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Frass from *Tenebrio Molitor* fed with polyurethane foams used as fertilizer determines the volatile organic compounds of *Bellis perennis* flowers

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Abstract. *Frass, the excrement of Tenebrio molitor larvae, contains significant amounts of nutrients and beneficial microorganisms, making it a promising biofertilizer. However, frass derived from larvae fed with polyurethane (PU) foams remains largely unexplored. This study evaluated the growth and volatile organic compound (VOC) profiles of Bellis perennis cultivated with frass from T. molitor fed either bran (BRAN) or PU foams (PU), compared to plants grown with conventional chemical fertilizer (FERT). Plant growth, flowering, nutrient content in both plants and floral VOC emissions were analyzed. PU-derived frass showed higher nitrogen and calcium levels than BRAN-frass and resulted in increased flower production throughout the experiment, although plant biomass did not differ significantly among treatments. VOC analysis revealed distinct profiles associated with BRAN- and PU-derived frass, including compounds with known antimicrobial properties. These findings suggest that PU-frass is a viable biofertilizer for ornamental plants and may influence flower-associated chemical signaling.*

Keywords: frass, poliurethane polymer, recycled, fertilizer

Introduction

The European Green Deal, which includes the “From Farm to Fork” strategy, aims to reduce the use of chemical pesticides by 50% and chemical fertilizers by 20% by 2030 (Montanarella and Panagos, 2021). Various managements allow farmers to reduce these agrochemicals without decreasing crop productivity. In some cases, productivity even increases. These management systems include organic agriculture, the use of organic amendments, biofertilizers, and biopesticides, integrated pest management, and the use of added-value tailored compost with an induced biofertilizer or biopesticide effect through composting management (Hernandez-Lara et al., 2021, 2022).

Edible insects are nothing new. They currently feed two billion people worldwide due to their rich protein content (Ordoñez-Araque et al., 2022). The mass production of the beetle *Tenebrio molitor* (mealworm) opens the possibility of obtaining new by-products for agricultural use, such as frass. This is the name given to insect excrement, which can be used in sustainable agriculture as an organic fertilizer and a biostimulant to increase plant tolerance under abiotic stresses and boost resistance to biotic stresses (Zunzunegui et al.,

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2024). In nature, insect frass is included in the nutrient cycle, forming a fundamental part of the exchange between plant material and soil (Poveda et al., 2019). It contains significant quantities of nutrients and beneficial microbes, making this product suitable for use in agriculture (Beesigamukama et al., 2023). Beesigamukama et al. (2022) compare the frass from nine insect species (*H. illucens, Gryllus bimaculatus, Scapsipedus icipe, Bombyx mori, Gonimbrasia krucki, T. molitor, Schistocerca gregaria, Pachnoda sinuate,* and *Oryctes rhinoceros*), reporting that not only does the frass of *H. illucens* and *T. molitor* have adequate contents of macronutrients and micronutrients, *T. molitor* frass also has the highest Mg and P content. Although the nutritional composition of frass mostly depends on the insects' diet, in general, it contains a large amount of organic matter and an abundance of nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), along with a narrow C/N ratio (Watson et al., 2022). The nitrogen supplied by frass is one of the most noteworthy aspects of its potential use as a fertilizer (Poveda et al., 2019). Frass has also been found to enhance the soil's microbial biomass and add biomolecules and microbes that promote plant's growth (Watson et al., 2022) and antagonistic capacity toward various pathogens (Barragán-Fonseca et al., 2022) .

In 2020, a research by the European Bedding Industry Association showed that 49% of mattresses were landfilled in the European Union, 33% were sent to energy plants, and 17% were recycled (EBIA, 2020) Therefore, the makers of flexible polyurethane foam (PU-foam), the principal material in mattresses, should develop existing and new recycling technologies to reduce the impact of their products on the environment.

The mealworms of *T. molitor* have an inherent ability to degrade various polymers, using them as energy and a source of nutrients with the assistance of their gut microbiota (Orts et al., 2022; Yang et al., 2018). Moreover, the intestinal tracts of yellow mealworms can secrete emulsifying substances to facilitate plastic biodegradation (Brandon et al., 2021). Using this insect to biodegrade plastic can reduce the cost of mattress disposal, which is a significant economic benefit, not only as a solution for polymer recycling but also for the production of added-value by-products such as frass. Nevertheless, the effect of mealworm food on the qualities of their frass for agricultural use has been little analyzed.

Using frass for agriculture is extensive. However, the frass from mealworms fed with biodegradable compounds such as PU-foam is not so common. Our research has focused on the use of frass for floral cropping. Soil composition and nutrient availability are known to alter floral traits (Caruso et al., 2019; Strauss and Whittall, 2006) by changing the plant's volatile organic compounds (VOCs). These are generally lipophilic compounds with low molecular weight and high vapor pressure. They represent about 1% of the secondary metabolites identified in the plant kingdom. VOCs are produced in all plant organs, such as roots, stems, leaves, fruits, seeds, and flowers, which have been found to release the greatest quantities and diversity of VOCs (Amorim et al., 2024; Lo et al., 2024). Their physical properties allow them to cross cell membranes and be released into the atmosphere or soil without a diffusion barrier (Pichersky et al., 2006). Recently, scientific advances have greatly improved our understanding of VOC biosynthesis and have shown that VOC emissions are also epigenetically regulated (Patrick et al., 2021; Vlachonasios, 2021). Depending on their biosynthetic pathway, floral VOCs can be classified into three major families: terpenoids, phenylpropanoids/benzenoids, and fatty acid and amino acid derivatives (Raguso, 2004; Knudsen, 2006). Although they have attracted attention for their perfume, cosmetic, aroma, and medicinal applications, the primary functions of floral volatiles are ecological (Dudareva and Pichersky, 2008). They mediate ecological interactions between flowers and visitors, including pollinators, florivores, and pathogens, and serve as signals in plant-to-plant communication (Levin et al., 2003). Some VOCs may have no function of their own but are necessary for metabolic processes (Tholl et al., 2021). Depending on the species and the influence of biotic and abiotic factors, the diversity and quantity of volatiles emitted by flowers can vary (Lo et al., 2024).

