dry weight, the highest values were found in the CAN and insect frass 2 % treatments. In addition, the application of insect frass significantly enhanced soil fertility, with the highest levels of P and K recorded in the insect frass 2 % treatment. In contrast, the CAN treatment resulted in the highest NO_3–N content in the soil (15.83 and 19.26 mg kg^{-1} in common sowthistle and bristly oxtongue, respectively). Moreover, both P and K content in the above-ground plant tissues had the highest values in the insect frass 2 % treatment, while the content of Mg, Mn, and Cu in plant tissues was not affected by the fertilization sources. Therefore, our findings indicate that insect frass can be an additional option in crop fertilization programs as it can improve both the soil fertility and growth of crops compared to conventional inorganic fertilizer sources. However, the effects of insect frass in mixtures with inorganic fertilizers needs to be taken into consideration in future studies.

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1. Introduction

Common sowthistle (*Sonchus oleraceus* L.) and bristly oxtongue [*Helminthotheca echioides* (L.) Holub; synonym *Picris echioides* L.] are broad-leaved weeds [1,2] that are known to infest various crops, such as common wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.) [3–6]. However, these species are also recently being studied as new crops due to their edible leaves [2,7] which have a nutritional value attributed to various bioactive compounds (e.g., flavonoids) contained in the aerial plant parts [8,9].

Undoubtedly, there is a growing interest in utilizing wild edible species in agriculture as new crops [2,10], due to their high nutritional value [2,11–13] and adaptability to abiotic stresses [10]. Consequently, the application of appropriate cultivation practices to increase their productivity is of paramount importance. Among these practices, fertilization is considered as one of the most crucial factors for maximizing both the yield and quality of crops. However, limited information in the literature exists on the effects of fertilization on the growth and yield of common sowthistle and bristly oxtongue, grown either under conventional or organic farming practices. In a previous study, the application of nitrogen inorganic fertilizer 26-0-0 ($N–P_2O_5–K_2O$) as top-dressing at 92 days after sowing (DAS) in a single dose (100 kg ha^{-1}) increased significantly both the rosette diameter and fresh weight of bristly oxtongue [2]. Additionally, weekly application of the inorganic fertilization treatment 600-100-100 mg L^{-1} (concentration of nutrient solution) with 100 ml of nutrient solution resulted in a 90.6 % and 63.6 % increase in fresh above-ground weight compared to the control treatment and the treatment with 100-100-100 mg L^{-1}, respectively [14]. The above-mentioned results clearly demonstrate that inorganic fertilization enhances plant growth for both species.

Concerning the impact of organic fertilizers like compost and manure on growth of wild edible species, in a recent study conducted in pots containing as a substrate a mixture of soil, sand, and vermiculite, the application of a compost extract (nitrogen concentration 300 mg L^{-1}) resulted in an 84.1 % increase in the total above-ground fresh dry weight of common sowthistle compared to the control treatment [14]. Similar results were observed in two common purslane (*Portulaca oleracea* L.) genotypes, where the total fresh above-ground weight increased up to 69.5 % in the compost extract treatment compared to the control [14]. The effects of manure on the growth of some wild leafy vegetables have not been extensively studied. In a field experiment, Polyzos and Petropoulos [10] found that the application of manure in a dose of 5 t ha^{-1} significantly increased both the leaf area index and leaf weight per plant of common sowthistle, spiny chicory (*Cichorium spinosum* L.), and common golden thistle (*Scolymus hispanicus* L.).

In recent years, the insect frass, a by-product of the insect farming industry [15,16], is evaluated as an alternative soil improvement agent or as an organic fertilizer [17–19]. The number of industries specializing in insect rearing for feed and food production are constantly increasing, resulting in an increase in the availability of frass [15]. This fertilizer consists of a mixture of insect faeces, residues from the various foods used in insect diets, and exuviae [19–22] and it contains a considerable amount of primary (N, P, and K) and secondary macronutrients (e.g., Mg and Ca) along with a lesser quantity of micronutrients (e.g., Zn, Mn, and Fe) [23]. The insect frass fertilizers vary in their chemical properties [e.g., pH (6.08–6.39, EC (4.35–9.89 mS cm^{-1}), C:N ratio (10.5–11.2), organic N (3.3–3.4 %), P (1.6–1.9 %), K (1.9 %), and Na (92.4–260.5 mg kg^{-1})] depending on the feed used in insect production [24]. Additionally, the frass fertilizers produced by the various insect species vary in its nutrient composition and other chemical properties such as pH, electrical conductivity, C:N ratio, and organic matter [25].

Certain commercial products based on the insect frass have been approved for use in organic agriculture [18], a growing sector worldwide [26]. Among the various insect species reared in insect farms and used as feed for animal nutrition and/or as food source, the yellow mealworm, *Tenebrio molitor* L. (Coleoptera, Tenebrionidae) is particularly important due to the high nutritional value of its larvae [27–30]. Mealworm frass application improves soil properties, enhances plant growth, and increases crop yields [17] since it contains a considerable amount of N, P, K, and other nutrients [31]. In a pot-based experiment, Antoniadis et al. [17] reported that the application of frass from *T. molitor* at a rate of 1 % resulted in increased organic matter, P, and K content in the soil compared to the control treatment where no fertilization was applied. In the same study, the highest values of above-ground dry weight (>2.5 g $plant^{-1}$) for spinach (*Spinacia oleracea* L.) plants were found in the 1 % frass treatment. Similarly, the insect frass from the same insect species significantly increased both the height and shoot fresh weight of tomato (*Solanum lycopersicum* L.) plants 6 weeks after the application compared to the control [23]. In another study, the mealworm frass application at a rate of 1 % increased the root and shoot fresh weight of lettuce (*Lactuca sativa* L.) plants as well as the N, P, and Mg content in the above-ground tissues. Moreover, when mealworm frass was applied at rates of 2.5 % or 5 % a significant decrease in both root and shoot weight was observed [32]. According to Chia et al. [33] the application of mealworm frass reduced both the leaf number and area of field mustard (*Brassica rapa* L.) plants in the early growth stages.

To the best of our knowledge, there are no studies available for the evaluation of the impact of insect frass on the growth and yield of common sowthistle and bristly oxtongue. The hypotheses tested in this study is whether insect frass can meet plants nutrient needs and improve plant growth of common sowthistle and bristly oxtongue. In this context, the aim of this study was to assess the impact of insect frass derived from larvae of *T. molitor* on growth parameters and nutrient content (P, K, Mg, Cu, Mn, and Zn) in the above-ground plant tissues of common sowthistle and bristly oxtongue. Additionally, the effect of insect frass applied at different rates on soil nutrient content (NO_3–N, NH_4–N, P, K, and Mg) was also evaluated.

