BRILL WAGENINGEN ACADEMIC

JOURNAL OF INSECTS AS FOOD AND FEED 11 (2025) 803–818

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Agronomic potential of *Hermetia illucens* frass in the cultivation of ryegrass in distinct soils

I. Rehan[1,2*], *I.G. Lopes*[3*], *D. Murta*[4,5], *F. Lidon*[2], *P. Fareleira*[6,7], *C. Esteves*[8], *O. Moreira*[1,9,10] *and R. Menino*[6,7]

[1]Instituto Nacional de Investigação Agrária e Veterinária (INIAV, IP.), Polo de Inovação da Fonte Boa, Unidade Estratégica de Investigação e Serviços de Produção e Saúde Animal (UEIS-PSA), Av. Professor Vaz Portugal, Quinta da Fonte Boa, 2005-048 Vale de Santarém, Portugal; [2]FCT-NOVA, GeoBioTec Research Center, Campus da Caparica, 2829-516 Caparica, Portugal; [3]Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Box 190, 23422 Alnarp, Sweden; [4]EntoGreen, Ingredient Odyssey SA, Industrial Zone of Santarém, 2005-079 Santarém, Portugal; [5]Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Quinta da Granja, 2829-511 Monte da Caparica, Portugal; [6]Instituto Nacional de Investigação Agrária e Veterinária (INIAV, IP.), Unidade Estratégica de Investigação e Serviços dos Sistemas Agrários e Florestais e Sanidade Vegetal (UEIS-SAFSV), Av. da República, Quinta do Marquês, 2780-159 Oeiras, Portugal; [7]ITQB NOVA, GREEN-IT Bioresources for Sustainability, 2780-157 Oeiras, Portugal; [8]ISA-UL, Terra Associate Laboratory, Linking Landscape, Environment, Agriculture and Food (LEAF) Research Centre, Tapada da Ajuda 1349-017 Lisboa, Portugal; [9]FMV-UL, Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Av. da Universidade Técnica, 1300-477 Lisboa, Portugal; [10]FMV-UL, AL4AnimalS, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal; *iryna.rehan@iniav.pt; iva.guidini.lopes@slu.se

Received 12 April 2024 | Accepted 29 April 2024 | Published online 10 October 2024 | Published in issue 28 March 2025

Abstract

Cropping systems are strongly dependent on mineral fertilisers, which are effective in achieving high crop productivities. However, these chemical inputs end up compromising soil quality in the long-term. Frass from black soldier fly (BSF) larvae is a novel organic fertiliser that is rich in organic matter and advocated as a material that can sustain crop productivity while increasing soil quality. This study aimed at evaluating distinct fertilisation regimes in the cultivation of ryegrass (*Lolium multiflorum* Lam. or annual ryegrass) in soils of different types (sandy, loamy and clay) and fertility levels. In a 7-month pot experiment conducted in a glass greenhouse, plants were cultivated with exclusive mineral (MT) or organic (OT) fertilisation, in addition to combinations between both (mineral and organic, MOTs) in different proportions (25:50; 50:50 and 75:25), considering a 140 kg per hectare N demand. Crop yield was favoured by the combination of organic and mineral fertilisers in all soils, which also had its fertility increased, especially regarding organic matter build-up and nutrient accumulation. In addition, the presence of frass in the sandy soil stimulated microbial activity, which was measured by the enzyme dehydrogenase. Frass derived from BSF larvae can be considered an adequate organic fertiliser in the cultivation of ryegrass in distinct soil types, when applied in partial (25% to 75%) replacement of mineral fertilisers, enabling high crop productivity and nutritional quality of the crop, while increasing soil fertility.

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-00001242
 | ISSN: 2352-4588 (online)

Keywords

black soldier fly – fertilisation – frass – waste

1 Introduction

The most recent projections of the United Nations indicated that the population might grow to roughly 8.5 billion by 2030 and reach 10.4 billion by 2100 (UN, 2022). Meeting the increasing population demands for food will require a significant boost in agricultural and livestock production in order to ensure food security (van Dijk *et al.*, 2021), leading to a consequent increase in fertiliser usage (Penuelas *et al.*, 2023). Such an increased food production is followed by several concerns, including an increased waste generation that should be managed properly in order to avoid environmental pollution and loss of resources (Kaza *et al.*, 2018). From a circular economy's perspective, waste management can and should be integrating agri-livestock systems with food production, increasing the general resilience of food systems (Bajželj *et al.*, 2020; Dou *et al.*, 2018).

Several technologies were developed and thoroughly improved for dealing with increasing volumes of varied waste streams. One of the most widespread technologies is thermophilic composting, which has been used for dealing with waste streams from agriculture and livestock production decades ago, generating safe products that are applied in cropland (Pergola *et al.*, 2018). Complementarily, a novel waste management technology has been gaining prominent attention over the last decade, namely the use of an insect species – the black soldier fly (*Hermetia illucens*, BSF) larvae for converting waste materials, which stands out for being a rapid process with potential to be more sustainable than composting or other technologies (Gold *et al.*, 2018; Smetana *et al.*, 2021). With this technology, waste is converted into two main by-products (a larval biomass and an organic fertiliser named frass), enabling the reintroduction of nutrients, that would be lost, into the food supply chain (Singh and Kumari, 2019).

Among several waste streams, generated in large volumes within food production systems, animal manures are considered sources of environmental pollution, especially in livestock-dense regions (Pratt *et al.*, 2015). Traditional processing techniques (e.g., thermophilic composting and anaerobic digestion) are many times not widely adopted due to logistic, economic and infrastructure constraints (Varma *et al.*, 2021). Thus, the treatment of manures with BSF larvae could facilitate proper treatment, especially considering that this process can be environmentally sustainable when waste is used as feedstock for the larvae (Bosch *et al.*, 2019). Several studies investigated the use of BSF larvae as tools for manure bioconversion (Chen *et al.*, 2019; Newton *et al.*, 2005; Parodi *et al.*, 2021), demonstrating the feasibility of this method for dealing with this waste stream. Manure bioconversion with BSF larvae results in the production of a high-quality fertiliser (frass) that, when applied to cropland, enables the build-up of soil organic matter and accumulation of nutrients in the soil (Veldkamp *et al.*, 2021). However, it is noteworthy that frass might not be adequate to be applied as single input for certain crops, considering that most of the plant nutrients in frass might be in organic forms. This was reported by Esteves *et al.* (2022) when evaluating frass as a fertiliser for lettuce, which is a short-cycle crop. Due to long N immobilisation periods, that are typically verified when frass is applied in the soil (Beesigamukama *et al.*, 2021), those authors verified reduced growth in lettuce in comparison to plants fertilised with a commercial NPK fertiliser. On the other hand, Romano *et al.* (2023) showed that replacing 1/2 of the synthetic fertiliser with frass from household organic waste resulted in greater biomass, stem diameter and height of the kale, as well as an increase in macro and micro nutrients with the use of frass. Chirere *et al.* (2021) used frass from different substrates such as human faeces, food waste and cow manure in Swiss chard production and showed that production was higher with these frasses compared to inorganic fertilisers. Frass can also be used to replace up to 20% of the peat-based for the soilless production of lettuce, basil and tomato plants in pots (Setti *et al.*, 2019). Increasing the fraction of frass resulted in a higher yield and quality of ryegrass, as well as an increase in soils organic matter (Menino *et al.*, 2021). On sweet potato slip production, there were no differences between the use of frass and inorganic fertiliser, but the cost-benefit ratio was higher when using frass (Romano *et al.*, 2022). In addition, there is a strong lack of knowledge on adequate application rates of frass, either alone or in combination with other inputs, as well as how frass exerts its effects in soils of distinct textures (Lopes *et al.*, 2022).

There is a strong need to reduce the use of less- or unsustainable inputs in agriculture, especially consider-

TABLE 1 Chemical characteristics of the soils used in this study, which were collected in distinct regions in Portugal. pH_{H2O} = pH measured in water; C/N = carbon to nitrogen ratio; N_t = total nitrogen; SOM = soil organic matter; P = phosphorus; K_2O = potassium oxide; Mg = magnesium; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; B = boron

	pH_{H2O}	C/N	N_t	SOM	P	K_2O	Mg	Fe	Mn	Zn	Cu	B
			%		mg/kg							
Podzol	7.2	9	0.037	0.55	17.5	47	35	46	12	15.3	2.8	<0.20
Calcisol	7.9	8	0.112	1.45	13.2	122	169	85	63	1.8	3.7	0.36
Fluvisol	7.9	8	0.160	2.08	50	371	635	297	266	2.1	6.1	1.30

ing that the nitrogen planetary boundary was exceeded and reactive nitrogen continues to be fixed for fertilisers' production (Schulte-Uebbing *et al.*, 2022). Additionally, soils have been degraded for decades globally due to exclusive and intense application of mineral fertilisers, highlighting the strong need to improve soil quality and resilience by the addition of organic matter in cropland, as this is one of the main factors affecting food production (Jie *et al.*, 2002). Organic amendments represent a simple and feasible alternative for this purpose. Organic fertilisers such as compost are known to build-up organic matter in the soil over time, promoting soil health (Scotti *et al.*, 2015). Such improvements can not only be observed when exclusive organic fertilisation is carried out, but also by combining organic and mineral inputs, which already represents a gain in terms of soil health and crop productivity (Esteves *et al.*, 2022).

Based on the aforementioned, this study aimed at evaluating the effects of frass derived from bovine slurry bioconversion with BSF larvae, alone and in combination with a commercial mineral fertiliser, using a widely used crop worldwide as a model (*Lolium multiflorum* Lam. or annual ryegrass), grown in soils of three distinct types (sandy, loamy and clay). It was hypothesised that regardless of soil type, frass would improve soil quality by means of fertility-related parameters and microbial activity, and that the standard mineral fertiliser could be at least partially replaced by the organic fertiliser while rendering similar crop productivity.