Here, we evaluate the use of frass from *T. molitor* fed with bran and with PU-foam for cropping daisies (*Bellis perennis*). We tested three hypotheses: 1) both frasses (bran and PU-foam) can substitute chemical fertilization partially or totally; 2) frass from *T. molitor*-fed PU-foam increases the number of flowers per plant and biomass; 3) frass from *T. molitor*-fed PU-foam induced more volatile compounds related to attracting pollinators, the plant's defenses, and communication with other plants.

Materials and methods

Frass and Plants

The frass was obtained from mealworms (*T. molitor*) fed with bran (frass-Bran) and with PU-foam (frass-PU), following the protocol in Ros et al. (2025). The characteristics of both frasses are shown in Table 1. The frass did not contain remains of PU foam. The daisies (*Bellis perennis*) were obtained from a nursery in Murcia (Spain) after 25 days grown in peat.

Pot experiment

The pot experiment was carried out in plastic pots filled with 500 g of soil mixed with peat (375 g soil + 125 g peat). The characteristics of the soil (pH 8.6; EC 217 µS cm^{-1}; TOC 17.5 g kg^{-1}; TN 1 g kg^{-1}) and peat (pH 6.5; EC 260 µS cm^{-1}) were as follows. Three treatments were implemented to provide the plants with the equivalent of 150 kg N ha^{-1}. The treatments were: (i) Frass from bran (BRAN), (ii) Frass from PU-foam (PU), and (iii) NPK-mineral fertilizer (FERT). Each experimental treatment was tested in six replicates using a completely randomized design with one plant per pot. The experiment was carried out in a growth chamber during the whole growth cycle, with 16:8 hours of light and 30ºC during the day and 20ºC at night, growing under artificial light with a PAR of 300 µmol photons m^{-2} s^{-1} provided by an Arize top light current 255VDC. The pots were regularly watered to maintain the soil moisture near 80% of water holding capacity and avoid leaching. The pots were moved inside the growth chamber every 2 days to ensure a random effect. After 12, 13, and 14 Weeks After Sowing (WAS), the flowers were weighed and counted. The plant weight (biomass) was recorded after 14 WAS. The plants were dried until they reached a constant weight at 60ºC for further analysis, and the flowers were kept in an airtight glass jar for Volatile Organic Compound (VOC) analysis.

Nutrient and human pathogen analysis

The total nitrogen (TN) and total organic carbon (TOC) were determined using an elemental CHNS-O analyzer (Truspec CN, Leco, St. Joseph, Mich., USA). Nutrients were measured using ICP-MS (7500CE, Agilent, Santa Clara, CA, USA). In addition, the human pathogens *Salmonell*a and *E. coli* were analyzed according to the proceeding PNT-04 (qPCR) by Microgaia Biotech (Murcia, Spain). The remains of the PU in the frass were measured following Ros et al. (2025).

Volatile Organic Compounds (VOCs)

The flowers were plucked, and 0.9 g of petals were placed in an airtight glass jar and stored in the refrigerator in their original trays. They were tested within 3 days of delivery.

SPME-GC–MS Analyses

The SPME/GC–MS analysis was performed following the study described by Martínez-Moreno et al. (2024), making the appropriate adjustments and slight modifications. Gas chromatographic analyses were performed on an HP 8890B gas chromatography (GC) system coupled to an HP 5977B quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Prior to extracting the volatiles, the samples were equilibrated at room temperature (25ºC) for 15 min. Two grams of sample were placed in 20 mL glass autosampler vials and sealed with blue PTFE/white silicone septum (Chromacol) from Merck KGaA (Darmstadt, Germany). The vials were automatically transferred to the autosampler oven thermostated at 40ºC for an incubation time of 5 min.

For the SPME, a DVB/Carboxen/PDMS 50/30, stable flex-gray fiber was used (Supelco, Bellefonte, PA, USA). The first step was to bake it for 3 min at 250ºC. After that, it was placed 28 mm into the headspace of the 20 mL vial for 10 min. Immediately after the exposure, the fiber was transferred to the GC injector for 5 min of thermodesorption at 270ºC with an inlet mode of split (ratio 1:20) through an ultra-inert liner (5190-2292-900 µL, Agilent Technologies). An analytical column of medium polarity (VF-WAXms, 30 m × 0.25 mm × 0.25 µm, Agilent Technologies) was used with the following temperature program: 40ºC for 5 min, with a temperature ramp of 4ºC min^{-1} up to 250ºC (holding time 1 min). The mass selective detection was performed in the scan mode (30–500 amu, EI (70 eV), detector temperature 230ºC, frequency of 1.6 scans/s, and a cycle time of 622.9 ms). The sample vials were interspersed with empty vials to avoid cross-contamination. The SPME fiber was thermally conditioned according to the manufacturer's instructions before use, and a 5-minute conditioning was performed at the end of each extraction to ensure no carryover.

Data were processed using the Mass Hunter Qualitative Analysis software (version B.10.00, Service Pack 1, Agilent Technologies Palo Alto, CA, USA). To find the compounds, the algorithm "compound discovery by deconvolution" was used to select peaks of the GC and MS spectra with an absolute height greater than or equal to 5000 counts. Tentative identification of volatile compounds was achieved by comparing mass spectra results (Wiley11N17 main/NIST20 mass spectral library; Wiley, Chichester, UK) with a score greater than 80% and comparing the calculated retention indices with those published in the literature. According to the accepted criteria defined by the Metabolomics Standards Initiative (Sumner et al. 2007), qualitative results based on spectral similarity to the commercial mass spectral database without the use of reference standards should be reported as putative compound identifications (Level 2).