2. Materials and methods

2.1. Experimental site and planting material

Two pot experiments were conducted at the School of Agricultural Sciences of the University of Thessaly (Volos, Greece) during the period of November 2022 to April 2023. Common sowthistle and bristly oxtongue were sown on November 2, 2022, at a depth of 1 cm, in pots with a capacity of 2 L. The pots were filled with slightly alkaline soil (pH = 7.4) and sandy clay loam (clay 26 %, sand 38 %, and silt 36 %) in texture. Seedlings emergence was completed on 7 and November 9, 2022 for common sowthistle and bristly oxtongue, respectively. Moreover, the plants were thinned twice on December 8, 2022 and January 13, 2023 and finally, four plants per pot remained. The seeds of both weed species were collected from fields in Agios Georgios (Domokos region, Central Greece) on 10 to June 20, 2022 and 10 to August 20, 2022 for sowthistle and bristly oxtongue, respectively. Irrigation was carried out when it was necessary based on plants needs and climatic conditions.

2.2. Experimental design and treatments

Each pot experiment was carried out according to the randomized complete (RCBD) block design with 5 treatments and a total of 75 pots (15 pots for each treatment). The treatments were as follows: a) control without fertilizer application, b) calcium ammonium nitrate (26-0-0) applied at a dose of 100 kg N ha^{-1} (0.82 g pot^{-1}), c) insect frass applied at a rate of 0.5 % w/w (3500 kg ha^{-1} or 7,5 g pot^{-1}), d) insect frass applied at a rate of 1 % w/w (7000 kg ha^{-1} or 15 g pot^{-1}), and d) insect frass applied at a rate of 2 % w/w (14,000 kg ha^{-1} or 30 g pot^{-1}). Calcium ammonium nitrate was applied twice on 10 January and February 15, 2023 in two equal doses (50 kg ha^{-1}). This inorganic fertilizer was used as positive control and the selection of the applied dose (100 kg N ha^{-1}) was based on the results of the previous study of Karkanis et al. [2]. The organic fertilizer (insect frass) was uniformly applied to all pots (Fig. 1a) and then incorporated into the soil. This organic fertilizer was collected from a *T. molitor* rearing maintained in the pilot-scale insect rearing unit of the Laboratory of Entomology and Agricultural Zoology at the Department of Agriculture Crop Production and Rural Environment of the University of Thessaly. Briefly, insects were kept in the dark at 27 ± 0.5 °C and 60 ± 5 % relative humidity. Larvae were fed on wheat bran and provided with agar (20 g L^{-1}) as a moisture source. A 500 μm sieve was used to separate frass from the larvae at harvest, whereas collected frass was stored in plastic bags at room temperature until further analysis and use. The insect frass was treated at 70 °C for 1 h one week before its application to the pots. The heat treatment is necessary to reduce pathogenic organisms in the insect frass according to Commission Regulation (EU) 2021/1925 [34]. The chemical properties of frass were as follows: EC: 33.6 mS cm^{-1}, pH: 5.5, C/N ratio: 13.2, organic matter: 90.4 %, total nitrogen (TN): 34,364 mg kg^{-1}, nitrate nitrogen (NO_3–N): 103 mg kg^{-1}, phosphorus (P): 3813 mg kg^{-1}, potassium (K): 22,339 mg kg^{-1}, magnesium (Mg): 6036 mg kg^{-1}, manganese (Mn): 107 mg kg^{-1}, iron (Fe): 39.1 mg kg^{-1}, zinc (Zn): 38.6 mg kg^{-1}, boron (B): 20.3 mg kg^{-1}, copper (Cu): 13.1 mg kg^{-1}, and sodium (Na): 486 mg kg^{-1}.

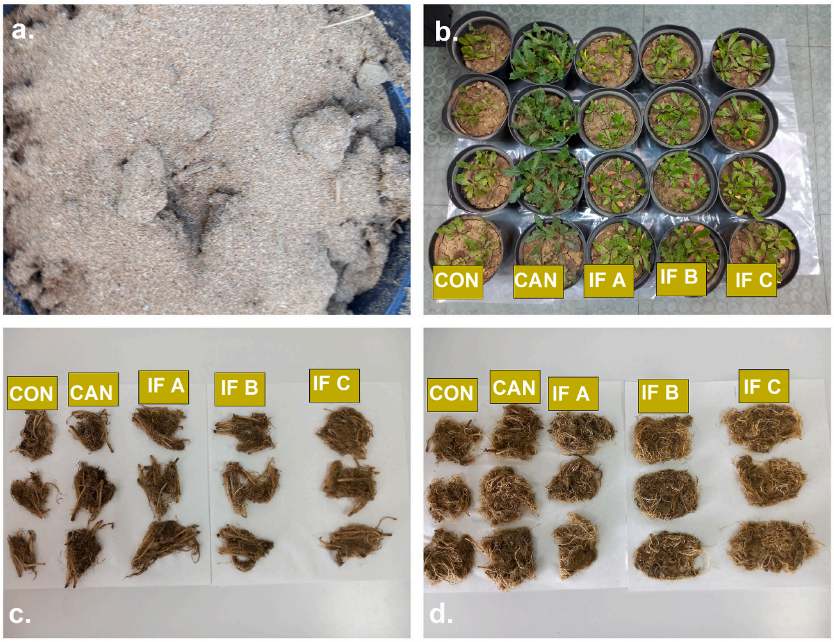

Fig. 1. a. Insect frass before its incorporation into the soil, b. Bristly oxtongue plants on April 3, 2023, c. Root growth of common sowthistle plants on April 3, 2023, and d. Root growth of bristly oxtongue plants on April 3, 2023. CON: control without fertilization, CAN: calcium ammonium nitrate, IF A: insect frass at 0.5 % w/w, IF B: insect frass at 1 % w/w, and IF C: insect frass at 2 % w/w.