2 Material and methods

Location, soils and fertilisers

The experiment was conducted in a glass greenhouse between October 2021 and May 2022, in Oeiras/Portugal, at the National Institute for Agrarian and Veterinary Research (INIAV). The soils used for the experiment were collected from the topsoil layer (0-50 cm), sampled at three different locations: Patacão (Algarve), Quinta da Fonte Boa (Santarém) and Lezíria de Vila Franca (Ribatejo). These soils were respectively classified as Gleyic podzol, Haplic calcisol and Haplic fluvisol, according to ISSS/ISRIC/FAO (2006).

A composite sample of each soil type was dried (40 °C), sieved to pass a 2 mm mesh and analysed for chemical properties. Soils' pH was determined by suspending samples in solution by adding deionized water in a soil:water ratio 1:5 and measuring it with a glass electrode pH meter, according to ISO 10390. The soil organic matter (SOM) was determined by the dichromate oxidation method (Walkley-Black method) and molecular absorption spectroscopy (MAS). The P was determined by P-Olsen method. The K_2O was extracted with ammonium lactate and measured by flame photometry (Egner-Riehm method), while Mg was extracted with ammonium acetate (Chapman, 1965) and measured by atomic absorption spectroscopy-flame (AAS-flame). As for Fe, Mn, Zn and Cu, these were extracted by EDTA (Lakanen and Ervio, 1971) and determined by AAS-flame, while B was extracted with boiled water (Berger and Truog, 1939) and measured by ICP-OES. The soil moisture was determined according to the method described in ISO 11465. The total-N (N_t) was determined by the dry combustion method with the aid of an elementary analyser (FlashSmart NC Soil, Thermo Fisher Scientific, Waltham, MA, USA). The characteristics of the soils are presented in Table 1.

The BSF frass used in this study derived from the bioconversion of bovine slurry, being experimentally produced by Entogreen® (Santarém, Portugal), according to the procedures forecasted in the Portuguese legislation (Costa *et al.*, 2018). Frass was used fresh without grinding and was analysed for selected physical and chemical properties. The analyses were carried out according to the methods established by the British Standards Institution (BSI) for the following properties: humidity (EN 13040:2007) and dry matter (DM), obtained by difference between before and after determination of humidity; pH (EN 13037:2011); electrical conductivity (EC) (EN 13038:2011); organic matter (OM) (EN 13039:2011); and K_2O, P_2O_5, CaO, MgO, NaO, Cu,

TABLE 2 Chemical traits of *Hermetia illucens* frass derived from the bioconversion of bovine slurry and a chemical fertiliser (Nitrolusal®). EC = electrical conductivity; N_t = total nitrogen; DM = dry matter; SOM = soils organic matter; P_2O_2 = phosphorous pentoxide; K_2O = potassium oxide; CaO = calcium oxide; MgO = magnesium oxide; NaO = sodium oxide; Zn = zinc; Cu = copper; B = boron

	pH	EC	N_t	DM	SOM	P_2O_5	K_2O	CaO	MgO	NaO	Zn	Cu	B
		mS/cm	%			g/kg					mg/kg		
Frass	7.6	2.3	15.8	70.0	67.6	23.0	21.0	36.9	5.4	3.0	516	144	15
Nitrolusal®	–	–	27.0	–	–	–	0	40.0	20.0	–	–	–	–

Zn and B (EN 13650:2001). In order to determine the N-Kjeldahl, the sample was digested with sulphuric acid, followed by distillation and titration. The obtained results for the characterisation of frass and Nitrolusal® (Borrego Leonor & Irmão S.A., Almeirim, Portugal) are presented in Table 2.

Greenhouse experiment

Cylindrical plastic pots with a surface area of 227 cm² (15 cm height, 12.5 cm bottom diameter and 17 cm top diameter) were filled with 3 kg of soil (previously sun dried and sieved to pass a 2 mm mesh) and the fertilisers respective to each treatment. The treatments were based on mineral (M) and organic (O) fertilisations, as well as mixed fertilisations with both types of fertiliser (MOT), and the rates of application of each fertiliser were calculated based on the N demand by the crop, which was estimated as 140 kg N per hectare. Each of the five treatments described below were established with five replicates, distributed in a randomised plot design:

MT (100% M + 0 % O): 1.2 g of Nitrolusal® (per pot);
MOT(75:25) (75% M + 25% O): 9.4 g of frass + 0.9 g of Nitrolusal® (per pot);
MOT(50:50) (50% M + 50% O): 18.8 g of frass + 0.6 g of Nitrolusal® (per pot);
MOT(25:75) (25% M + 75% O): 28.2 g of frass + 0.3 g of Nitrolusal® (per pot);
OT (0% M + 100% O): 42.9 g of frass (per pot).

The amount of frass relative to the amount of soil per pot corresponds to 0.31, 0.63, 0.94, 1.43% for MOT(75:25), MOT(50:50); MOT(25:75) and OT, respectively. The fertilisers were thoroughly mixed into the soil before sowing. The ryegrass was sown at a rate of 40 kg seeds/ha, which corresponded to 0.10 g of seeds per pot. Throughout the plant growth cycle, the register temperature inside the greenhouse was between 18 and 25 °C, and the pots were regularly watered with deionized water, in order to maintain the soil moisture near to 60% of water holding capacity, estimated by weight difference. Lighting and temperature were ensured naturally, and shading screens were used on sunny days.

Sampling

The samples of plant material were collected at three harvesting events (January 12th 2022, March 23rd, 2022 and May 9th, 2022), seen that this forage crop is commonly cut more than once. In each of the cuts, the plant material was cut 2 cm from the ground. Plants biomass, fresh and dry weight (FW and DW, respectively), and its chemical composition were evaluated for each cut and analysed according to the methods used routinely in the laboratories of INIAV in Oeiras, Portugal. Regarding plant chemical analyses, the total biomass of each pot was washed with deionized water and oven dried at 65 °C for about 48 hours, until reaching constant weight. Dry weight (DW) was evaluated by weighting the dry material. Subsequently, the dry tissues were ground to pass a 0.5 mm sieve using a bench mill, for further chemical analysis. Due to the reduced amount of plant material in the 2nd and 3rd cuts, composite samples were assembled with the plants collected from all replicates and then analysed. Plant samples were analysed for N by the macro Kjeldahl method. Samples were burned in a muffle oven (450 °C), digested with HCl (3 M) and analysed for P, K, Ca, Mg, Na, B, Zn, Cu, and Mn by ICP-OES.

At the end of the growth cycle, a soil sample was collected from each replicate for chemical analyses according to the methods mentioned above, as well as for cation exchange capacity (CEC) by ammonium acetate, pH7 method and for soil dehydrogenase activity (DHA). However, the enzyme's activity was measured in three replicates per treatment, randomly selected. The activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF), using a modification of the method described by Casida *et al.* (1964). Soil aliquots (300 µg) were suspended in 200 µl of 1 M Tris-HCl (pH 7.5). After the addition of 100 µl of TTC (3%), samples were vortexed and incubated in the dark at 37 °C for 24 hours. Then, the TPF produced by each sample was quantitatively extracted by four washes with 500 µl-increments of methanol, each followed by centrifugation. Finally, the combined supernatants were analysed with the aid of a spectrophotometer at 485 nm. The amount of TPF was calculated by reference to a calibration curve prepared from TPF standards. DHA of each soil (expressed as µg TPF per g of dry soil and hour) corresponds to the average of six replicate samples obtained from one replicate (pot), previously corrected with the mean value of two blank samples, treated the same way but to which no TTC was added before incubation. Since tetrazolium salts are light-sensitive (Tabatabai, 1994), all procedures were performed avoiding direct exposure to light.

Data analysis

Soil and plant characteristics were evaluated by means of both a *one-way* and *two-way* analysis of variance (ANOVA). The assumptions of the method, namely the normal distribution and the homogeneity of variances were assessed, respectively, with the Kolmogorov-Smirnov and the Levene tests ($P > 0.5$). A multiple comparison of means *post*-hoc test of Tukey was used when the variances were homogeneous, while the Games-Howell test was adopted in case the variances were heterogeneous. The two ANOVAs were carried out due to the need of evaluating the effects of treatments in each soil, separately, as well as respecting the factorial design that was established with three soils and five fertilisation regimes. When variables had a distribution that was very different from normal, extremely skewed (absolute asymmetry values greater than 2), or flat (absolute kurtosis values greater than 7) (Marôco, 2014), non-parametric tests (Kruskal-Wallis) were used. In case the values did not differ, the ANOVA results were presented. All statistical tests were conducted using SPSS Statistics 27 software, considering a type I error probability (α) of 0.05. The plots were made using the package "ggplot2" in RStudio (RStudio Team, 2020).

3 Results

Soil analysis at the end of the cultivation period

A generally lower fertility level was observed in the podzol in comparison to the other two soils, regardless of fertiliser application. The fluvisol had the highest fertility level, which was observed by distinct parameters, especially regarding SOM that was 4-fold higher in the fluvisol in relation to the podzol after the experiment. Similarly, the macronutrients (N, P and K_2O) content in the fluvisol were up to 10-fold higher in that soil in relation to the other soils. This was also observed for CEC, which did not exceed 2.73 $cmol_c$/kg in the podzol, while in the calcisol it ranged between 14.3 and 15.5 $cmol_c$/kg and in the fluvisol it reached up to 24.88 $cmol_c$/kg when only the mineral fertiliser was applied (Table 3). The OT (exclusive organic fertilisation treatment) resulted in the greatest accumulation of SOM and nutrients such P and K_2O in all soils. The same treatment resulted in a higher concentration of N_t in the calcisol (0.14%), while in both podzol and fluvisol no differences were verified between treatments.