Statistical Analysis

The statistical analyses were conducted in R v4.1.2 (R Development Core Team, 2021). Prior to the analyses, we set a random seed for reproducibility using the *set.seed* (123) command. A principal component analysis (PCA) was conducted using the *FactoMineR* v2.11 package (Husson and Pages, 2024), using the arguments *scale.unit = TRUE* and *ncp = 4 in* the PCA function. The PCA results were visualized using the *fviz_pca* function from the *factoextra* v1.0.7 package (Kassambara and Mundt, 2020). Normality and homoscedasticity assumptions were assessed using the *rstatix* v0.7.2 package: a Shapiro test using shapiro_test() and a Levene test using levene_test() (Kassambara and Mundt, 2020). Once the assumptions were confirmed, a one-way analysis of variance (ANOVA) was conducted, followed by a post hoc Tukey's HSD test with the *agricolae* v1.3-6 package (de Mendiburu, 2023).

Results

Frass characterization

Both frasses showed high TOC and nutrient content (Table 1). The content of TOC was higher in frass-PU than in frass-Bran. Neither of the frasses showed human pathogens (*Salmonella* and *E. coli*). Frass-PU showed significantly higher amounts of the macronutrients N and Ca than frass-Bran, while frass-Bran showed significantly higher amounts of other nutrients such as P, K, and Mg, as well as micronutrients such as Cu, Fe, Mn, and Zn (Table 1).

Table 1. Elemental composition of frass from polyurethane foams and bran

Chemical properties	Frass-PU	Frass-Bran
Total N (TN) (g kg^{-1})	84±0.4b	57±1.0a
Total organic Carbon (TOC) (g kg^{-1})	470±0.5b	410±0.8a
Total Phosphorous (P) (g kg^{-1})	9.1±0.1a	12.2±0.2b
Total Potassium (K) (g kg^{-1})	11.7±0.1a	19.8±0.4b
Total Magnesium (Mg) (g kg^{-1})	3.5±0.1a	5.3±0.1b
Total Calcium (Ca) (g kg^{-1})	14±0.1b	1.6±0.0a
Total Copper (Cu) (mg kg^{-1})	14.95±0.10a	27.40±0.30b
Total Iron (Fe) (mg kg^{-1})	112.18±2.51a	149.00±18.50b
Total Manganese (Mn) (mg kg^{-1})	68.42±0.79a	155.06±1.74b
Total Zinc (Zn) (mg kg^{-1})	95.29±1.44a	99.06±3.21a
C/N	5.7±0.02a	7.21±0.14b

Letters indicated significant differences($p<0.05$) between treatments for each parameter

Promotion of plant growth, productivity, and nutritional content

In the *Bellis perennis* plant biomass, the fresh and dry weights of BRAN, PU, and FERT did not show differences at 14WAS (Table 2). Likewise, the macro- and micro-nutrients of the plant at 14WAS (Table 3) in general, did not show significant differences among the treatments.

Table 2. *Bellis perennis* biomass

Treatments	Fresh weight (gr)	Dry weight (gr)
BRAN	13.50±2.08a	4.85±0.49a
PU	13.50±1.73a	4.17±1.12a
FERT	12.66±1.15a	4.33±0.25a

Bran: Frass from bran feed; PU: Frass from PU feed; FERT: only fertilization

Letters indicated the significant differences ($p<0.05$) between treatments for each parameter

Table 3. Chemical properties of *Bellis perennis* after 14WAS

Treatments	TN (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Fe (mg kg^{-1})	Na (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
BRAN	6.1±0.4a	0.5±0.1ab	10.3±0.7b	8.1±1.0b	1.3±0.1a	28.6±9.1b	0.10±0.01a	52.3±2.82a	9.09±1.08a	2.00±0.24a
PU	6.0±0.7a	0.5±0.1a	8.0±0.4a	6.4±0.4a	1.2±0.2a	15.4±1.8a	0.12±0.05a	42.5±1.08a	10.40±2.28a	1.73±0.27a
FERT	7.1±0.1a	0.8±0.2b	10.4±1.2b	8.3±0.2b	1.4±0.2a	27.0±4.5b	0.15±0.06a	48.4±2.11a	14.06±2.70a	2.48±0.54a

WAS: weeks after sowing; Bran: Frass from bran feed; PU: Frass from PU feed; FERT: only fertilization. Letters indicated the significant differences ($p<0.05$) between treatments for each parameter

Since the *Bellis perennis* seedlings were sown (14WAS), the *Bellis* flowers were counted and weighed at 12WAS, 13WAS, and 14WAS (Table 4). The number of flowers per plant increased during the experiment for BRAN and PU but not for FERT. PU, followed by BRAN, showed the maximum number of flowers per plant during the experiment (Table 4).

Table 4. Number of *Bellis perennis* flowers per plant

Treatments/ sampling Timing	12WAS	13WAS	14WAS
BRAN	1.00±0.57	3.6±2.00	5.75±1.00
PU	2.33±1.15	5.00±1.00	7.33±1.50
FERT	2.00±1.00	5.33±2.5	1.53±0.57

WAS: weeks after sowing; Bran: Frass from bran feed; PU: Frass from PU feed; FERT: only fertilization

VOCs of Bellis perennis flowers

Across the different treatments, 39 VOCs were detected for *Bellis perennis* flowers. BRAN and PU showed 49% (19) and 59% (23) of the total VOCs, respectively, while FERT showed 85% (33) (Table S1). Out of the total VOCs, 14 metabolites were common to FERT, BRAN, and PU, and only two were common to BRAN and PU. Of the VOCs detected, only three were characteristic of only BRAN, while only one VOC was characteristic of PU (Table S1).