2.3. *Sampling and measured parameters*

To evaluate the effects of the five fertilization treatments, the parameters of leaf number, rosette diameter, fresh and dry above-ground weight, fresh and dry root dry weight, and relative chlorophyll content were measured. The measurements of these parameters took place on 15 February, 1 March, 13 March, and 3 April (105, 119, 131, and 152 DAS, respectively). At each sampling date, the above-mentioned parameters were measured on all plants grown in each pot (Fig. 1b) and on a total of 15 pots (3 pots per treatment). The SPAD index (relative chlorophyll content) was recorded using the portable chlorophyll meter SPAD-502 (Konica Minolta Optics, Osaka, Japan) on fully expanded leaves. Root measurements were made only at the last sampling. Initially, the roots were separated from the soil and then, the samples were weighed with a precision scale after removing the excess water using a filter paper (Fig. 1c and d). Both above-ground and root dry weight were measured after drying the samples from each pot on an oven at 60 °C for 4 days.

2.4. *Soil analysis*

Soil analyses were performed at the Laboratory of Vegetable Production of the Agricultural University of Athens. The analysis of nitrate (NO_3–N) and ammonium nitrogen (NH_4–N) was conducted on frozen soil samples. Following crop termination, soil samples were collected using zip plastic bags and stored at −20 °C until nutrient determination. To assess nitrate (NO_3–N) and ammonium nitrogen (NH_4–N), the frozen soil samples were allowed to defrost at room temperature and then analyzed for nitrate (NO_3–N) and ammonium nitrogen (NH_4–N) content. Subsequently, the soil samples were oven-dried at 40 °C for 3–4 days until a constant weight was achieved, were sieved using a 2 mm sieve, and subsequently the dry soil weight was recorded and results (in terms of mineral N) were expressed as mg of mineral N per kg of dry soil. The dried soil was then utilized for determining other nutrients. To determine the concentration of nitrate (NO_3–N) and ammonium nitrogen (NH_4–N), the soil samples were extracted with 1M KCl solution and then, the NO_3–N and NH_4–N concentrations were measured in the obtained extracts using the Vanadium chloride (VCl3) reduction method [35] and Indophenol Blue method [36], respectively. Moreover, the same samples were extracted using the Mehlich III method as described by Mehlich [37]. The obtained extracts were used to determine the available P using the molybdate colorimetric method [38] and the exchangeable K using a flame photometer [39], while in the same extract the concentrations of Mg were measured using an atomic absorption spectrophotometer (AA-7000, Shimadzu Co., Tokyo, Japan).

2.5. *Plant tissue mineral analysis*

Plant tissue nutrient analyses were carried out at the Laboratory of Vegetable Production of the Agricultural University of Athens. Dried above-ground plant samples were ground in a Wiley mill to pass through a 20-mesh screen and 0.5 g of the ground samples was analyzed for the macronutrients P, K, Mg and the micronutrients Mn, Zn, Cu.

P, K, Mg, Zn, Mn, and Cu concentrations were determined by dry ashing at 550 °C for 8 h. Then, extraction for the measurement of nutrients was carried out with a solution of HCl 1N placed into the capsule. The solution contained in the capsule was filtered with Whatman No 42 filters into 100 ml volumetric flasks and distilled water was added up to 100 ml. Potassium was measured by placing, diluted or undiluted extraction solution, in the flame photometer (Sherwood 410, Cambridge, UK), while P was determined as phosphomolybdate blue complex at 880 nm using a spectrophotometer (Anthos Zenyth 200; Biochrom, Cam-bridge, United Kingdom). The above plant extract was used for determination of Mg which was assessed using an atomic absorption spectrophotometer (AA-7000, Shimadzu Co., Tokyo, Japan) [40].

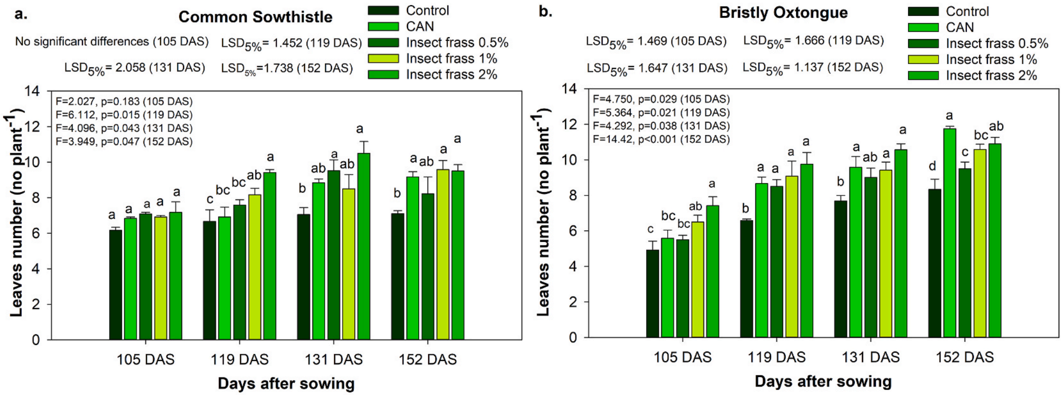

Fig. 2. Fertilization treatments (control, CAN, and insect frass) effects on leaf number of common sowthistle (a) and bristly oxtongue (b) plants. For each measurement, mean values followed by the same letters do not differ significantly according to Fisher's Least Significant Difference (LSD) test at p = 0.05. Error bars show the standard error of means. DAS denotes days after sowing.

2.6. Statistical analysis

The data of all measured parameters were submitted to Analysis of Variance (ANOVA) and when differences were detected among the five fertilization treatments, the Fisher's Least Significant Difference (LSD) post hoc test at the significant level (p) of 5 % was used for multiple comparisons of means. Means comparison for agronomic parameters (leaf number, rosette diameter, fresh and dry above-ground weight, and SPAD values) was performed separately for each sampling date. Normality of our data for all measured parameters was checked using the Shapiro-Wilk test. The data analysis was conducted using the statistics package SigmaPlot 12.0 (Systat Software, Inc.). The standard error of means was calculated using the Excel spreadsheet (Microsoft Office 365), while the figures were created using the SigmaPlot 12.0 software.

3. Results

3.1. Agronomic parameters

3.1.1. Leaves number and rosette diameter

The number of leaves ranged from 6.9 to 10.5 per plant in common sowthistle and from 4.9 to 11.8 per plant in bristly oxtongue (Fig. 2). In both species, the number of leaves was affected by the fertilization treatments, with the lowest values recorded in the control treatment and the highest values recorded in the 2 % frass treatment. For example, at the final measurement conducted at 152 DAS, the number of leaves in the 2 % frass treatment was 25.4 and 29.1 % higher compared to the control treatment in common sowthistle and bristly oxtongue, respectively. In all cases, no significant differences were found between the CAN treatment and the 2 % frass treatment.