Considering soil type and fertilisation regime as factors, significant differences were found among treatments. The type of fertilisation exerted strong effects ($P < 0.001$) in the soils' fertility level, especially for SOM, CEC and for the levels of P and K_2O, while soil type also influenced the results obtained (Table S1). Regarding the activity of DHA in the soil, significant differences among treatments ($P < 0.05$) were only verified in the pots containing the podzol, while no differences were observed for the calcisol or the fluvisol (Figure 1). Distinct values for DHA activity were also observed in each soil type ($P < 0.001$), regardless of fertilisation regime (Supplementary Table S1). A higher activity of this enzyme was verified when an exclusive organic fertilisation with BSF frass was adopted (OT), reaching 1.016 µg TPF/g dry soil/h, while the other treatments showed an average activity of 0.304 µg TPF/g dry soil/h.

Analysis of plant material

Considering the effects on ryegrass FW production, both the treatments and soil types influenced the productivity of the crop, with significant interactions ($P < 0.001$) also being verified for this parameter (Supplementary Table S2). Within the same soil type, the plants cultivated in the podzol rendered the lowest yield, considering individual cuts as well as total yield (Table 4). Generally, for the 1st cut, the treatments that received a combination of mineral and organic fertilisers (MOTs) displayed the highest yields in all soil types, in comparison to exclusive fertilisation (either mineral or organic) (Table 4). Regarding the 2nd and 3rd cuts, the treatment that yielded the highest production in the podzol was OT (20.4-25.4 g/pot in the 2nd cut and 7.0-10.4 g/pot in the 3rd cut) in relation to others. In the calcisol, there were no differences ($P > 0.05$) among treatments in both the 2nd and 3rd cuts for fresh weight (Table 3). In addition, even though no differences were verified in the 2nd cut for the fluvisol, the OT treatment

TABLE 3 Chemical analysis of soils fertilised with distinct fertilisers in the cultivation of ryegrass (Lolium multiflorum Lam.). Data are presented as mean ± SD. MT = soil fertilised exclusively with a mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers (BSF frass); MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75) = soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised exclusively with BSF frass

	Podzol	Calcisol	Fluvisol
	Soil organic matter (%)		
MT	0.36 ± 0.05^{cC}	1.28 ± 0.05^{bB}	2.01 ± 0.03^{aC}
MOT(75:25)	0.39 ± 0.09^{cBC}	1.32 ± 0.15^{bB}	2.19 ± 0.05^{aB}
MOT(50:50)	0.46 ± 0.01^{cBC}	1.27 ± 0.02^{bB}	2.22 ± 0.16^{aBC}
MOT(25:75)	0.47 ± 0.05^{cB}	1.25 ± 0.08^{bB}	1.95 ± 0.09^{aC}
OT	0.59 ± 0.04^{cA}	1.78 ± 0.11^{bA}	2.57 ± 0.14^{aA}
	Cation exchange capacity ($cmol_c$/kg)		
MT	2.10 ± 0.07^{cB}	15.48 ± 0.93^{b}	24.88 ± 1.97^{aA}
MOT(75:25)	2.01 ± 0.33^{cB}	14.26 ± 0.46^{b}	18.00 ± 0.90^{aB}
MOT(50:50)	2.21 ± 0.31^{bB}	15.31 ± 0.82^{a}	17.87 ± 2.48^{aB}
MOT(25:75)	2.24 ± 0.21^{cB}	15.39 ± 1.70^{b}	18.62 ± 0.84^{aB}
OT	2.73 ± 0.23^{cA}	14.32 ± 0.75^{b}	24.04 ± 2.08^{aA}
	pH		
MT	8.23 ± 0.04^{AB}	8.50 ± 0.24	8.38 ± 0.15^{AB}
MOT(75:25)	8.26 ± 0.11^{cAB}	8.76 ± 0.09^{a}	8.56 ± 0.09^{bAB}
MOT(50:50)	8.24 ± 0.03^{cAB}	8.60 ± 0.10^{a}	8.41 ± 0.05^{bB}
MOT(25:75)	8.11 ± 0.08^{bB}	8.62 ± 0.09^{a}	8.56 ± 0.05^{aA}
OT	8.35 ± 0.10^{A}	8.48 ± 0.14	8.37 ± 0.10^{B}
	N_t (%)		
MT	0.03 ± 0.01^{c}	0.11 ± 0.02^{bB}	0.15 ± 0.02^{a}
MOT(75:25)	0.04 ± 0.02^{c}	0.12 ± 0.00^{bAB}	0.14 ± 0.01^{a}
MOT(50:50)	0.03 ± 0.01^{b}	0.11 ± 0.01^{aB}	0.15 ± 0.04^{a}
MOT(25:75)	0.04 ± 0.01^{c}	0.10 ± 0.01^{bB}	0.15 ± 0.02^{a}
OT	0.04 ± 0.01^{c}	0.14 ± 0.02^{bA}	0.17 ± 0.01^{a}
	P (mg/kg)		
MT	9.15 ± 1.05^{bBC}	6.66 ± 0.47^{cC}	49.84 ± 1.55^{aB}
MOT(75:25)	8.04 ± 1.22^{bC}	7.26 ± 1.22^{bC}	51.72 ± 3.99^{aB}
MOT(50:50)	8.68 ± 0.83^{bC}	8.10 ± 0.87^{bBC}	59.83 ± 4.92^{aB}
MOT(25:75)	12.45 ± 3.13^{bB}	10.69 ± 1.96^{bB}	56.27 ± 9.10^{aB}
OT	19.98 ± 2.25^{bA}	21.88 ± 2.41^{bA}	70.12 ± 4.71^{aA}
	K_2O (mg/kg)		
MT	12.77 ± 2.11^{cB}	88.07 ± 8.44^{bB}	203.4 ± 14.96^{aC}
MOT(75:25)	14.24 ± 0.52^{cB}	75.47 ± 3.16^{bB}	238.2 ± 9.40^{aBC}
MOT(50:50)	15.97 ± 1.69^{cB}	74.58 ± 4.77^{bB}	237.5 ± 12.86^{aBC}
MOT(25:75)	15.20 ± 3.58^{cB}	83.32 ± 6.29^{bB}	253.1 ± 27.77^{aB}
OT	98.95 ± 13.1^{bA}	128.61 ± 18.90^{bA}	352.1 ± 30.18^{aA}

Distinct lowercase superscript letters indicate significant differences of soil characteristics within the same line for each variable (comparison between soil types); and distinct uppercase superscript letters indicate significant differences of soil characteristics within the same column (comparison between treatments within the same soil type), for each variable presented ($P < 0.05$).

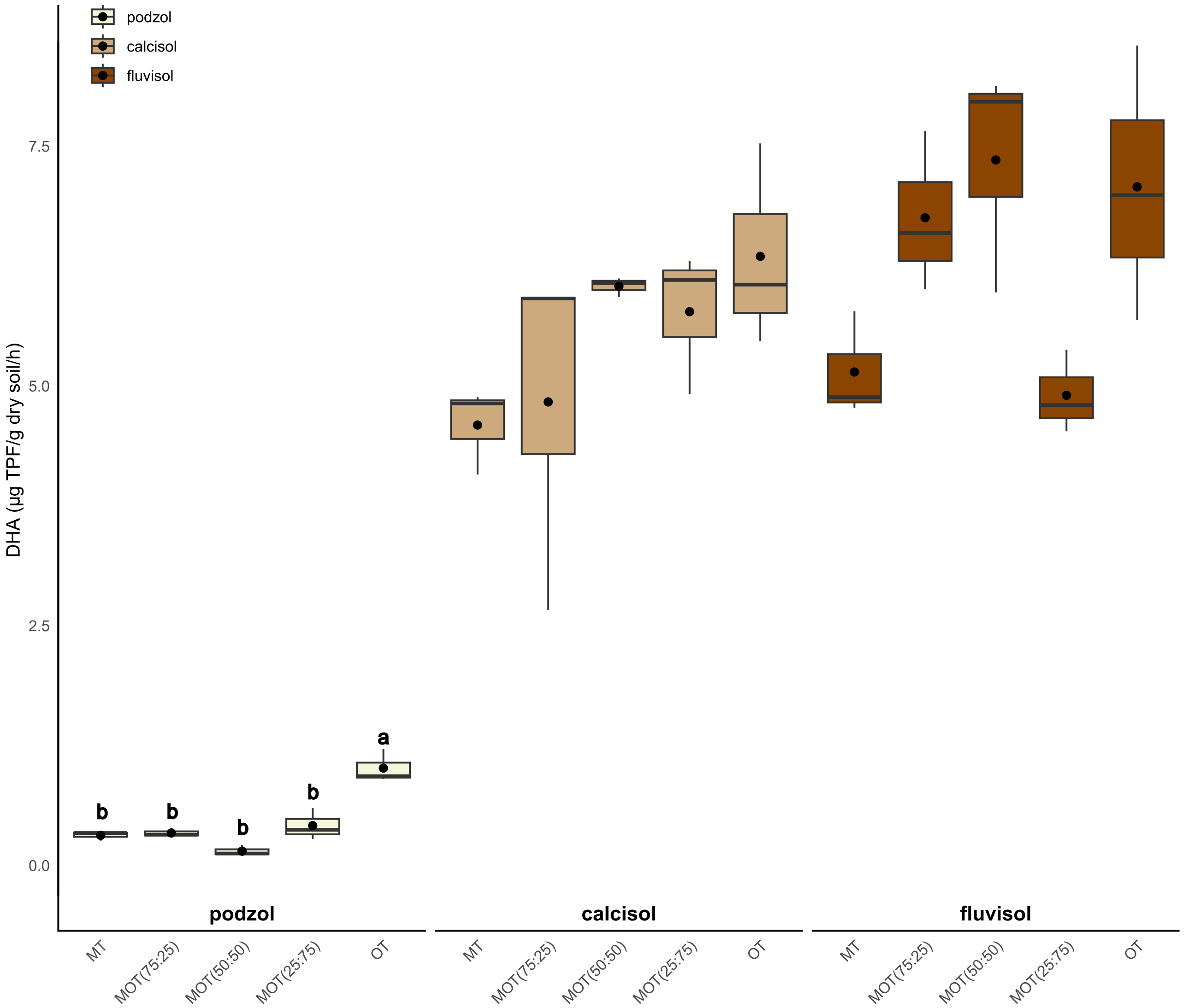

FIGURE 1 Dehydrogenase activity (µg TPF/g dry soil/h) in three soils (Podzol, Calcisol and Fluvisol) that received different fertilisation regimes. Error bars represent the standard error of the mean (SEM). Distinct letters indicate significant differences among treatments within the same soil type ($P < 0.05$). MT = soil fertilised exclusively with mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers (BSF frass); MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75) = soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised exclusively with organic fertiliser.

was the one that resulted in the highest yield in the 3rd cut. Considering the total production of the three cuts, it was observed that the mixtures of organic and mineral fertilisers in different proportions resulted in higher ryegrass production in the three types of soil.