The PCA of the VOCs explained 60.2% (axe 1) and 17.8% (axe 2) of the total variance (Figure 1; Table S2). The first principal component (axe1) grouped the treatments into three groups: FERT, PU, and BRAN. Three metabolites [(+)-4-Carene; (E)-2, 6-Dimethylocta-2, 5, 7-trien-4-one; 2-(Bromomethyl)-1, 3, 3-trimethylcyclohexene; gamma-Terpinene] separated BRAN from PU and FERT (Figure 1). Four metabolites [2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene); Bicyclo [3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl) and Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha., 7. alpha.,8a. beta.)]- contributed to the separation of PU from BRAN and FERT (Figure 1).

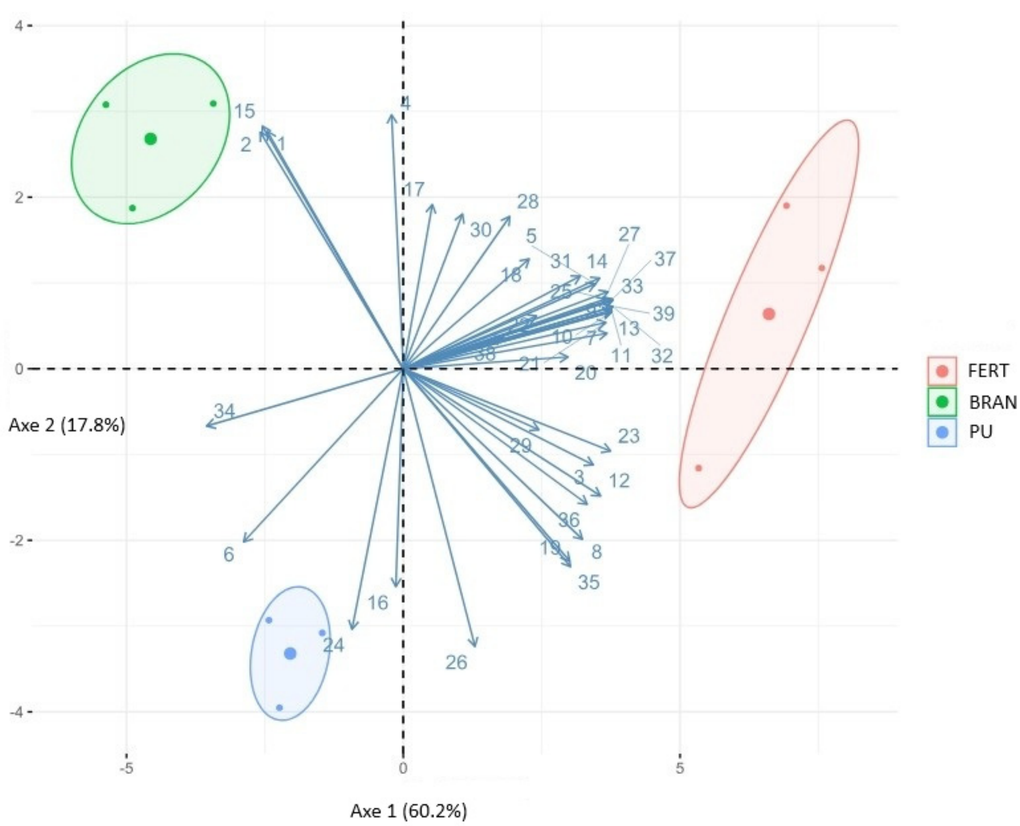

Figure 1. Principal component analysis of VOCs from *Bellis perennis* flowers

Table 5. Principal metabolites associated to BRAN and PU treatments

Metabolite code	Name	FERT	BRAN	PU
1	(+)-4-Carene	0 ± 0 b	1529196 ± 605060 a	0 ± 0 b
2	(E)-2,6-Dimethylocta-2,5,7-trien-4-one (Cis-tagetone)	0 ± 0 b	5450638 ± 518218 a	0 ± 0 b
15	2-(Bromomethyl)-1,3,3-trimethylcyclohexene	0 ± 0 b	1415540 ± 360403 a	0 ± 0 b
6	gamma-Terpinene	0 ± 0 c	4036022 ± 1847646 b	7414496 ± 414456 a
16	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	4200365 ± 3602703	3391690 ± 2702235	6273254 ± 466846
24	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl) (Beta-Thujene)	0 ± 0 a	0 ± 0 a	2894698 ± 2070512 a
34	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha. 7. alpha., 8a.beta.)] (beta-seline)	0 ± 0 b	822456 ± 261362 a	876194 ± 84556 a

Different letters indicate differences between treatment ($p<0.001$) for each metabolite

Discussion

Frass as fertilizer to grow Bellis perennis

Insect frass has nutrients easily assimilated by the roots due to the insect's physiological mechanisms for shaping its body waste. According to Poveda et al. (2021), the nutritional content of frass and its possible capacity as a fertilizer are closely linked to the feed received by the insect. The insect frass of the *T. molitor* mealworm produces frass rich in macro- and micro-nutrients that can be used as organic fertilizer (Amorim et al., 2024). PU-foam as feed for mealworms from *T. molitor* can be a sustainable environmental alternative to convert PU-foams (Orts et al., 2022) into frass that can be used as organic fertilizer. This topic has been scarcely addressed in the literature. The frass used in this study did not show human pathogens or traces of PU-foams (Ros et al., 2025). We observed that frass-Bran and frass-PU were rich in macronutrients, although frass-PU showed higher N and Ca content than frass-Bran. This could be because PU foams are used as polymers composed of repeating units containing urethane linkages formed by the reaction between isocyanates and polyols. This urethane linkage includes additional functional groups (like triazine and azanediyl), contributing to the overall nitrogen content of the polymers (Nanbedeh and Faghihi, 2021). The amount of N in polyurethane varies depending on its specific chemical structure, particularly the types and ratios of isocyanates and polyols used in its synthesis. In our case, frass-PU reached 7% of total nitrogen. Furthermore, in the formulation of our PU foam, carbonate calcium was incorporated as an additive to increase the viscoelastic properties of the foam (Donate-Robles and Martín-Martínez, 2011). Although the larvae use as much of the ingested nutrients as possible, their efficacy in absorbing nitrogen or other nutrients from the gut to maintain the body's stoichiometric homeostasis is not perfect. This forces the larvae to excrete these nutrients in the frass (Zhang et al., 2014), which results in higher amounts of N and Ca in the frass-PU.

