

Original Article



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Bioconversion of Scotch broom into a high-quality organic fertiliser: Vermicomposting as a sustainable option

Jorge Domínguez¹, María Gómez-Brandón¹, Hugo Martínez-Cordeiro¹ and Marta Lores²

Abstract

Wild Scotch broom (*Cytisus scoparius* (L.) Link) shrubs are widely distributed throughout the world and, in some countries, are considered to be a threat to other plant species. The use of plant biomass from Scotch broom as a fertiliser seems to be the optimum solution for its disposal because it contains considerable amounts of macronutrients. However, its direct application to soils may cause phytotoxicity due to the release of polyphenols, which could negatively affect crop growth. This study evaluated the efficiency of vermicomposting in processing this leguminous plant on an industrial scale. Vermicomposting substantially reduced the biomass of Scotch broom (by 84%), mainly as a result of the loss of volatile solids. Simultaneously, the initial population of earthworms (*Eisenia andrei*) increased remarkably throughout the process, offering the possibility of obtaining earthworm protein for animal feed. A nutrient-rich and stabilised peat-like material without polyphenol-associated phytotoxicity was obtained after 42 days of vermicomposting. Lower values of microbial biomass and activity, indicative of stabilised materials, were recorded at the end of the trial. These findings suggest that vermicomposting is an environmentally sound management system for Scotch broom and could easily be scaled up for industrial application.

Keywords

Cytisus scoparius, vermicomposting, Eisenia andrei, microbial activity, phenolic compounds, vermicompost

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Introduction

Scotch broom (*Cytisus scoparius* (L.) Link), a leguminous shrub belonging to the family Fabaceae and native to the Mediterranean Basin, has become an invasive plant of ecological concern in several parts of the world, including France, Canada, Chile, India, Iran, South Africa, Australia, New Zealand and North America (Peterson and Prasad, 1998; Prévosto et al., 2004; Potter et al., 2009; Herrera-Redy et al., 2012). Its invasive nature is mainly due to the prolific production and prolonged viability of its seeds, along with the formation of dense stands, the production of alkaloid compounds, and its ability to reduce soil water and the availability of light (Shaben and Myers, 2010; Slesak et al., 2016).

This plant is considered to be a symbiotic N-fixing species (Pérez-Fernández et al., 2017) and can fix as much as 100 kg N ha⁻¹ per year. It is also characterised by a high content of P, K and Ca and a high polyphenol content (Barros et al., 2012). Bearing this in mind, the potential use of Scotch broom plant biomass as a fertiliser in agricultural soils seems to be an optimum solution for its disposal because it contains considerable amounts of macronutrients. However, its direct application into soils may also cause phytotoxicity due to the release of polyphenols, which could negatively affect crop growth (Inderjit, 1996). Vermicomposting offers the possibility of minimising or

reducing these agronomic problems (Domínguez et al., 2010; Gómez-Brandón and Domíguez, 2014) because earthworms can at least partly digest polyphenols (Hättenschwiler and Vitousek, 2000; Domínguez et al., 2014).

Vermicomposting involves the biostabilisation of organic matter under aerobic and mesophilic conditions through the joint action of earthworms and microorganisms, thus facilitating the management of large amounts of hazardous organic wastes rapidly and at low cost by transforming them into safe and valuable products called vermicomposts (Domínguez and Edwards, 2011a; Lazcano and Domínguez, 2011; Ali et al., 2015). Although a wide variety of organic wastes have been successfully managed by vermicomposting (Domínguez and Gómez-Brandón, 2012;

Corresponding author:

María Gómez-Brandón, Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Campus As Lagoas Marcosende, Vigo, E-36310, Spain.

Email: mariagomez@uvigo.es

¹Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Spain

²Laboratorio de Investigación y Desarrollo de Soluciones Analíticas, Departamento de Química Analítica, Nutrición y Bromatología, Universidade de Santiago de Compostela, Facultad de Química, Spain



Figure 1. Overview of the industrial scale vermireactor containing the Scotch broom (*Cytisus scoparius* (L.) Link) plant material at the beginning of the trial (day 0) and after 28 and 42 days of vermicomposting.