Similar trends were observed in relation to DW, namely a higher production in both calcisol and fluvisol in comparison to the podzol (Table 5), with a strong effect of soil type ($P < 0.001$) and fertilisation regime ($P < 0.001$) affecting this parameter (Supplementary Table S2). As for differences between treatments, in the 1st cut there were only differences in the podzol, with higher production observed in the mixed treatments (6.40-7.80 g/pot). In the 2nd cut, for the same type of soil, production was higher in the OT (5.08 g/pot) and lower in the MT (2.08 g/pot). The opposite was observed in the fluvisol soil with 4.78 g/pot in the OT and 8.74 g/pot in MT. No differences were observed in the calcisol. The highest yields in the 3rd cut were observed in the OT treatment in both the podzol (3.40 g/pot) and calcisol (4.02 g/pot). For the fluvisol, the mixed treatments (MOTs) – with an exception for MOT(25:75) – and the OT treatment resulted in the highest ryegrass yields (on average 3.73 g/pot). Considering the total production of the three cuts, there were significant differences only for the podzol, with higher production in the mixed or OT treatments (on average 11.90 g/pot).

TABLE 4 Productivity (grams of fresh biomass per pot) of ryegrass cultivated in distinct soils (Podzol, Calcisol and Fluvisol) under distinct fertilisation regimes. Data are presented for the first, second and third cuts, as well as for the total biomass obtained with all three cuts. Data are presented as mean ± SD. MT = soil fertilised exclusively with mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers (BSF frass); MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75): soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised exclusively with organic fertiliser

	Podzol	Calcisol	Fluvisol
	1st cut (g/pot)		
MT	18.74 ± 5.27^{bB}	57.00 ± 10.30^{aBC}	70.40 ± 14.71^{aAB}
MOT(75:25)	45.20 ± 4.55^{bA}	77.60 ± 4.88^{aA}	77.00 ± 12.08^{aAB}
MOT(50:50)	45.60 ± 9.63^{bA}	73.80 ± 3.42^{aAB}	73.60 ± 7.44^{aA}
MOT(25:75)	48.60 ± 17.47^{bA}	61.00 ± 20.03^{abAC}	77.60 ± 10.92^{aAB}
OT	25.16 ± 5.44^{cB}	45.60 ± 6.19^{bC}	56.80 ± 3.11^{aB}
	2nd cut (g/pot)		
MT	12.32 ± 2.57^{bB}	25.12 ± 4.23^{a}	33.58 ± 9.49^{a}
MOT(75:25)	12.68 ± 1.72^{bB}	23.72 ± 1.80^{a}	28.88 ± 8.61^{a}
MOT(50:50)	16.10 ± 1.51^{bBA}	22.40 ± 3.05^{b}	29.58 ± 6.49^{a}
MOT(25:75)	13.42 ± 4.86^{bB}	21.98 ± 5.09^{ab}	26.06 ± 5.50^{a}
OT	20.44 ± 4.94^{A}	25.24 ± 4.32	24.00 ± 1.89
	3rd cut (g/pot)		
MT	2.88 ± 1.05^{cB}	6.62 ± 0.82^{b}	8.32 ± 0.93^{aB}
MOT(75:25)	2.80 ± 0.80^{cB}	7.38 ± 0.92^{b}	9.12 ± 1.20^{aAB}
MOT(50:50)	2.46 ± 0.61^{cB}	7.08 ± 0.61^{b}	9.30 ± 0.60^{aAB}
MOT(25:75)	2.54 ± 0.27^{bB}	6.56 ± 0.89^{a}	7.76 ± 0.90^{aB}
OT	6.98 ± 1.54^{bA}	8.98 ± 2.12^{ab}	10.36 ± 0.76^{aA}
	Total of cuts (g/pot)		
MT	33.94 ± 8.29^{bB}	88.74 ± 11.59^{aAB}	112.3 ± 22.72^{aAB}
MOT(75:25)	60.68 ± 5.70^{bA}	108.7 ± 5.21^{aA}	115 ± 13.25^{aAB}
MOT(50:50)	64.16 ± 10.57^{bA}	103.28 ± 5.56^{aA}	112.48 ± 10.92^{aA}
MOT(25:75)	64.56 ± 21.83^{bA}	89.54 ± 21.43^{abAB}	111.42 ± 9.52^{aA}
OT	52.58 ± 3.32^{cAB}	79.82 ± 7.79^{bB}	91.16 ± 3.59^{aB}

Distinct lowercase superscript letters indicate significant differences of fresh weight production within the same line for each variable (comparison between soil types); and distinct uppercase superscript letters indicate significant differences of fresh weight production within the same column (comparison between treatments within the same soil type) for each cut ($P < 0.05$).

The concentrations of macro- and micronutrients in the plant material harvested in the first cut of the ryegrass are shown in Figure 2 and Table 6. In both podzol and calcisol, the accumulation of N in the plants was significantly higher in the MT treatment in comparison to OT (approximately 4.5-fold higher in the podzol and 2.5-fold higher in the calcisol), and a trend was observed regarding N accumulation in the MOTs treatments, in which the higher the concentration of frass, the lower the N accumulation. However, an opposite trend was verified for P, which was generally found at slightly higher concentrations in the mixed treatments. This was also observed for K, especially in the podzol. Both macro- and micronutrients were also analysed in a factorial design and it was observed that both the fertilisation regimes ($P < 0.05$) and soil types ($P < 0.001$, expect for Cu that $P < 0.05$) influenced the nutrient concentration in the ryegrass biomass (Supplementary Table S2). Generally, all plants cultivated in the podzol had lower uptake of nutrients, with some exceptions such as N, Ca, Mg and some micronutrients. In addition, the MT treatment resulted generally in the highest accumulation of nutrients such as N, Ca, Mg and micronutrients (but not for P and K), however statistically similar to MOT(75:25) and MOT(50:50) (Supplementary Table S2). As previously mentioned, the total

TABLE 5 Productivity (grams of dry biomass per pot) of ryegrass cultivated in distinct soils (Podzol, Calcisol and Fluvisol) under distinct fertilisation regimes. Data are presented for the first, second and third cuts, as well as for the total biomass obtained with all three cuts. Data are presented as mean ± SD. MT = soil fertilised exclusively with mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers (BSF frass); MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75) = soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised exclusively with organic fertiliser

	Podzol	Calcisol	Fluvisol
	1st cut (g/pot)		
MT	2.40 ± 0.89^{bB}	8.00 ± 0.71^{a}	10.0 ± 2.92^{a}
MOT(75:25)	6.40 ± 2.07^{AC}	10.6 ± 4.77	9.40 ± 2.70
MOT(50:50)	7.40 ± 1.82^{A}	9.40 ± 0.89	9.40 ± 2.07
MOT(25:75)	7.80 ± 2.28^{bA}	9.40 ± 1.14^{ab}	11.0 ± 1.58^{a}
OT	3.40 ± 1.14^{bBC}	6.20 ± 2.39^{ab}	7.00 ± 1.73^{a}
	2nd cut (g/pot)		
MT	2.08 ± 0.50^{bC}	6.50 ± 1.11^{a}	8.74 ± 2.63^{aA}
MOT(75:25)	3.14 ± 0.32^{bBC}	6.30 ± 0.43^{a}	7.02 ± 1.75^{aAB}
MOT(50:50)	4.36 ± 0.39^{bAB}	5.68 ± 0.70^{ab}	7.88 ± 2.63^{aAB}
MOT(25:75)	3.56 ± 1.35^{bAB}	5.50 ± 1.53^{ab}	6.10 ± 1.41^{aAB}
OT	5.08 ± 0.91^{A}	5.98 ± 0.86	4.78 ± 0.48^{B}
	3rd cut (g/pot)		
MT	0.88 ± 0.28^{bB}	2.58 ± 0.40^{aB}	2.88 ± 0.48^{aB}
MOT(75:25)	0.98 ± 0.26^{bB}	2.72 ± 0.48^{aB}	3.30 ± 0.27^{aAB}
MOT(50:50)	0.96 ± 0.15^{cB}	2.54 ± 0.18^{bB}	3.90 ± 0.37^{aA}
MOT(25:75)	1.10 ± 0.07^{bB}	2.26 ± 0.35^{aB}	2.78 ± 0.38^{aB}
OT	3.40 ± 0.35^{bA}	4.02 ± 0.13^{aA}	3.98 ± 0.31^{aA}
	Total of cuts (g/pot)		
MT	5.36 ± 1.44^{bB}	17.08 ± 1.42^{a}	21.62 ± 5.26^{a}
MOT(75:25)	10.52 ± 2.10^{bA}	19.62 ± 4.90^{a}	19.72 ± 2.39^{a}
MOT(50:50)	12.72 ± 1.88^{bA}	17.62 ± 1.37^{a}	21.18 ± 3.97^{a}
MOT(25:75)	12.46 ± 3.57^{bA}	17.16 ± 1.79^{a}	19.88 ± 2.00^{a}
OT	11.88 ± 2.09^{bA}	16.20 ± 2.60^{a}	15.76 ± 1.90^{a}

Distinct lowercase superscript letters indicate significant differences of dry weight production within the same line for each variable (comparison between soil types); and distinct uppercase superscript letters indicate significant differences of dry weight production within the same column (comparison between treatments within the same soil type) ($P < 0.05$).

biomass harvested from each pot at the second and third cuts was not sufficient for analysing the nutrients in individual replicates of those cuts; therefore, all replicates were put together and analysed as a composite sample (Supplementary Table S3).