The rosette diameter of the plants showed an increasing trend over time. However, this trend was more pronounced in the fertilization treatments compared to the control (Fig. 3). In all measurements, statistical analysis revealed significant differences among treatments, with the lowest values recorded in the control treatment. However, at 131 DAS and 152 DAS, no significant differences among the frass rates (0.5, 1, and 2 %) were recorded in both species. Notably, until the second measurement, the highest rosette diameter was recorded in the 2 % frass treatment, while in the subsequent measurements the highest values for this trait was found in the CAN treatment. For example, at the last measurement, in bristly oxtongue the rosette diameter was 27 % higher compared to the 2 % frass treatment.

3.1.2. SPAD index

The SPAD index (relative chlorophyll content) was influenced by the fertilization regimes. In both plant species, the highest values of this parameter were recorded in the CAN treatment (Fig. 4), while in most measurements, there were no significant differences between the frass treatments and the control treatment. For example, in the final measurement, the SPAD index in the CAN fertilization treatment was 28.4 and 41.5 % higher compared to the control in common sowthistle and bristly oxtongue, respectively, while the SPAD index in the CAN fertilization treatment was 25 and 33.3 % higher compared to the 2 % frass treatment in common sowthistle and bristly oxtongue, respectively.

In both plant species, a noticeable difference in the intensity of the green color of the leaves among the inorganic fertilizer treatment and the other treatments (Fig. 1b) was noted. Moreover, in most sampling dates and for the same fertilization treatments, the SPAD index was higher in bristly oxtongue compared to common sowthistle. It is also important to note that the SPAD index values for the same fertilization treatments were lower at 105 and 152 DAS compared to those at 119 and 131 DAS.

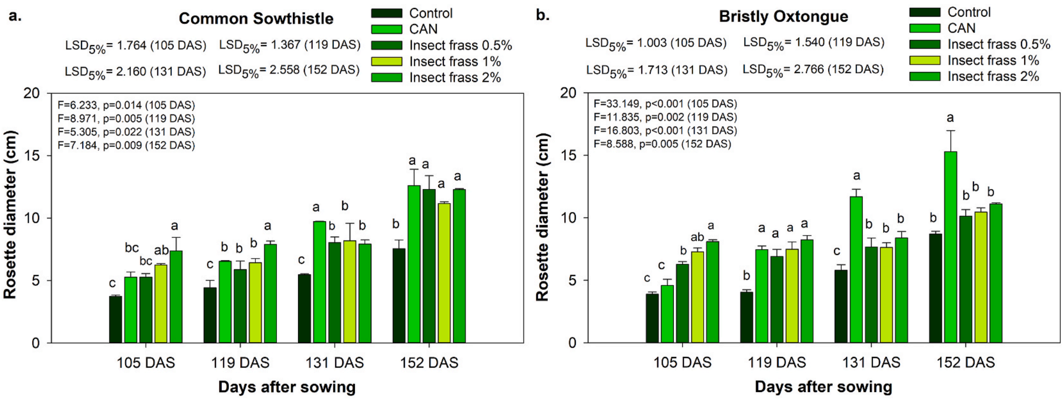

Fig. 3. Fertilization treatments (control, CAN, and insect frass) effects on rosette diameter of common sowthistle (a) and bristly oxtongue (b) plants. Statistical explanation as in Fig. 2.

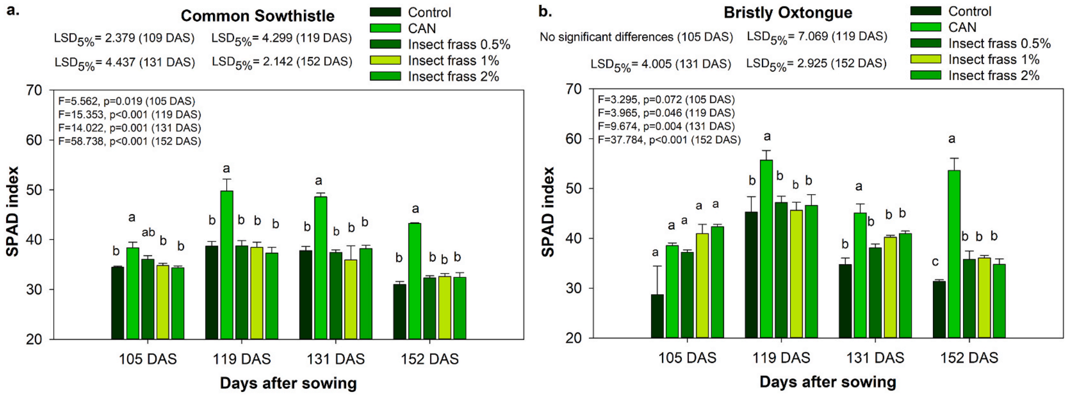

Fig. 4. Fertilization treatments (control, CAN, and insect frass) effects on SPAD index in leaves of common sowthistle (a) and bristly oxtongue (b) plants. Statistical explanation as in Fig. 2.

3.1.3. *Fresh and dry weight of above-ground parts of plants*

The fresh and dry weight of above-ground parts of plants were notably affected by the fertilization treatments (Figs. 5 and 6). In all measurements and for both parameters, the lowest values were recorded in the control treatment. At the final measurement, in common sowthistle, significant differences between the control treatment and the other fertilization treatments were recorded, while there were no differences between the insect frass treatments and the inorganic fertilizer. In bristly oxtongue, the highest dry weight values were measured in the CAN treatment (0.79 g plant^{-1}) followed by that in the 2 % frass treatment (0.45 g plant^{-1}).

3.1.4. *Root weight (fresh and dry) and root to above-ground dry weight ratio*

The root fresh weight ranged from 1.88 to 4.01 g plant^{-1} in common sowthistle (Fig. 1a) and from 2.33 to 5.61 g plant^{-1} in bristly oxtongue (Fig. 1b). In common sowthistle, the highest values of this trait were measured in the insect frass treatments, and there were significant differences among these treatments and the CAN treatment (Fig. 7). In contrast, in bristly oxtongue, there were no significant differences among the CAN, 1 % frass, and 2 % frass treatments. Similar results were also recorded for root weight, with the lowest values (0.45 g plant^{-1} and 0.28 g plant^{-1} in common sowthistle and bristly oxtongue, respectively) measured in the control treatment. In the case of root to above-ground dry weight ratio, there were no significant differences among the five experimental treatments. However, the values of this ratio varied between the two species, with the highest values (2.04–3.68) found in common sowthistle.