Using insect frass from different origins (including PU-foams) as an alternative source of nutrients can reduce dependence on mineral and synthetic fertilizers and potentially lower agricultural production costs. However, the use of insect frass as fertilizer in agriculture is novel, and research on frass composition is still incipient, particularly compared with other commonly used animal manures or composts of different origins (Amorim et al., 2024). In addition to providing nutrients, insect frass can offer other important compounds for plant development. Frass also provides sugars, alkaloids, and phenols, as well as beneficial microorganisms that favor better plant adaptation and protection against biotic and abiotic stresses by activating tolerance and defense responses and fixing atmospheric nitrogen and/or solubilizing phosphates (Ji et al. 2014). Although, like compost, not all frasses show the same results in all crops in terms of plant defense (Acevedo et al., 2017; Poveda, 2021), similar results were observed by Kagata and Ohgushi (2012), where frass from the cabbage moth (*Mamestra brassicae*) promoted the growth of cabbage plants (*Brassica rapa* var. *perviridis*). van de Zande et al. (2024) observed that after 8 weeks, *Tenebrio molitor* frass, at 5 g kg^{-1}, exhibited higher biomass than the control plants and those with mineral fertilizer. Nyanzira et al. (2023) observed an increase in plant growth promotion and yield in wheat with 2% frass. Antoniadis et al. (2023) observed an increase in plant growth promotion of spinach with 1% v/v frass.

Regarding the fresh and dry plant biomass weight of *Bellis perennis*, PU and BRAN showed similar weight to FERT after 14 WAS, while the number of flowers increased during the experiment, principally in PU. This could be due to the release of nutrients from the frass, which had a similar effect on plant development as mineral fertilizer (Blakstad et al., 2023; Houben et al., 2020; Hénault-Ethier et al., 2023). Currently, there is no research available on the use of frass from *T. molitor* fed PU-foam for crop cultivation.

It is probable that the frass, and principally frass-PU, contains some microorganisms acting as biofertilizers, biostimulants, or biocontrol agents (Porto de Souza Vandenberghe et al., 2017), as well as chitin and other compounds that may stimulate beneficial soil microbes (Poveda, 2021) and enhance the number of flowers and plant growth. This should be studied more in-depth in the future.

Emission of Volatile organic compounds (VOCs) in response to frasses

More than 50% of the VOCs found in this experiment were terpenoids. Terpenes are highly aromatic compounds that, along with benzenoid VOCs, determine the smell of many plants and herbs and show defense functions in plants. Pollinator attraction is mediated by benzenoids (Schiestl, 2010). Thus, terpenoids and essential oils hold great insecticidal potential and can act as alternatives to synthetic pesticides (Siddiqui et al., 2024).

FERT produced the highest number of VOCs (33), with only ten unique to FERT. This was followed by PU (23), with only one characteristic VOC (*Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)*), and BRAN (19), with only three specific VOCs ((+)-*4-Carene; (E)-2,6-Dimethylocta-2,5,7-trien-4-one; and 2-(Bromomethyl)-1,3,3-trimethylcyclohexene*). This suggests that FERT stimulated a broader array of metabolic pathways than BRAN and PU, which showed limited specificity in VOC induction. These differences align with the chemical composition of the fertilizers, which often contain readily available nutrients that can enhance secondary metabolite production (Kaur et al., 2017).

On the other hand, among the 39 detected VOCs, 14 were common across all the treatments, reflecting a core set of metabolites that plants may consistently produce under varying nutrient and compound inputs. The VOCs that contributed to the separation in the PCA between BRAN and the others were (+)-*4-Carene; (E)-2,6-Dimethylocta-2,5,7-trien-4-one* or *cis-tagetone;* and 2-*(Bromomethyl)-1,3,3-trimethylcyclohexene, VOCs* associated with plant odor. VOCs such as *Gamma-Terpinene; 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene); Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)* or *β-thujene*; and *Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.α.,7.α.,8a.β)]* or *β-selinene* were associated with PU. *Gamma-Terpinene* and *β-thujene* are related to plant odor. *β-Thujene* is a molecule that gives a spicy odor

(Xiao et al., 2016), while *Gamma-Terpinene* is a natural product found in medicinal and aromatic plants, such as thyme, and is used in perfume and flavoring. In addition, it possesses antioxidant and antibacterial properties (Nabavi et al., 2015). *Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.α.,7α.,8a.β)]* or *β-selinene* is a natural product found in essential oils from a variety of plants with functional antioxidant, antiradical, and antimicrobial properties (Sacchetti et al., 2006).

Other metabolites common to the three treatments had different effects on the daisy flowers. *Bisabolenes* are present in essential oils, and various derivatives function as pheromones in insects such as stink bugs and fruit flies. *1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R,S*)]** and *Caryophyllene* smell spicy and have anti-inflammatory and insecticide effects (Huang et al., 2012). *Benzaldehyde, 4-(1-methylethyl)* is a component of floral scents (Farré-Armengol et al., 2020) and is a product of the plant's metabolism, which can have antibacterial activity against some pathogens (Neto et al., 2021). Finally, *Germacrene D* has antibacterial and antifungal properties (Montanari et al., 2011).