A higher concentration of Ca was observed in the plant material produced in the podzol and calcisol in the MT (2.80% and 1.40%, respectively) (Table 6). The concentration of Mg in ryegrass decreased with increasing proportions of organic fertiliser in the mixed treatments across all soil types. As for Na, its concentration varied unevenly. While the plant material produced in the podzol had a higher concentration of Na in the OT (0.34%), the same treatment in the other soils resulted in a lower concentration of 0.18% in the calcisol and 0.23% in the fluvisol. In general, the concentration of Mn in the plants' biomass was lower in the OT treatment, regarding all soil types. As for Zn, the results were different for the three soils: in the podzol, the MT treatment showed the highest concentration (78.46 mg/kg), while in the calcisol it was the MOT(75:25) treatment (46.64 mg/kg) and in the fluvisol it was the MOT(75:25) and OT treatments (on average 24.69 mg/kg).

The lowest plant Cu concentration was observed in the OT (5.28 mg/kg) and MT (6.36 mg/kg) treatments in both the podzol and fluvisol, respectively,

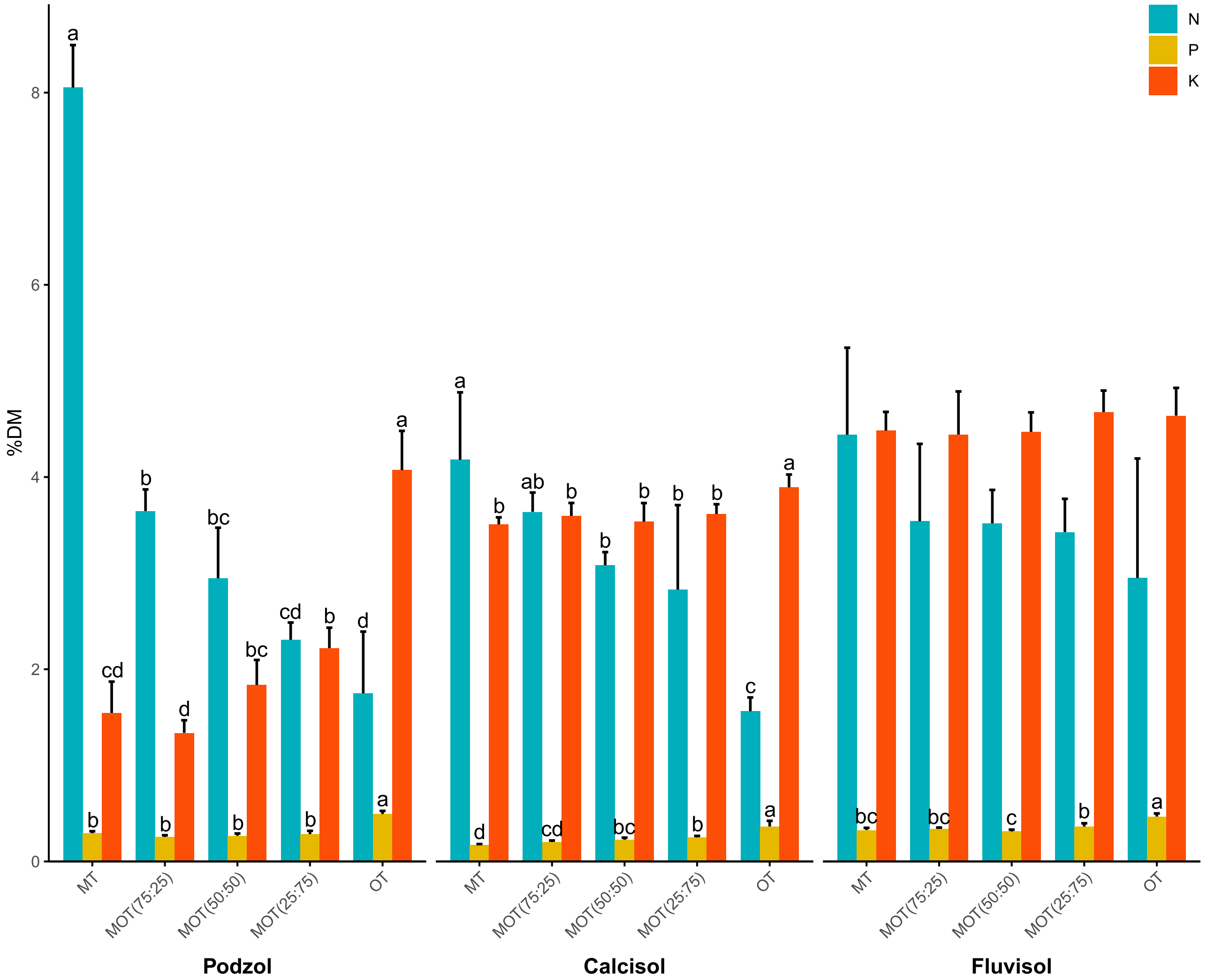

FIGURE 2 Macronutrients in plant material from the 1st cut of different soils. Error bars represent the standard error of the mean (SEM). Distinct letters indicate significant differences among treatments within the same soil type ($P < 0.05$). MT = soil fertilised with mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers; MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75) = soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised with organic fertiliser.

while in the calcisol the MT and OT treatments were responsible for the lowest Cu concentration (on average 5.49 mg/kg). With regard to B, this micronutrient was found with the lowest concentration in the podzol in the OT treatment (4.66 mg/kg) and the highest in the fluvisol (5.36 mg/kg). There were no differences in B concentration in the calcisol (Table 6).

4 Discussion

As the soils used in this study were significantly different in relation to their sand, silt and clay contents and consequently their natural fertility, as shown in Table 1, the obtained results were mainly discussed from a perspective that compares the distinct treatments (fertilisation regimes) within the same soil type. Nevertheless, it is noteworthy that both fertilisation regime and soil type exerted significant effects in the growth of ryegrass (Supplementary Tables S2 and S3). As expected, most of the fertility traits evaluated in this study were found to be higher in the fluvisol, especially in relation to the podzol, due to its organic nature. Even though the podzol is a typical sandy soil that displayed lower fertility in relation to the calcisol and fluvisol, it had some of its fertility traits highly influenced by the presence of the organic fertiliser, frass, as indicated by higher SOM accumulation and enzymatic activity, demonstrating that organic fertilisation benefits not only organic soils but also low-fertility soils.

The lower concentrations of SOM, CEC, N, P and K_2O in the podzol at the end of the experiment in comparison to both calcisol and fluvisol reflected its lower fertility. This is likely due to the fact that the

TABLE 6 Macro and micronutrients in plant material from 1st cut of different soils. Data are presented as mean ± SD. MT = soil fertilised with mineral fertiliser; MOT(75:25) = soil fertilised with 75% mineral and 25% organic fertilisers; MOT(50:50) = soil fertilised with 50% mineral and 50% organic fertilisers; MOT(25:75) = soil fertilised with 25% mineral and 75% organic fertilisers; OT = soil fertilised with organic fertiliser