3.2. *Soil nutrient content*

The nitrate content (NO_3–N) in the soil was affected by the fertilization treatments with the highest values recorded in the CAN treatment (15.83 g kg^{-1} and 19.26 g kg^{-1} in common sowthistle and bristly oxtongue, respectively). However, there were no significant differences between the control and the insect frass treatments (Table 1). In contrast, the highest values of ammonium (NH_4–N) (15.65 mg kg^{-1} and 20.86 mg kg^{-1} in common sowthistle and bristly oxtongue, respectively) were recorded in the highest rate of insect frass and were 71.9–74.6 % and 41.2–45.5 % higher compared to the control treatment and the CAN treatment,

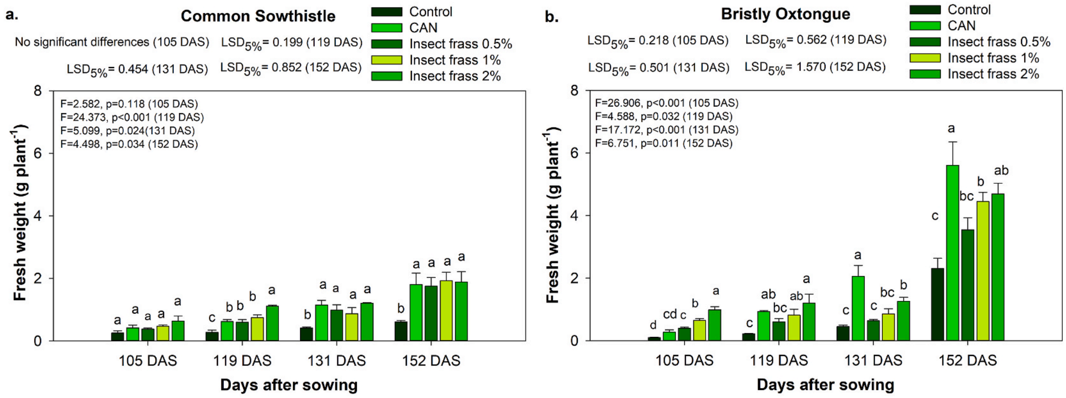

Fig. 5. Fertilization treatments (control, CAN, and insect frass) effects on above-ground fresh weight of common sowthistle (a) and bristly oxtongue (b) plants. Statistical explanation as in Fig. 2.

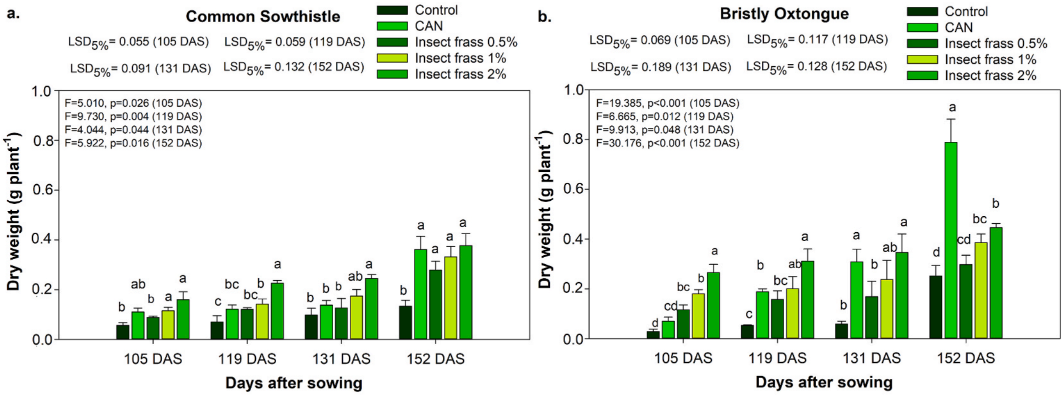

Fig. 6. Fertilization treatments (control, CAN, and insect frass) effects on above-ground dry weight of common sowthistle (a) and bristly oxtongue (b) plants. Statistical explanation as in Fig. 2.

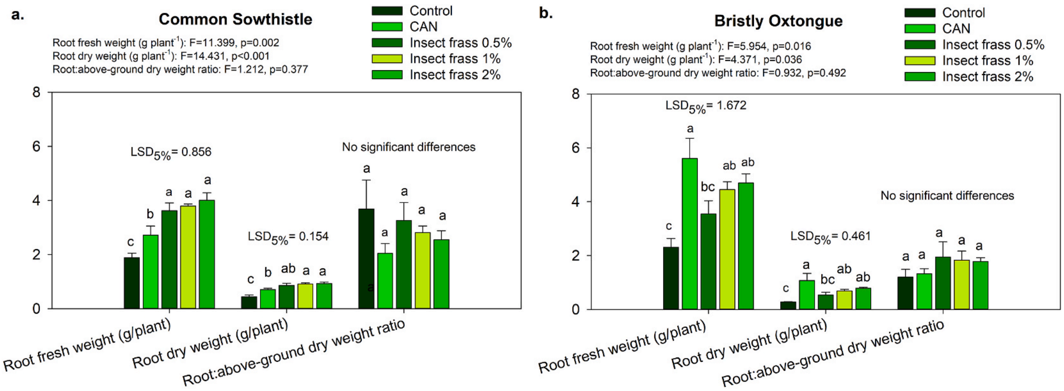

Fig. 7. Fertilization treatments (control, CAN, and insect frass) effects on weight (fresh and dry) and root:above-ground dry weight ratio of common sowthistle (a) and bristly oxtongue (b). Statistical explanation as in Fig. 2.

respectively. In both species, no significant differences were found between the control treatment and the inorganic fertilizer treatment for soil NH_4–N content.

Moreover, P and K soil content was affected by the fertilization regime with the highest values (P: 96.09 and 100.33 mg kg^{-1} in common sowthistle and bristly oxtongue, respectively; K: 361.7 and 418.3 mg kg^{-1} in common sowthistle and bristly oxtongue, respectively) recorded in the highest rate of insect frass. Specifically, in the common sowthistle experiment, there were significant differences between the 2 % frass treatment and the other treatments, while in the bristly oxtongue experiment significant differences between 2 % frass and 1 % frass were recorded. It is also important to point out that the P content for all the fertilization treatment was higher in the pots of bristly oxtongue compared to common sowthistle. In the case of Mg content in the soil, no significant differences among the fertilization treatments were found. Magnesium content in soil ranged from 1323.3 to 1594.1 mg kg^{-1} and from 1093.3 to 1393.7 mg kg^{-1} in the pots of common sowthistle and bristly oxtongue, respectively.