Huang et al., 2014; García-Sánchez et al., 2017; Suleiman et al., 2017), to our knowledge there is no previous study of the use of vermicomposting for processing plant material derived from the leguminous shrub *C. scoparius* (L.) Link on either a laboratory or industrial scale. As such, the objective of this study was to evaluate whether plant material from this leguminous shrub could be processed by vermicomposting on an industrial scale to yield a high-quality, polyphenol-free organic vermicompost that could be used as an environmentally friendly fertiliser.

Material and methods

Plant material and earthworm species

Scotch broom was used as the food source for the earthworms during the vermicomposting process. Plants were hand-harvested in spring 2014 from a natural population in Galicia (NW Spain) during the flowering stage. Prior to vermicomposting, young

branches of Scotch broom were cut-milled into pieces ranging between 3 and 6 mm; the flowers and leaves were not subjected to any pre-treatment.

Individuals of the earthworm species *E. andrei* were used in the vermicomposting system and were obtained from a stock culture reared in the laboratory. This earthworm species is widely used for vermicomposting (Domínguez and Edwards, 2011b; Suleiman et al., 2017; García-Sánchez et al., 2017).

Vermireactor set-up and sampling strategy

A rectangular metal pilot-scale vermireactor ($4 \times 1.5 \times 1$ m; 6 m³; Aira et al., 2011) housed in a greenhouse with no temperature control was used as the vermicomposting system as previously described by Domínguez et al. (2014). The vermireactor consisted of a 12 cm layer of mature vermicompost on which earthworms (E. andrei) were placed at an initial density of 280 ± 9 individuals m⁻², including 111 ± 10 mature individuals m⁻², 169 ± 7 juveniles m⁻² and 120 ± 3 cocoons m⁻², representing an initial earthworm biomass of 79.1 ± 5.2 g m⁻² (Figure 1). A 12 cm layer containing the Scotch broom plant biomass was placed over a plastic mesh (5 cm mesh size) to avoid sampling the bedding (Figure 1). To prevent desiccation, the moisture content of this plant material was kept at about 85% by covering the vermireactor with a shade cloth.

The density and biomass of the earthworm population (adults, juveniles and cocoons) were determined every 14 days by randomly collecting 10 samples of the Scotch broom plant material (five samples from above and five samples from below the plastic mesh), each 6 cm thick, with a core sampler (7.5 cm diameter and 12 cm height) during the vermicomposting trial. The high rate of consumption, digestion and assimilation of organic matter by *E. andrei* resulted in the substrate being processed by the earthworms in 42 days.

To analyse the polyphenols and the physico-chemical and microbiological properties of the Scotch broom plant biomass, five samples of 10 g of this plant material were randomly collected every 7 and 14 days, respectively, throughout the process using the core sampler. The samples were stored in plastic bags at -20° C until analysis.

Physico-chemical analyses

The moisture content was assessed drying the samples for 24 h at 105°C. The volatile solids content was determined from the weight loss after ignition in a Carbolite CWF 1000 muffle furnace at 550°C for 5 h. The electrical conductivity and pH were measured in aqueous extracts (1:10 weight to volume) using a Crison CM35 conductivity meter and a Crison MicropH 2000 pH meter, respectively. Total C and N contents were determined in ovendried (60°C) samples in a Carlo Erba EA 1108 CHNS-O 1500 C/N analyser. The total P, K, Ca, Mg, S, Fe, Mn, B and Mo contents were determined in oven-dried (60°C) samples, previously

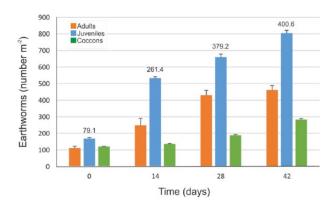


Figure 2. Earthworm density (number of adults, juveniles and cocoons per square metre) and earthworm biomass (g m⁻² fw, numbers on top of the bars) during vermicomposting of the Scotch broom plant material (*Cytisus scoparius* (L.) Link). Values are mean \pm SE values (n = 5).

subjected to microwave-assisted acid digestion, by inductively coupled plasma optical emission spectrometry. The cellulose, hemicellulose and lignin contents were assessed by detergent fibre methods (Goering and Van Soest, 1970) using the FibreBag System (Gerhardt, Königswinter, Germany) (Aira et al., 2006).