		Ca	Mg	Na	Mn	Zn	Cu	B
		%			mg/kg			
Podzol	MT	2.80 ± 0.59^{A}	0.31 ± 0.02^{A}	0.17 ± 0.04^{B}	28.98 ± 1.83^{A}	78.46 ± 12.21^{A}	7.76 ± 1.27^{A}	5.72 ± 0.64^{A}
	MOT(75:25)	2.00 ± 0.07^{A}	0.27 ± 0.01^{AB}	0.17 ± 0.01^{B}	25.06 ± 4.23^{AB}	56.82 ± 2.29^{B}	9.48 ± 1.08^{A}	4.92 ± 0.41^{BC}
	MOT(50:50)	1.48 ± 0.07^{B}	0.28 ± 0.02^{A}	0.21 ± 0.03^{B}	23.42 ± 1.48^{AB}	50.70 ± 5.70^{B}	7.96 ± 1.22^{A}	5.04 ± 0.27^{ABC}
	MOT(25:75)	1.08 ± 0.07^{C}	0.24 ± 0.03^{B}	0.20 ± 0.04^{B}	26.20 ± 4.44^{AB}	43.96 ± 3.88^{B}	7.78 ± 0.83^{A}	5.52 ± 0.30^{AB}
	OT	0.64 ± 0.04^{D}	0.17 ± 0.01^{C}	0.34 ± 0.07^{A}	22.50 ± 3.55^{B}	47.22 ± 7.17^{B}	5.28 ± 1.78^{B}	4.66 ± 0.33^{C}
Calcisol	MT	1.40 ± 0.11^{A}	0.27 ± 0.03^{A}	0.37 ± 0.05^{A}	43.76 ± 2.73^{A}	41.56 ± 2.14^{AB}	5.82 ± 0.46^{BC}	3.60 ± 1.37
	MOT(75:25)	1.20 ± 0.04^{B}	0.27 ± 0.02^{A}	0.45 ± 0.07^{A}	40.00 ± 2.07^{A}	46.64 ± 2.12^{A}	9.00 ± 0.25^{A}	4.06 ± 0.73
	MOT(50:50)	1.10 ± 0.06^{BC}	0.25 ± 0.01^{AB}	0.45 ± 0.05^{A}	39.68 ± 2.13^{A}	41.34 ± 3.92^{AB}	6.26 ± 2.70^{AB}	3.22 ± 1.12
	MOT(25:75)	1.05 ± 0.03^{C}	0.22 ± 0.01^{B}	0.49 ± 0.12^{A}	34.30 ± 1.24^{B}	38.88 ± 2.62^{B}	6.92 ± 1.16^{AC}	3.60 ± 0.37
	OT	1.11 ± 0.05^{BC}	0.18 ± 0.01^{C}	0.18 ± 0.04^{B}	29.68 ± 3.02^{C}	32.42 ± 3.04^{C}	5.16 ± 0.27^{BC}	3.54 ± 0.28
Fluvisol	MT	1.06 ± 0.07^{A}	0.30 ± 0.03^{A}	0.51 ± 0.05^{A}	46.18 ± 3.38^{A}	24.26 ± 3.67^{AB}	6.36 ± 0.32^{B}	4.56 ± 0.86^{AB}
	MOT(75:25)	0.95 ± 0.09^{AB}	0.28 ± 0.04^{AB}	0.33 ± 0.06^{C}	34.58 ± 0.82^{B}	24.54 ± 2.14^{A}	7.66 ± 1.24^{AB}	4.38 ± 0.41^{AB}
	MOT(50:50)	0.87 ± 0.03^{B}	0.26 ± 0.02^{AB}	0.33 ± 0.02^{C}	43.44 ± 5.02^{A}	19.60 ± 2.83^{B}	7.64 ± 0.48^{A}	3.56 ± 1.01^{B}
	MOT(25:75)	1.03 ± 0.09^{A}	0.29 ± 0.02^{A}	0.42 ± 0.04^{B}	44.30 ± 4.06^{A}	22.14 ± 1.55^{AB}	7.58 ± 1.54^{AB}	4.82 ± 0.70^{AB}
	OT	0.94 ± 0.03^{A}	0.23 ± 0.01^{B}	0.23 ± 0.02^{D}	36.32 ± 3.96^{B}	24.84 ± 1.71^{A}	6.96 ± 1.63^{AB}	5.36 ± 0.40^{A}

Distinct uppercase superscript letters indicate significant differences of plant characteristics within the same column (comparison between treatments within the same soil type) ($P < 0.05$).

podzol is coarser compared to the other soils included in this study, as suggested by Huang and Hartemink (2020). However, according to Centeno *et al.* (2017), such a low fertility could also be correlated with other traits, such as the type of soil management as well as the crop being cultivated. Nevertheless, organic amendments are known to contribute to soil fertility especially in sandy soils. Considering the differences of fertility traits observed between treatments within the same soil type (Table 3), SOM was found to be higher with the exclusive use of organic fertiliser in all soils evaluated, as also verified by other studies with similar designs (Esteves *et al.*, 2022; Menino *et al.*, 2021; Scotti *et al.*, 2015). The MT treatment resulted in the lowest concentration of SOM in all soils in comparison to the initial concentration before applying the fertilisers (Table 1, Supplementary Table S1). This may have occurred due to an increased microbial activity and respiration after the fertilisers were applied, as suggested by Gomes *et al.* (2021). Additionally, considering that mineral fertilisers do not contribute with SOM provision, it is feasible to assume that this treatment would not result in an increase of this parameter in the soils. However, the mixed treatments (MOTs) led to increases in SOM as the incorporation of frass increased in the podzol and fluvisol (with the exception of MOT(25:75) for the latter), as also observed by Esteves *et al.* (2022).

Before applying the fertilisers all soils had low C/N ratios (and also the frass used in this study), meaning that the tendency for humus build-up might have been reduced and consequently it would require a longer period for increasing SOM significantly, as suggested by (Chen *et al.*, 2014). Nevertheless, other studies also demonstrated increases in SOM and humus formation under greenhouse conditions with soils of low C/N ratio and under low humidity conditions (Zhou *et al.*, 2019). The temperature and humidity in this study, conducted inside a glass greenhouse, are certainly much favourable in comparison to field conditions, which also contributes to increasing SOM over time, and it is important to highlight that such SOM build-up herein observed (Table 3) is most commonly observed in greenhouse conditions, while in field conditions, increases in SOM to that extent would usually take at least a few years to be verified (Chen *et al.*, 2014). Further studies should investigate the differences between the effects of frass under greenhouse and field conditions.

Organic matter build-up is related to several traits in cropland, including higher nutrient availability due to increased water holding capacity causing nutrient priming, as well as increased enzymatic activity (Kumar *et al.*, 2024). The DHA in the soils followed a similar trend as for its general fertility, with the podzol having the lowest activity in relation to the calcisol and the fluvisol. Enzymatic activity in soils is typically correlated with the presence of SOM and the presence of humus (a resistant fraction of SOM), with organic fertilisation typically stimulating such activity (Kotroczó *et al.*, 2014). That was verified in the present study for the podzol, which had displayed a 3.3-fold higher DHA in relation to other treatments when receiving frass as sole input. Differently, such an increase was not verified in the calcisol and in the fluvisol (Figure 1). The DHA of the MOT(25:75) in the fluvisol was 4.90 μg TPF/g dry soil/h, 31% lower than in MOT(75:25) and in MOT(50:50), even though no significant differences were found for this parameter in this soil.

In addition to promoting microbial and enzymatic activity, increases in SOM also typically result in higher CEC, as thoroughly discussed by Bhatt *et al.* (2019). The increased CEC in the podzol with exclusive organic fertilisation (2.73 ± 0.23 $cmol_c$/kg) in relation to MT (2.10 ± 0.07 $cmol_c$/kg) can be attributed to a direct effect of the presence of the fertiliser in the pots. However, such an increase was also expected in both the calcisol and the fluvisol, which had significantly higher SOM at the end of the experiment when comparing OT and MT (Table 3). The CEC is commonly used as an indicator of soil stability and it is a measure of the quantity of readily exchangeable cations neutralizing negative charges in the soil (Rhoades, 1982). Thus, even though increased SOM can be observed, the amount of cations existing in a soil system can result in distinct CEC, which in turn affects the availability of nutrients for the plants being cultivated in that soil.

The CEC is also directly correlated with the abundance of negative charges in the colloids constituting SOM, which are more abundant in clayey soils in relation to sandy soils and are built-up by organic fertilisation (Pernes-Debuyser and Tessier, 2004). This might explain the fact that the MT resulted in a CEC as high as 24.88 ± 1.97 $cmol_c$/kg, comparable to 24.04 ± 2.08 $cmol_c$/kg in the OT, and no clear pattern of increase was visualized in both calcisol and fluvisol, as the pattern observed in the podzol (Table 2). Such concentrations might also be attributed to the fact that the mineral fertiliser used in this study contained 4% CaO and 2% MgO, which could result in increased CEC when analysing this parameter in the soil. This is also reflected in the nutrient concentration of the plant material (Table 6), in which it was found that the plants from the MT in the fluvisol had a higher content of Ca, Mg and Na, respectively 1.06, 0.30 and 0.51%. It is important to highlight that both fluvisol and calcisol have higher organic matter content and consequently higher CEC, which *per se* might hamper a clear visualisation of CEC changes under different fertilisation regimes.

The N_t of the soils remained very close to the initial values (0.04, 0.11 and 0.16% in the podzol, calcisol and fluvisol, respectively) and showed differences only in the calcisol. In this soil a higher concentration of N_t was observed in the OT, corresponding to an increase of 0.02% of the N_t when compared to the other treatments. An exception was the MOT(75:25), which did not differ from OT. The lack of changes in soil N following fertilisation is expected, especially when the fertiliser used (in this case frass) has a low C/N ratio, as no immobilisation of N by soil microorganisms is expected (Chen *et al.*, 2014). In addition, in the absence of immobilisation, plants constantly absorb available N, thus leading to no accumulation in the soil. Conversely, the N accumulation in plants in distinct soils was highly variable among treatments, with a reduced trend for accumulation of this nutrient when increasing amounts of frass were added (Figure 2), which might be a result of mineralisation. It was demonstrated by Lopes *et al.*, (2024) that BSF frass is many times an unstable organic material that could benefit from post-processing methods, in order to improve its fertilising potential by reducing its phytotoxicity and increasing its nutrient availability to plants. This is due to the fact that a great share of N in frass is still in organic form, thus unavailable to plants. Even though mineralisation typically occurs in conditions similar to the ones verified in this study (e.g., adequate soil moisture, abundance of oxygen, low C/N ratio and others), this process might be longer than 60 or 90 days according to Beesigamukama *et al.* (2021), thus justifying such a lower absorption of N by the plants. Additionally, the higher N concentrations in the plants observed with MT were expected, as all N existing in the mineral fertiliser is promptly available in mineral form, which stimulates absorption by plants (Bhatt *et al.*, 2019).