3.3. Nutrient content in plant tissues

Phosphorus and potassium content in above-ground plant tissues of common sowthistle and bristly oxtongue were affected by the fertilization regimes (Table 2). For both species, the highest values of P content (10.36–11.07 g kg^{-1} and 7.15–9.21 g kg^{-1} in common sowthistle and bristly oxtongue, respectively) were found in the treatments with 1 and 2 % frass, while there were no significant differences between the control treatment and the CAN treatment. Moreover, the lowest K content (10.87 g kg^{-1} and 11 g kg^{-1} in common sowthistle and bristly oxtongue, respectively) was observed in the control treatment, while there were no significant differences between the CAN treatments and insect frass regardless of its application rate. In the case of Zn content, no significant differences in common sowthistle were found among the treatments, while in bristly oxtongue the highest Zn content (19.80 mg kg^{-1}) was recorded in the highest application rate of frass. Moreover, for all fertilization treatments, Zn content was higher in common sowthistle compared to bristly oxtongue. Finally, our results indicated that Mg, Mn, and Cu content in the above-ground tissues of both species.

Table 1

Fertilization treatments (control, CAN, and insect frass) effects on soil nutrient content (NO$_3$–N, NH$_4$–N, P, K, and Mg) at 152 days after sowing. Statistical explanation as in Fig. 2 n.s.: non-significant.

Treatments	NO$_3$–N (mg kg^{-1})	NH$_4$–N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})
	Common sowthistle				
Control	1.97 ± 0.10 b	4.39 ± 0.43 b	12.34 ± 1.03 c	231.7 ± 26.82 b	1387.6 ± 8.61 a
CAN	15.83 ± 5.51 a	9.20 ± 0.62 b	12.58 ± 0.44 c	252.5 ± 33.97 b	1354.3 ± 105.75 a
Insect frass 0.5 %	1.67 ± 0.32 b	8.54 ± 0.15 b	20.72 ± 3.12 bc	220.0 ± 22.91 b	1323.3 ± 64.68 a
Insect frass 1 %	1.86 ± 0.53 b	9.42 ± 0.40 b	41.06 ± 14.59 b	276.7 ± 21.28 b	1364.8 ± 87.37 a
Insect frass 2 %	1.76 ± 0.28 b	15.65 ± 3.79 a	96.09 ± 15.03 a	361.7 ± 19.22 a	1594.1 ± 91.94 a
LSD$_{5\%}$	7.923	5.497	26.576	63.827	n.s.
Analysis of Variance					
F-values	6.656	5.717	18.735	9.437	1.600
p-values	0.012	0.018	<0.001	0.004	0.265
Treatments	NO$_3$–N (mg kg^{-1})	NH$_4$–N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})
	Bristly oxtongue				
Control	1.15 ± 0.04 b	5.31 ± 0.72 c	19.82 ± 3.58 b	223.3 ± 13.64 b	1166.5 ± 92.62 a
CAN	19.26 ± 4.98 a	11.38 ± 2.05 bc	16.30 ± 1.85 b	210.0 ± 2.82 b	1093.3 ± 177.84 a
Insect frass 0.5 %	1.13 ± 0.20 b	10.69 ± 2.23 bc	30.02 ± 5.40 b	256.7 ± 19.65 b	1293.7 ± 168.13 a
Insect frass 1 %	1.47 ± 0.16 b	16.73 ± 4.38 ab	68.82 ± 33.26 ab	283.3 ± 36.09 b	1337.7 ± 104.85 a
Insect frass 2 %	1.22 ± 0.04 b	20.86 ± 6.93 a	108.33 ± 18.69 a	418.3 ± 30.60 a	1396.7 ± 104.49 a
LSD$_{5\%}$	7.332	9.142	56.959	77.241	n.s.
Analysis of Variance					
F-values	12.844	4.543	5.076	12.384	1.253
p-values	0.001	0.033	0.025	0.002	0.363

Table 2

Fertilization treatments (control, CAN, and insect frass) effects on nutrient content (NO$_3$–N, NH$_4$–N, P, K, and Mg) in above-ground plant tissues of common sowthistle and bristly oxtongue at 152 days after sowing. Statistical explanation as in Fig. 2 and Table 1.

Treatments	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
	Common sowthistle					
Control	3.16 ± 0.46 c	10.87 ± 0.90 b	4.77 ± 0.09 a	43.32 ± 4.06 a	21.28 ± 1.28 a	10.77 ± 0.12 a
CAN	1.90 ± 0.48 c	26.00 ± 2.53 a	3.76 ± 0.48 a	35.17 ± 1.11 a	19.37 ± 5.05 a	9.83 ± 1.71 a
Insect frass 0.5 %	6.96 ± 0.31 b	25.47 ± 1.74 a	3.97 ± 0.10 a	36.57 ± 2.29 a	20.69 ± 0.58 a	10.26 ± 2.08 a
Insect frass 1 %	10.36 ± 0.28 a	27.33 ± 0.93 a	3.69 ± 0.14 a	36.07 ± 2.33 a	22.53 ± 0.31 a	8.33 ± 0.30 a
Insect frass 2 %	11.07 ± 1.79 a	24.27 ± 3.57 a	4.07 ± 0.15 a	30.12 ± 3.09 a	24.55 ± 1.83 a	9.35 ± 1.03 a
LSD$_{5\%}$	3.038	7.912	n.s.	n.s.	n.s.	n.s.
Analysis of Variance						
F-values	19.610	7.749	2.593	2.961	0.649	0.845
p-values	<0.001	0.007	0.117	0.089	0.643	0.535
Treatments	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
	Bristly oxtongue					
Control	2.36 ± 0.30 c	11.00 ± 0.64 b	4.48 ± 0.40 a	52.57 ± 3.33 a	12.87 ± 0.64 c	17.84 ± 2.20 a
CAN	1.66 ± 0.13 c	17.27 ± 1.52 a	3.95 ± 0.07 a	55.54 ± 7.18 a	15.60 ± 0.62 bc	21.57 ± 6.86 a
Insect frass 0.5 %	6.28 ± 0.14 b	17.13 ± 1.46 a	4.32 ± 0.07 a	51.73 ± 4.15 a	16.57 ± 1.24 abc	12.53 ± 0.72 a
Insect frass 1 %	7.15 ± 0.72 ab	16.93 ± 1.88 a	3.68 ± 0.05 a	50.89 ± 1.51 a	18.03 ± 2.25 ab	17.90 ± 4.64 a
Insect frass 2 %	9.21 ± 1.25 a	18.27 ± 0.87 a	4.30 ± 0.13 a	54.21 ± 2.93 a	19.80 ± 0.92 a	16.61 ± 5.42 a
LSD$_{5\%}$	2.281	4.657	n.s.	n.s.	3.703	n.s.
Analysis of Variance						
F-values	21.214	4.147	2.597	0.231	5.267	0.445
p-values	<0.001	0.041	0.117	0.913	0.022	0.774