Conclusions

The study highlights the influence of new fertilizers, such as the frasses from *Tenebrio molitor* fed with bran and, especially, with PU-foam, for producing ornamental flowers such as *Bellis perennis* as efficiently as chemical fertilizer but with different VOC patterns. Frass-PU contained higher N and Ca content than frass-Bran and produced more flowers during the experiment, although no differences in plant weight were observed among frass fertilization (BRAN and PU) and fertilization (FERT). The VOCs produced by *Bellis perennis* flowers with frass fertilization (BRAN and PU) showed lower VOCs than FERT but with specific functions, e.g., antimicrobial function. This study highlights the need to integrate the ecological functions of VOCs into the evaluation of fertilization strategies, promoting sustainable practices that enhance plant health and ecosystem services.

Ethics declarations

The authors declare no competing interests.

The 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.

Availability of data and material

Under request

Code availability

R v4.1.2 (R Development Core Team, 2021).

Credit authorship contribution statement

Margarita Ros and Jose Antonio Pascual: Funding acquisition, Conceptualization. Margarita Ros: Writing – original draft; review & editing. Margarita Ros and Jose Antonio Pascual: Writing – review and editing. Paula Lidon and Jose Maria Orts: Data curation, Methodology and editing.

Consent to participate and Consent for publication.

All authors consent to participate and publicate this work.

References

Acevedo FE, Peiffer M, Tan CW, Stanley BA, Stanley A, Wang J, … Felton G, 2017. Fall Armyworm-Associated Gut Bacteria Modulate Plant Defense Responses. Molecular Plant-Microbe Interactions Journal, 30, 127-137. https://doi.org/10.1094/MPMI-11-16-0240-R

Amorim HCS, Ashworth AJ, Arsi K, Rojas MG, Morales-Ramos JA, Donoghue A and Robinson K, 2024. Insect Frass Composition and Potential Use as an Organic Fertilizer in Circular Economies. Journal of Economic Entomology,117, 1261-1268. https://doi.org/10.1093/jee/toad234

Antoniadis V, Molla A, Grammenou A, Apostolidis V, Athanassiou CG, Rumbos CI and Levizou E, 2023. Insect frass as a novel organic soil fertilizer for the cultivation of spinach (*Spinacia oleracea*): Effects on soil properties, plant physiological parameters, and nutrient status. Journal of Plant Nutrition and Soil Science, 23, 5935-5944. https://doi.org/10.1007/s42729-023-01451-9.

Barragán-Fonseca KY, Nurfikari A, van de Zande EM, Wantulla M, van Loon JJ, de Boer W and Dicke M, 2022. Insect Frass and Exuviae to Promote Plant Growth and Health. Trends in Plant Science, 27, 646-654. https://doi.org/10.1016/j.tplants.2022.01.007

Beesigamukama D, Subramanian S and Tanga CM, 2022. Nutrient Quality and Maturity Status of Frass Fertilizer from Nine Edible Insects. Scientific Reports, 12, 7182. https://doi.org/10.1038/s41598-022-11336-z

Beesigamukama D, Tanga CM, Sevgan S, Ekesi S and Kelemu S, 2023. Waste to Value: Global Perspective on the Impact of Entomocomposting on Environmental Health, Greenhouse Gas Mitigation and Soil Bioremediation. Science of The Total Environment, 902, 166067. https://doi.org/10.1016/j.scitotenv.2023.166067

Blakstad JI, Strimbeck R, Poveda J, Bones AM and Kissen R, 2023. Frass from yellow mealworm (*Tenebrio molitor*) as plant fertilizer and defense priming agent. Biocatal. Agric. Biotechnol, 53, 102862. https://doi.org/10.1016/j.bcab.2023.102862.

Brandon AM, Garcia AM, Khlystov NA, Wu WM and Criddle CS, 2021. Enhanced Bioavailability and Microbial Biodegradation of Polystyrene in an Enrichment Derived from the Gut Microbiome of *Tenebrio molitor* (Mealworm Larvae). Environmental Science & Technology, 55, 2027-2036. https://doi.org/10.1021/acs.est.0c04952

Caruso CM, Eisen KE, Martin RA and Sletvold NA, 2019. Meta-Analysis of the Agents of Selection on Floral Traits. Evolution 73, 4-14. https://doi.org/10.1111/evo.13639

de Mendiburu F, 2023. Agricolae: Statistical Procedures for Agricultural Research, R Package Version 1.3-7, https://CRAN.R-project.org/package=agricolae

Donate-Robles J and Martín-Martínez JM, 2011. Addition of Precipitated Calcium Carbonate Filler to Thermoplastic Polyurethane Adhesives. International Journal of Adhesion and Adhesives, 31, 795-804. https://doi.org/10.1016/j.ijadhadh.2011.07.008

Dudareva N and Pichersky E, 2008. Metabolic Engineering of Plant Volatiles. Current Opinion in Biotechnology, 19, 181-189. https://doi.org/10.1016/j.copbio.2008.02.011

EBIA, 2020. A study on the European mattress market and state of play with Extended Producer Responsibility.

Farré-Armengol G, Fernández-Martínez M, Filella I, Junker RR and Peñuelas J, 2020. Deciphering the Biotic and Climatic Factors That Influence Floral Scents: A Systematic Review of Floral Volatile Emissions. Frontiers in Plant Science,11. https://doi.org/10.3389/fpls.2020.01154.

Hénault-Ethie L, Reid B, Hotte N, Paris N, Quinche M and Lachance C, 2023.Growth trials on vegetables, herbs, and flowers using mealworm frass, chicken manure, and municipal compost. Journal of Agricultural Science and Technology, 3, 249-259. https://doi.org/10.1021/acsagscitech.2c00217.s001.