It is noteworthy that the presence of frass exerted positive effects in relation to the concentrations of P in the soils, especially at higher application rates (Table 3). Increasing the P content of soils through waste-derived organic amendments and fertilisers is highly desirable, considering that many soils around the globe are depleted in this macronutrient (Alewell *et al.*, 2020) and P reserves are considered a limited, finite resource (de Boer *et al.*, 2019). Similarly, the concentration of K increased with higher applications of frass, as also verified by Boudabbous *et al.* (2023) in wheat production, which was also reflected accumulation of those nutrients in the plant material. The presence of frass resulted in an increased concentration of K both in the soils (analysed after the experiment) and in the plants, which might suggest a positive correlation between both. McDonnell *et al.* (2018) demonstrated that the maximum of 1.6% of K must be guaranteed in ryegrass so that its growth is not affected. The plant material analysed in the 1st cut presented values generally above that (1.5-5.0%). In the aforementioned study it also demonstrated that, as the concentration of K in the forage increased, the concentrations of Ca, Mg and Na decreased, which was also verified in this study (with the exception of Ca in the fluvisol). The concentrations of plant N_t, P, Ca, Mg and Mn are similar to those presented by the McDonnell *et al.* (2018).

Ryegrass can either be used as a pasture, for which the production in fresh weight is considered, or for the production of silage, when the dry matter content of the production is mostly accounted for. Generally, crop yield was reduced in both FW and DW from the 1st to the 2nd cut (around 2.5-fold and 1.4-fold, respectively), as well as from the 2nd cut to the 3rd cut (around 3.6-fold and 2.3-fold, respectively). These trends were also verified by Vinther (2006) and Menino *et al.* (2021), which can be fully attributed to the fact that this experiment was conducted inside a greenhouse, differing from field conditions. In the study developed by Kemešytė *et al.* (2023), in which the authors followed ryegrass production in Italy, a Mediterranean country such as Portugal, between 2009 and 2022, it was verified that the first, second, third and even fourth cuts of ryegrass in field conditions vary over time, with the second cut even generating higher productivities sometimes in relation to the first cut. Such variations are correlated with the temperature and rainfall registered throughout the years, conditions that are more or less irrelevant under greenhouse conditions. Therefore, considering the conditions of the present study in which the first cut generated the highest plant biomass, followed by the second and third cuts, it is possible to understand which fertilisation regime would be beneficial for achieving high productivities under certain climatic conditions, which might or not reflect similar trends under varied conditions. For instance, under extreme temperatures and drought periods, the productivity of ryegrass can be severely affected (Kemešytė *et al.*, 2023), which in turn could be mitigated by certain fertilisation regimes. Future studies should investigate the effect of extreme climatic events in the cultivation of ryegrass with waste-derived fertilisers.

It is noteworthy that the FW crop production in the all treatments was the highest in all soils in the 1st cut when compared to the OT treatment with an increase of 1.6-fold, 1.5-fold and 1.3-fold in the podzol, calcisol and fluvisol, respectively. However, the FW observed in the 2nd and 3rd cuts with exclusive organic fertilisation generated 1.5-fold and 2.6-fold higher yields in the podzol in relation to the others treatments, which might indicate the mineralisation process as described by Beesigamukama *et al.* (2021). Finally, considering the total FW production of the three cuts for all soils, it was verified that the mixture of fertilisers in different proportions originated higher production compared to the exclusive treatments, whether organic or mineral. This is a strong evidence of the premise of this study, which supports the fact that partial replacements of mineral fertiliser by organic amendments can result in an equal (as generally verified in the calcisol and fluvisol) or greater production, as also demonstrated by Bhatt *et al.* (2019), and also that frass derived from a waste stream can be considered a potential organic fertiliser from that perspective.

5 Future perspectives

The productivity of grassland species such as ryegrass is an important component for animal feeding worldwide, and the possibility of fertilizing grasslands with waste-derived fertilisers such as BSF larvae frass can represent a major gain in the sustainability of the whole food production system. Based on the results obtained in this study, it is clear that mineral fertilisers can be at least partially replaced by frass, maintaining high productivities in distinct types of soil. In addition, such replacements would represent a great opportunity for building SOM in the long-term, maintaining or even increasing the fertility of soils over time. Future studies should verify the effect of BSF frass in ryegrass cultivated over time under field conditions and how this organic fertiliser could help plants to thrive under distinct scenarios of abiotic stresses, such as extreme temperatures, flooding and drought.

6 Conclusions

Organic fertilisation with frass derived from the bioconversion of bovine slurry by black soldier fly larvae is feasible for cultivating ryegrass in different soils and crop productivity is favoured by the combined application of frass and a mineral fertiliser. The presence of increasing amounts of frass resulted in the accumulation of organic matter and nutrients such as P and K in the soil, while a higher enzymatic activity and CEC were only verified in the sandy soil. This is an interesting finding, considering that sandy soils lack organic matter and it was demonstrated that frass can support higher productivity under such a challenging soil type. Frass can successfully replace up to 75% of mineral fertilisation when cultivating ryegrass in soils of distinct types in order to maintain similar productivities, resulting in an increased sustainability of the production as a whole.

Supplementary material

Supplementary material is available online at: https://doi.org/10.6084/m9.figshare.27153621

Acknowledgements

This study was supported by the Recovery and Resilience Program of Portugal (PRR), as part of the project (C632327738-00466948: InsectERA).

References

Alewell, C., Ringeval, B., Ballabio, C., Robinson, D.A., Panagos, P. and Borrelli, P., 2020. Global phosphorus shortage will be aggravated by soil erosion. Nature Communications 11: 4546. https://doi.org/10.1038/s41467-020-18326-7

Bajželj, B., Quested, T.E., Röös, E. and Swannell, R.P.J., 2020. The role of reducing food waste for resilient food systems. Ecosystem Services 45: 101140. https://doi.org/10.1016/j.ecoser.2020.101140

Beesigamukama, D., Mochoge, B., Korir, N., Ghemoh, C.J., Subramanian, S. and Tanga, C.M., 2021. *In situ* nitrogen mineralization and nutrient release by soil amended with black soldier fly frass fertilizer. Scientific Reports 11: 14799. https://doi.org/10.1038/s41598-021-94269-3

Berger, K.C. and Truog, E., 1939. Boron determination in soils and plants using quinalizarin reaction. Industrial and Engineering Chemistry Analytical Edition 11: 540-545. https://doi.org/10.1021/ac50138a007

Bhatt, M.K., Labanya, R. and Joshi, H.C., 2019. Influence of long-term chemical fertilizers and organic manures on soil fertility – a review. Universal Journal of Agricultural Research 7: 177-188. https://doi.org/10.13189/ujar.2019.070502

Bosch, G., van Zanten, H.H.E., Zamprogna, A., Veenenbos, M., Meijer, N.P., van der Fels-Klerx, H.J. and van Loon, J.J.A., 2019. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. Journal of Cleaner Production 222: 355-363. https://doi.org/10.1016/j.jclepro.2019.02.270

Boudabbous, K., Hammami, S.B.M., Toukabri, W., Bouhaouel, I., Ayed, S., Fraihi, W., Gastli, M., Chaalala, S. and Labidi, S., 2023. Black soldier fly (*Hermetia illucens*) larvae frass organic fertilizer improves soil quality and the productivity of durum wheat. Communications in Soil Science and Plant Analysis 54: 2491-2507. https://doi.org/10.1080/00103624.2023.2227208

Casida, L.E., Klein, D.A. and Santoro, T., 1964. Soil dehydrogenase activity. Soil Science 98: 371-376. https://doi.org/10.1097/00010694-196412000-00004

Centeno, L.N., Guevara, M.D.F., Cecconello, S.T., Sousa, R.O. de and Timm, L.C., 2017. Textura do solo: conceitos e aplicações em solos arenosos. Revista Brasileira de Engenharia e Sustentabilidade 4: 31. https://doi.org/10.15210/rbes.v4i1.11576

Chapman, H.D., 1965. Total exchangeable bases. In: Norman, A.G. (ed.) Methods of soil analysis, Part 2 – Chemical and microbiological properties. American Society of Agronomy, Inc., Madison, WI, USA, Series Agronomy n° 9, pp. 902-904. https://doi.org/10.2134/agronmonogr9.2.c7

Chen, B., Liu, E., Tian, Q., Yan, C. and Zhang, Y., 2014. Soil nitrogen dynamics and crop residues. A review. Agronomy for Sustainable Development 34: 429-442. https://doi.org/10.1007/s13593-014-0207-8

Chen, J., Hou, D., Pang, W., Nowar, E.E., Tomberlin, J.K., Hu, R., Chen, H., Xie, J., Zhang, J., Yu, Z. and Li, Q., 2019. Effect of moisture content on greenhouse gas and NH_3 emissions from pig manure converted by black soldier fly. Science of The Total Environment 697: 133840. https://doi.org/10.1016/j.scitotenv.2019.133840

Chirere, T.E.S., Khalil, S. and Lalander, C., 2021. Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. Journal of Insects as Food and Feed 7: 457-470. https://doi.org/10.3920/JIFF2020.0120

Costa, J.M., Murta, D. de M. and Magalhães, T.O., 2018. Good practice manual on insect production, processing and use in animal feeding. Direção-Geral de Alimentação e Veterinária, Lisbon, Portugal. Available at: https://docs.wixstatic.com/ugd/84b720_b250c2433e474e419dfeca3fc2ac4407.pdf (Accessed 20 March 2024).

de Boer, M.A., Wolzak, L. and Slootweg, J.C., 2019. Phosphorus: reserves, production, and applications. In: Ohtake, H. and Tsuneda, S. (eds.) Phosphorus recovery and recycling. Springer, Singapore, pp. 75-100. https://doi.org/10.1007/978-981-10-8031-9_5

Dou, Z., Toth, J.D. and Westendorf, M.L., 2018. Food waste for livestock feeding: feasibility, safety, and sustainability implications. Global Food Security 17: 154-161. https://doi.org/10.1016/j.gfs.2017.12.003

Esteves, C., Fareleira, P., Castelo-Branco, M.A., Lopes, I.G., Mota, M., Murta, D. and Menino, R., 2022. Black soldier fly larvae frass increases the soil's residual nutrient content and enzymatic activity – a lettuce production trial. Journal of Insects as Food and Feed 8: 1431-1440. https://doi.org/10.3920/JIFF2022.0005

Gold, M., Tomberlin, J.K., Diener, S., Zurbrügg, C. and Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. Waste Management 82: 302-318. https://doi.org/10.1016/j.wasman.2018.10.022

Gomes, D. da S., Barbosa, A. da S., Santos, T. de M., Santos, S.K. dos, Silva, J.H.C.S. and Aquino, Í. de S., 2021. Release kinetics of CO_2 and phytomass decomposition in systems of use and soil management. Research, Society and Development 10: e9810111413. https://doi.org/10.33448/rsd-v10i1.11413

Huang, J. and Hartemink, A.E., 2020. Soil and environmental issues in sandy soils. Earth-Science Reviews 208: 103295. https://doi.org/10.1016/j.earscirev.2020.103295

ISSS/ISRIC/FAO, 2006. World reference base for soil resources 2006. FAO, Rome, Italy Available at: https://www.fao.org/fileadmin/templates/nr/images/resources/pdf_documents/wrb2007_red.pdf (Accessed 21 March 2024).