4. Discussion

A dose-dependent impact of the frass originated from *T. molitor* on soil fertility was clearly profound in the present study. Indeed, the 2 % frass treatment resulted in higher P and K soil content compared to the control, 0.5 % frass, and 1 % frass treatments. This is ascribed to the high P and K soil content delved from the 2 % frass treatment, thereby contributing to enhanced availability of these essential nutrients in the soil and the concomitant increase in soil fertility. Similar results were reported by Antoniadis et al. [17], who also investigated the impact of *T. molitor* frass on soil fertility by conducting pot experiments. In that study, the authors reported that the application of frass from *T. molitor* at the rates of 0.5 and 1 % resulted in increased P and K content in the soil, while the low rate of 0.25 % had no significant impact on the concentration of these elements in the soil. Therefore, the above consistent results reveal the importance of the right dose application of *T. molitor* frass in maximizing the benefits of this alternative fertilizer on soil fertility enhancement.

The above-mentioned significant increase in soil P and K content is mainly attributed to the substantial amounts of these nutrients (P: 3813 mg kg^{-1} and K: 22,339 mg kg^{-1}) contained in the *T. molitor* frass of the present study. A comparison with similar research outcomes clearly shows variations in nutrient content of the frass derived from the different insect farms where these species are bred. For instance, in the study of Blakstad et al. [23] the values of available P and K in frass from *T. molitor* were reported to be approximately 13,000 mg P kg^{-1} and 23,000 mg K kg^{-1}. On the other hand, the N, P, and K content in a commercial frass fertilizer (HexaFrass™) from the black soldier fly, *Hermetia illucens* L. (Diptera, Stratiomyidae), was 3, 2, and 1 %, respectively [41]. In a recent study, the P content in two insect frass fertilizers (IF–C and IF-L) fluctuated between 1.6 and 1.9 %, while differences between the frass fertilizers were also found for other macro- and micronutrients [24]. These differences in mineral content are due to the different substrates used to rear the insects [24]. It is also important to point out that mineral composition varies among the frass fertilizers derived by different insect species [25].

In contrast to P and K content, Mg levels in the soils were not affected by the application of insect frass, although a slight increase (13–16.5 %) in the 2 % frass treatment was observed. This can be ascribed to the high initial Mg concentration of the soil used as substrate in our experiment. Nevertheless, the Mg content in the insect frass itself was also high (6036 mg kg^{-1}). Similarly, a considerable amount of Mg (8700 mg kg^{-1}) was also found in *H. illucens* frass, currently commercialized as HexaFrass™ [41]. Therefore, all the above data point to the nutrient-rich profile of insect frass fertilizers, particularly for P and K macronutrients, constituting them as valuable organic fertilizers regardless of their origin.

Concerning nitrate (NO_3–N) and ammonium (NH_4–N) nitrogen (N) content in the soil, our results show that the highest NO_3–N content was recorded in the CAN treatment, while the application of insect frass at the high rate of 2 % increased NH_4–N content up to 74.5 % compared to the other treatments. Similarly, Antoniadis et al. [17] observed the highest values of NO_3–N in the inorganic fertilization treatment, while the insect frass application had no significant impact in this parameter. Moreover, Jenkins et al. [42] reported that the NH_4–N content in the soil was significantly higher in *H. illucens* frass treatment compared to the unfertilized control after 1, 7, 14 or 28 days of incubation, while no significant differences for NO_3–N content were found. Ammonium content increase in the soil in the insect frass treatments was also reported by Zou et al. [43] and Huang et al. [44]. These varying findings across the aforementioned studies in terms of the different N forms in the soil suggest the occurrence of dissimilar composition and decomposition dynamics of nitrogen-containing compounds in different insect frass types in the soil. In addition, other studies have also shown that the application of insect mulch improves various soil properties. Huang et al. [44] reported that the dissolved organic carbon (DOC) and nitrogen (DON) content were significantly higher in black soldier fly frass treatments compared to the control treatment. Similarly, Zou et al. [23] observed an improvement in soil fertility in the treatment of larval frass vermicompost derived from larvae of *Protaetia brevitarsis* Lewis, 1879 (Coleoptera, Scarabaeidae) as both DOC and DON content increased up to 25 % and 34.3 %, respectively, compared to the control treatment. Thus, soil amendment with this unique fertilizer can enrich soil fertility, and subsequently improve both the root and above-ground growth of plants.

Concerning root growth, our results show that bristly oxtongue plants formed a rich root system, characterized by many lateral roots (Fig. 1d). Indeed, the fresh root weight in bristly oxtongue was 2.07-fold higher in the CAN treatment compared to the equivalent treatment in common sowthistle. Moreover, both inorganic and insect frass fertilizers resulted in increased fresh and dry weight of the root system in both plant species. Similarly, Antoniadis et al. [17] reported that the application of insect frass at rates of 0.25, 0.5, and 1 %, significantly increased the root weight of spinach plants compared to the control treatment, while inorganic fertilization increased the root weight to a lesser extent than the insect frass treatments. In another study, Borkent and Hodge [41] reported that the application of HexaFrass™ at relatively low rates increased root dry weight in lettuce and basil (*Ocimum basilicum* L.) crops. Moreover, an increase of 30.2 % in fresh root weight of zucchini (*Cucurbita pepo* L.) plants in the *T. molitor* frass treatment compared to the control was also observed in the study of Zim et al. [45]. All the above suggest a strong interaction of insect frass with the plant roots directly through the functional properties of the frass as a nutrient source.