Hernández-Lara A, Ros M, Cuartero J, Bustamante MÁ, Moral R, Andreu-Rodríguez FJ and Pascual JA, 2022. Bacterial and Fungal Community Dynamics during Different Stages of Agro-Industrial Waste Composting and Its Relationship with Compost Suppressiveness. Science of The Total Environment, 805, 150330. https://doi.org/10.1016/j.scitotenv.2021.150330

Hernández-Lara A, Ros M, Pérez-Murcia MD, Bustamante MÁ, Moral R, Andreu-Rodríguez FJ and Pascual JA, 2021. The Influence of Feedstocks and Additives in 23 Added-Value Composts as a Growing Media Component on *Pythium irregulare* Suppressivity. Waste Manage, 120, 351-363. https://doi.org/10.1016/j.wasman.2020.11.053

Houben D, Daoulas G, Faucon MP and Dulaurent AM, 2020. Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Scientific Reports, 10, 4659. https://doi.org/10.1038/s41598-020-61765-x.

Huang M, Sanchez-Moreiras AM, Abel C, Sohrabi R, Lee S, Gershenzon J and Tholl D, 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytologist, 193, 997-1008. https://doi.org/10.1111/j.1469-8137.2011.04001.x.

Husson F, Lê S and Pagès J, 2024. FactoMineR: Multivariate Exploratory Data Analysis and Data Mining with R, Version 2.11.

Ji SH, Gururani MA and Chun SC, 2014. Isolation and Characterization of Plant Growth Promoting Endophytic Diazotrophic Bacteria from Korean Rice Cultivars. Research in Microbiology, 169, 83-98. https://doi.org/10.1016/j.micres.2013.06.003

Kagata H and Ohgushi T, 2012. Positive and Negative Impacts of Insect Frass Quality on Soil Nitrogen Availability and Plant Growth. Population Ecology, 54, 75-82. https://doi.org/10.1007/s10144-011-0281-6

Kassambara A and Mundt F, 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses, R Package Version 1.0.7. https://CRAN.R-project.org/package=factoextra

Kaur A, Scarborough P and Rayner MA, 2017. Systematic review, and meta-analyses, of the impact of health-related claims on dietary choices. International Journal of Behavioral Nutrition and Physical Activity, 14, 93. https://doi.org/10.1186/s12966-017-0548-1.

Knudsen J, Eriksson R, Gershenzon J and Ståhl B, 2006. Diversity and Distribution of Floral Scent. Bot. Rev, 72, 1-120. https://doi.org/10.1663/0006-8101(2006)72[1:DADOFS]2.0.CO;2

Levin RA, McDade LA and Raguso RA, 2003. The Systematic Utility of Floral and Vegetative Fragrance in Two Genera of Nyctaginaceae. Systematic Biology, 52, 334-351. https://doi.org/10.1080/10635150390196975

Lo MM, Benfodda Z, Molinié R and Meffre P, 2024. Volatile Organic Compounds Emitted by Flowers: Ecological Roles, Production by Plants, Extraction, and Identification. Plants, 13, 417. https://doi.org/10.3390/plants13030417

Martínez-Moreno A, Toledo-Gil R, Bautista-Ortín AB, Gómez-Plaza E, Yuste JE and Vallejo F, 2024. Exploring the Impact of Extended Maceration on the Volatile Compounds and Sensory Profile of Monastrell Red Wine. Fermentation, 10, 343. https://doi.org/10.3390/fermentation10070343

Montanarella L and Panagos P, 2021. The Relevance of Sustainable Soil Management within the European Green Deal, Land Use Policy, 100. 104950. https://doi.org/10.1016/j.landusepol.2020.104950

Montanari RM, Barbosa LCA, Demuner AJ, Silva CJ, Carvalho LS and Andrade NJ, 2011 Chemical composition and antibacterial activity of essential oils from *Verbenaceae* species: alternative sources of (E)-caryophyllene and germacrene-D. Química Nova, 34, 1550-1555. https://doi.org/10.1590/S0100-40422011000900013.

Nabavi SM, Marchese A, Izadi M, Curti V, Daglia M and Nabavi SF, 2015. Plants belonging to the genus *Thymus* as antibacterial agents: From farm to pharmacy. Food Chemistry, 173, 339-347. https://doi.org/10.1016/j.foodchem.2014.10.042.

Nanbedeh S and Faghihi K, 2021. Synthesis and Characterization of New Mesoporous Polyurethane-Nitrogen Doped Carbon Dot Nanocomposites: Ultrafast, Highly Selective and Sensitive Turn-Off Fluorescent Sensors for Fe^{3+} Ions. Journal of Fluorescence, 31, 517-539. https://doi.org/10.1007/s10895-020-02680-2

Neto LJ, de L, Ramos AGB, Freitas TS, de Barbosa CR, dos S, de Sousa Júnior DL, Siyadatpanah A and da Cunha FAB, 2021. Evaluation of Benzaldehyde as an Antibiotic Modulator and Its Toxic Effect against *Drosophila melanogaster*. Molecules, 26, 5570. https://doi.org/10.3390/molecules26185570.

Nyanzira A, Machona O, Matongorere M, Chidzwondo F and Mangoyi R, 2023. Analysis of frass excreted by *Tenebrio molitor* for use as fertilizer. Entomology and Applied Science Letters, 10, 29-37. https://doi.org/10.51847/xBw1ooFqXN.