Jie, C., Jing-zhang, C., Man-zhi, T. and Zi-tong, G., 2002. Soil degradation: a global problem endangering sustainable development. Journal of Geographical Sciences 12: 243-252. https://doi.org/10.1007/BF02837480

Kaza, S., Yao, L.C., Bhada-Tata, P. and Van Woerden, F., 2018. What a waste 2.0: a global snapshot of solid waste management to 2050. World Bank, Washington, DC, USA. Available at: http://hdl.handle.net/10986/30317 (Accessed 21 March 2024).

Kemešytė, V., Statkevičiūtė, G., Norkevičienė, E. and Jaškūnė, K., 2023. Italian ryegrass as a forage crop for the Baltics: opportunities and challenges in light of climate change. Plants 12: 3841. https://doi.org/10.3390/plants12223841

Kotroczó, Z., Veres, Z., Fekete, I., Krakomperger, Z., Tóth, J.A., Lajtha, K. and Tóthmérész, B., 2014. Soil enzyme activity in response to long-term organic matter manipulation. Soil Biology and Biochemistry 70: 237-243. https://doi.org/10.1016/j.soilbio.2013.12.028

Kumar, N., Basak, B.B., Patel, V.J., Senapati, N., Ramani, V.P., Gajbhiye, N.A. and Kalola, A.D., 2024. Enriched soil amendments influenced soil fertility, herbage yield and bioactive principle of medicinal plant (*Cassia angustifolia Vahl.*) grown in two different soils. Heliyon 10: e24874. https://doi.org/10.1016/j.heliyon.2024.e24874

Lakanen, E. and Erviö, R., 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agralia Fennica 123: 223-232.

Lopes, I.G., Wiklicky, V., Vinnerås, B., Yong, J.W.H. and Lalander, C., 2024. Recirculating frass from food waste bioconversion using black soldier fly larvae: impacts on process efficiency and product quality. Journal of Environmental Management 366: 121869. https://doi.org/10.1016/j.jenvman.2024.121869

Lopes, I.G., Yong, J.W. and Lalander, C., 2022. Frass derived from black soldier fly larvae treatment of biodegradable wastes. A critical review and future perspectives. Waste Management 142: 65-76. https://doi.org/10.1016/j.wasman.2022.02.007

Marôco, S., 2014. Análise estatística com o SPSS Statistics. Pêro Pinheiro.

McDonnell, R.P., Staines, M. vH. and Bolland, M.D.A., 2018. Determining the critical plant test potassium concentration for annual and Italian ryegrass on dairy pastures in south-western Australia. Grass and Forage Science 73: 112-122. https://doi.org/10.1111/gfs.12286

Menino, R., Felizes, F., Castelo-Branco, M.A., Fareleira, P., Moreira, O., Nunes, R. and Murta, D., 2021. Agricultural value of Black Soldier Fly larvae frass as organic fertilizer on ryegrass. Heliyon 7: e05855. https://doi.org/10.1016/j.heliyon.2020.e05855

Newton, L., Sheppard, C., Watson, D.W., Burtle, G. and Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Available at: https://insectinnovation.org/wp-content/uploads/2015/05/A2.pdf (Accessed 20 March 2024).

Parodi, A., Gerrits, W.J.J., Van Loon, J.J.A., De Boer, I.J.M., Aarnink, A.J.A. and Van Zanten, H.H.E., 2021. Black soldier fly reared on pig manure: Bioconversion efficiencies, nutrients in the residual material, greenhouse gas and ammonia emissions. Waste Management 126: 674-683. https://doi.org/10.1016/j.wasman.2021.04.001

Penuelas, J., Coello, F. and Sardans, J., 2023. A better use of fertilizers is needed for global food security and environmental sustainability. Agriculture & Food Security 12: 5. https://doi.org/10.1186/s40066-023-00409-5

Pergola, M., Persiani, A., Palese, A.M., Di Meo, V., Pastore, V., D'Adamo, C. and Celano, G., 2018. Composting: the way for a sustainable agriculture. Applied Soil Ecology 123: 744-750. https://doi.org/10.1016/j.apsoil.2017.10.016

Pernes-Debuyser, A. and Tessier, D., 2004. Soil physical properties affected by long-term fertilization. European Journal of Soil Science 55: 505-512. https://doi.org/10.1111/j.1365-2389.2004.00614.x

Pratt, C., Redding, M., Hill, J., Shilton, A., Chung, M. and Guieysse, B., 2015. Good science for improving policy: greenhouse gas emissions from agricultural manures. Animal Production Science 55: 691. https://doi.org/10.1071/AN13504

Rhoades, J.D., 1982. Cation exchange capacity. In: Methods of soil analysis: part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, WI, USA, pp. 149-157.

Romano, N., Datta, S.N., Sinha, A.K. and Pande, G.S.J., 2023. Partially replacing synthetic fertilizer with black soldier fly (*Hermetia illucens*) larvae frass enhances kale (*Brassica oleracea* var. *sabellica*) production. Technology in Horticulture 3: 8. https://doi.org/10.48130/TIH-2023-0008

Romano, N., Fischer, H., Powell, A., Sinha, A.K., Islam, S., Deb, U. and Francis, S., 2022. Applications of black soldier fly (*Hermetia illucens*) larvae frass on sweetpotato slip production, mineral content and benefit-cost analysis. Agronomy 12: 928. https://doi.org/10.3390/agronomy12040928

RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, Inc.

Schulte-Uebbing, L.F., Beusen, A.H.W., Bouwman, A.F. and de Vries, W., 2022. From planetary to regional boundaries for agricultural nitrogen pollution. Nature 610: 507-512. https://doi.org/10.1038/s41586-022-05158-2

Scotti, R., Bonanomi, G., Scelza, R., Zoina, A. and Rao, M., 2015. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. Journal of Soil Science and Plant Nutrition 15: 333-352. https://doi.org/10.4067/S0718-95162015005000031

Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L. and Ronga, D., 2019. Use of black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae processing residue in peat-based growing media. Waste Management 95: 278-288. https://doi.org/10.1016/j.wasman.2019.06.017.

Singh, A. and Kumari, K., 2019. An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: a review. Journal of Environmental Management 251: 109569. https://doi.org/10.1016/j.jenvman.2019.109569

Smetana, S., Spykman, R. and Heinz, V., 2021. Environmental aspects of insect mass production. Journal of Insects as Food and Feed 7: 553-571. https://doi.org/10.3920/JIFF2020.0116

Tabatabai, M.A., 1994. Soil Enzymes. Methods of soil analysis. Part 2, microbiological and biochemical properties. Madison, WI, USA, pp. 775-833. https://doi.org/10.2136/sssabookser5.2.c37

United Nations, 2022. World population prospects 2022. Available at: https://population.un.org/wpp

van Dijk, M., Morley, T., Rau, M.L. and Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010-2050. Nature Food 2: 494-501. https://doi.org/10.1038/s43016-021-00322-9

Varma, V.S., Parajuli, R., Scott, E., Canter, T., Lim, T.T., Popp, J. and Thoma, G., 2021. Dairy and swine manure management – challenges and perspectives for sustainable treatment technology. Science of The Total Environment 778: 146319. https://doi.org/10.1016/j.scitotenv.2021.146319

Veldkamp, T., van Rozen, K., Elissen, H., van Wikselaar, P. and van der Weide, R., 2021. Bioconversion of digestate, pig manure and vegetal residue-based waste operated by black soldier fly larvae, *Hermetia illucens* L. (Diptera: Stratiomyidae). Animals 11: 3082. https://doi.org/10.3390/ani11113082

Vinther, F.P., 2006. Effects of cutting frequency on plant production, N-uptake and N_2 fixation in above and below ground plant biomass of perennial ryegrass–white clover swards. Grass and Forage Science 61: 154-163. https://doi.org/10.1111/j.1365-2494.2006.00519.x

Zhou, G., Xu, S., Ciais, P., Manzoni, S., Fang, J., Yu, G., Tang, X., Zhou, P., Wang, W., Yan, J., Wang, G., Ma, K., Li, S., Du, S., Han, S., Ma, Y., Zhang, D., Liu, J., Liu, S., Chu, G., Zhang, Q., Li, Y., Huang, W., Ren, H., Lu, X. and Chen, X., 2019. Climate and litter C/N ratio constrain soil organic carbon accumulation. National Science Review 6: 746-757. https://doi.org/10.1093/nsr/nwz045