In our experiment, the application of insect frass at a high rate of 2 % resulted in increased root growth. However, application rates exceeding those of 2 % may negatively impact root growth due to its elevated electrical conductivity (EC), as it has been already reported by previous studies. For instance, Zim et al. [45] reported a 7.3 and 11.4-fold higher EC in frass fertilizers from *H. illucens* and *T. molitor* compared to that in cattle manure and vermicompost, respectively. In some cases, it has been observed that the application of organic fertilizers (e.g., animal manures) in high doses can impede root growth, subsequently reducing the above-ground growth of plants. In a recent study, Siddiqui et al. [46] observed a significant decrease in both root and above-ground weight of lettuce plants with the application of poultry manure at a rate of 15 %. Our results also indicate that the root to above-ground dry ratio remained unaffected by both inorganic and insect frass treatments. In contrast, the application of an inorganic basal fertilizer (15-5-20) applied at a rate of 250 kg ha^{-1} or 500 kg ha^{-1}, in combination with CAN (50 or 100 kg ha^{-1}) applied as top-dressing in two equal doses, significantly increased the root to shoot ratio of bristly oxtongue compared to the control treatment [2].

Concerning the nutrient content in above-ground tissues, the results of this study revealed that the lowest values of P and K were found in the control treatment. For P content, the highest values were recorded in the insect frass 2 % treatment, while for K content, no differences between the insect frass treatments were found. The higher content of P and K in the soil along with the improved root growth in the frass treatments resulted in increased uptake by the roots and consequently, the rise of their content in above-ground tissues of both species. Similarly, Gärttling et al. [47] found that the application of frass from *H. illucens* significantly increased P (P_2O_5) content in maize plants compared to an organic fertilizer. Nevertheless, Antoniadis et al. [17] reported no increase in the levels of both nutrients in the above-ground parts of spinach, which can be partially attributed to the different plant species and experimental conditions.

The relative chlorophyll content (SPAD index) ranged from 31.0 to 49.8 in common sowthistle and from 31.4 to 55.7 in bristly oxtongue. In both species, the application of CAN fertilizer increased SPAD index values in the leaves, while significant differences between the control and the frass treatments were observed only in bristly oxtongue cultivation. In contrast, Antoniadis et al. [17] observed that at the final measurement the highest values of total chlorophyll content were observed in 0.5 % frass, 1 % frass, and inorganic fertilization treatments, with no significant differences among them. Moreover, increase in SPAD values of barley (*Hordeum vulgare* L.) plants in the HexaFrass™ treatment compared to the control treatment was also observed in the study of Carroll et al. [48].

The contrasting SPAD index results between common sowthistle and bristly oxtongue can be ascribed to the higher N uptake of the former in the early growth stages, evident by the lower concentration of soil NH_4 at harvest. According to Rummel et al. [49], N mineralization from *H. illucens* frass is rapid thereby constituting N readily available to plants. While rapid mineralization of N is an advantage for initial growth stages, it may become a burden in meeting plants' demands at later growth stages. Thus, the application of additional N either as top-dressing or a second frass dose, becomes imperative for maximizing plant growth and productivity. Fast mineralization of nutrients from the insect frass fertilizer, however, may have adverse effects on plant growth. According to Chiam et al. [50] the application of *H. illucens* frass at high rates (e.g., 20 and 30 %) retarded the growth of lettuce plants, leading to reduced dry weight.

Growth and fresh weight yield in both species was significantly enhanced by the application of *T. molitor* frass compared to the control treatment. However, agronomic parameters including leaf number, rosette diameter, and above-ground weight at harvest for both crops had the highest values in the CAN treatment, followed by the 2 and 1 % frass treatments. Antoniadis et al. [17] found the highest values of above-ground weight of spinach plants in the 1 % frass treatment, with no differences among the 0.25 % frass, 0.5 % frass, and inorganic fertilization treatments. Moreover, the application of HexaFrass™ in lettuce, resulted in an 84.8 and 93 % increase in its fresh above-ground weight compared to the control, depending on the nutrient level (high or low) of the substrate used [41]. In our study, it appears that during the rapid growth period (119–152 DAS), plants' N requirements were fully met by the inorganic fertilizer compared to insect frass, regardless of its application rate. Remarkably, at harvest, the total N content (NO_3–N plus NH_4–N) in the soil was higher by 23.4–27.9 % in the CAN treatment compared to 2 % frass. However, the increased nitrate and ammonia content in the soil can cause pollution of ground water with nitrates. Phillips et al. [51] reported that N loss through runoff was greater in the inorganic nitrogen fertilizer (ammonium sulfate) treatment compared to that in the combined application of organic and inorganic fertilizers.

5. Conclusions

The results of this study clearly demonstrate that the application of insect frass as organic amendment significantly improves soil fertility and plant growth, for both plant species tested. In the frass-treated soil a high increase in P and K content was achieved, compared to the unfertilized control treatment, constituting this fertilizer an effective source of soil enrichment. Concerning the NO_3 and NH_4 content in the soil, the 2 % frass and calcium ammonium nitrate treatments showed an opposite trend, since the CAN treatment exhibited the highest values of NO_3, while the lowest NH_4 content was found in the 2 % frass treatment. Moreover, the root weight (fresh and dry) of both species reached the highest values in the CAN and 2 % frass treatments.

At harvest, the above-ground dry weight of both species was significantly lower in unfertilized control. For common sowthistle, no significant differences between the CAN and frass treatments were recorded while for bristly oxtongue CAN application increased the above-ground dry weight. Concerning the nutrient content in the above-ground plant, our results indicate that the application of both inorganic and organic fertilization had no impact on Mg, Mn, and Cu content, while frass application increased P and K content compared to the control treatment. In conclusion, our results show that *T. molitor* frass can be considered a promising organic fertilizer the application of which significantly elevates soil P and K content, thereby improving common sowthistle and bristly oxtongue growth performance.

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The data included in this article.

CRediT authorship contribution statement

Anestis Karkanis: Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Angeliki Charitomeni Asprogeraka:** Investigation, Formal analysis. **Efthymios Paouris:** Investigation, Formal analysis. **Theodora Ntanasi:** Investigation, Formal analysis. **Ioannis Karavidas:** Investigation, Formal analysis, Data curation. **Christos I. Rumbos:** Writing – review & editing, Visualization, Methodology, Data curation. **Christos G.**

Athanassiou: Writing – review & editing, Visualization, Resources, Methodology, Data curation, Conceptualization. **Georgia Ntatsi:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Georgia Ntatsi, the coresponding author of this manuscipt, is Associate Editor for Heliyon Agriculture (a section of Heliyon), Christos Athanassiou, one of the co-authors of this manuscipt, is Associate Editor for Heliyon Agriculture (a section of Heliyon) and Christos Rumbos, one of the co-authors of this manuscipt, is Associate Editor for Heliyon Agriculture (a section of Heliyon). The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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