Ordoñez-Araque R, Quishpillo-Miranda N and Ramos-Guerrero L, 2022. Edible Insects for Humans and Animals: Nutritional Composition and an Option for Mitigating Environmental Damage. Insects, 13, 944. https://doi.org/10.3390/insects13100944

Orts JM, Parrado J, Pascual JA, Orts A, Cuartero J, Tejada M and Ros M, 2022. Polyurethane Foam

Residue Biodegradation through the *Tenebrio molitor* Digestive Tract: Microbial Communities and Enzymatic Activity. Polymers, 15, 204. https://doi.org/10.3390/polym15010204

Patrick RM, Huang XQ, Dudareva N and Li Y, 2021. Dynamic Histone Acetylation in Floral Volatile Synthesis and Emission in Petunia Flowers. Journal of Experimental Botany, 72, 3704-3722. https://doi.org/10.1093/jxb/erab020

Pichersky E, Noel JP and Dudareva N, 2006. Biosynthesis of Plant Volatiles: Nature's Diversity and Ingenuity. Science, 311, 808-811. https://doi.org/10.1126/science.1118510

Porto de Souza Vandenberghe L, Marcela Blandon Garcia L, Rodrigues C, Cândido Camara M, Vinícius de Melo Pereira G, de Oliveira J and Soccol CR, 2017. Potential applications of plant probiotic microorganisms in agriculture and forestry. AIMS Microbiology, 3, 629-648. https://doi.org/10.3934/microbiol.2017.3.629.

Poveda J, Jiménez-Gómez A, Saati-Santamaría Z, Usategui-Martín R, Rivas R and García-Fraile P, 2019. Mealworm Frass as a Potential Biofertilizer and Abiotic Stress Tolerance-Inductor in Plants. Applied Soil Ecology, 142, 110-122. https://doi.org/10.1016/j.apsoil.2019.04.016

Poveda J, 2021. Insect Frass in the Development of Sustainable Agriculture: A Review. Agronomy for Sustainable Development, 41, 5. https://doi.org/10.1007/s13593-020-00656-x

R Development Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing (4.1.2). https://www.Rproject.org/

Raguso RA, 2004. Why Are Some Floral Nectars Scented? Ecology, 85, 1486-1494. https://doi.org/10.1890/03-0410

Ros M, Lidon P, Carrascosa A, Muñoz M, Navarro MV, Orts JM and Pascual JA, 2025. Polyurethane Foam Degradation Combining Ozonization and Mealworm Biodegradation and Its Exploitation. Environmental Science and Pollution Research, 2, 5332-5346. https://doi.org/10.1007/s11356-025-36029-8

Sacchetti G, Guerrini A, Noriega P, Bianchi A and Bruni R, 2006. Essential oil of wild *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) leaves from Amazonian Ecuador. Flavour and Fragrance Journal, 21, 674-676. https://doi.org/10.1002/ffj.1648.

Schiestl FP, 2010. The evolution of floral scent and insect chemical communication. Ecology Letters, 13, 643-656. https://doi.org/10.1111/j.1461-0248.2010.01451.x.

Siddiqui T, Khan MU, Sharma V and Gupta K, 2024. Terpenoids in essential oils: Chemistry, classification, and potential impact on human health and industry. Phytomed. Plus, 100549. https://doi.org/10.1016/j.phyplu.2024.100549.

Strauss S and Whittall J, 2006. Non-Pollinator Agents of Selection on Floral Traits. In: Ecology and Evolution of Flowers. Harder, L.; Barrett, S., Eds. Oxford University Press: Oxford, 120-138.

Sumner LW, Amberg A, Barrett D, Beale MH, Beger R and Daykin CA, 2007. Proposed Minimum Reporting Standards for Chemical Analysis. Metabolomics, 3, 211-221. https://doi.org/10.1007/s11306-007-0082-2

Tholl D, Hossain O, Weinhold A, Röse USR and Wei Q, 2021. Trends and Applications in Plant Volatile Sampling and Analysis. Plant Journal, 106, 314-325. https://doi.org/10.1111/tpj.15176

Van de Zande EM, Wantulla M, van Loon JJA and Dicke M, 2024. Soil amendment with insect frass and exuviae affects rhizosphere bacterial community, shoot growth, and carbon/nitrogen ratio of a brassicaceous plant. Plant Soil, 495, 631-648. https://doi.org/10.1007/s11104-023-06351-6.

Vlachonasios KE, 2021. Histone Acetylation: A Requirement for Petunia Floral Scent. Journal of Experimental Botany, 72, 3493-3495. https://doi.org/10.1093/jxb/erab092

Watson C, Houben D and Wichern F, 2022 Editorial: Frass: The Legacy of Larvae – Benefits and Risks of Residues from Insect Production. Frontiers in Sustainable Food Systems, 6, 889004. https://doi.org/10.3389/fsufs.2022.889004

Xiao Z, Fan B, Niu Y, Wu M, Liu J and Ma S, 2016. Characterization of odor-active compounds of various Chrysanthemum essential oils by gas chromatography–olfactometry, gas chromatography–mass spectrometry and their correlation with sensory attributes. Journal of Chromatography B, 1009-1010, 152-162. https://doi.org/10.1016/j.jchromb.2015.12.029.

Yang SS, Brandon AM, Flanagan JCA, Yang J, Ning D, Cai SY and Wu WM, 2018. Biodegradation of Polystyrene Wastes in Yellow Mealworms (Larvae of *Tenebrio molitor*): Factors Affecting Biodegradation Rates and the Ability of Polystyrene-Fed Larvae to Complete Their Life Cycle. Chemosphere, 191, 979-989. https://doi.org/10.1016/j.chemosphere.2017.10.117

Zhang J, Lu A, Kong L, Zhang Q and Ling E, 2014. Functional Analysis of Insect Molting Fluid Proteins on the Protection and Regulation of Ecdysis. Journal of Biological Chemistry, 289, 35891-35906. https://doi.org/10.1074/jbc.M114.599597

Zunzunegui I, Martín-García J, Santamaría Ó and Poveda J, 2024. Analysis of Yellow Mealworm (*Tenebrio molitor*) Frass as a Resource for a Sustainable Agriculture in the Current Context of Insect Farming Industry Growth. Journal of Cleaner Production, 460, 142608. https://doi.org/10.1016/j.jclepro.2024.142608