Microbiological analyses

Microbial activity was determined by measuring the oxygen consumption using a WTW OxiTop Control System (Weilheim, Germany) according to ISO 16072 (2002). The chloroform fumigation-extraction method (Vance et al., 1987) was used to measure the microbial biomass concentration ($C_{\rm mic}$) on moist samples (5 g fresh weight), with some minor modifications (Aira et al., 2006).

Determination of polyphenols

Total and individual polyphenols were determined by pressurised solvent extraction in a solvent extractor (ASE 150, Dionex, Sunnyvale, CA, USA) in which water:MeOH (1:1) was used as the extractant (Álvarez-Casas et al., 2014). The samples were previously ground in a mortar with the selected dispersant (sand) at a ratio of 1:2 to facilitate the extraction of the phenolic compounds by breaking down the plant tissues. The concentration of total polyphenols in the Scotch broom extracts was performed following the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) and the absorbance values were measured at 760 nm by spectrophotometry (UVmini-1240, Shimazdu, Tokyo, Japan). Total phenols were quantified from a calibration graph prepared with gallic acid standard solutions and expressed as mg gallic acid equivalents in the liquid extract (mg L⁻¹ GAE). The final concentrations of total phenols were expressed as mg gallic acid per g dry weight (mg gallic g⁻¹ dw).

These extracts were further analysed by liquid chromatography—mass spectrometry/mass spectrometry (LC-MS/MS) to accurately identify the major polyphenols. The liquid chromatographic system

used was a Thermo Scientific (San Jose, CA, USA) system coupled with an electrospray ionisation (ESI) mass spectrometer based on a TSQ Quantum Discovery triple-stage quadrupole mass spectrometer. The experimental conditions included the use of a Thermo Scientific Hypersil Gold aQ column (1.9 m long, 100 mm × 2.1 mm i.d.) in a high-performance liquid chromatography system equipped with an Accela autoinjector. The entire high-performance liquid chromatography run time was 19 min with a flow rate of 300 mL min⁻¹ and a 30°C column temperature.

The identities of seven of 18 phenolic compounds were confirmed by LC-MS/MS: protocatechuic acid (*m/z* parent ion 152; *m/z* product ions 108, 109; ESI–); 3,4 dihydroxybenzaldehyde (*m/z* parent ion 137; *m/z* product ions 91, 92, 136; ESI–); caffeic acid (*m/z* parent ion 179; *m/z* product ions 134, 135; ESI); quercentin-3-glucuronide (*m/z* parent ion 479; *m/z* product ions 303, 461; ESI+); rutin (*m/z* parent ion 609; *m/z* product ions 179, 271, 300; ESI–); apigenin (*m/z* parent ion 269; *m/z* product ions 117, 149, 151, 225; ESI–); and chrysin (*m/z* parent ion 253; *m/z* product ions 209, 145, 143, 63; ESI–).

Statistical analyses

The data were analysed by repeated measures analysis of variance (ANOVAR) in which the vermireactor represented the subject and the sampling time was fixed as the within-subject factor. All the variables met the normality (Shapiro–Wilks test) and sphericity condition (Mauchly's test), except for the C to N ratio, K, Mg and hemicellulose. In these cases the sphericity violation was corrected with the Geisser–Greenhouse procedure (Potvin et al., 1990). Significant differences in the main effects were analysed by paired comparisons with the Tukey HSD test. All statistical analyses were performed with the SPSS software programme version 9.

Results and discussion

Population dynamics of earthworms during vermicomposting of Scotch broom plant material

The vermireactor had an initial population density of *E. andrei* of 280 ± 9 individuals m⁻², including 111 ± 10 mature earthworms m⁻², 169 ± 7 juveniles m⁻² and 120 ± 3 cocoons m⁻², with a mean biomass of 79.1 ± 5.2 g live weight m⁻² (Figure 2). The total number of earthworms ($F_{3,12} = 296.01$, p < 0.001) and their biomass ($F_{3,12} = 349.57$, p < 0.001) as well as the number of mature earthworms, juveniles and cocoons, increased significantly throughout the vermicomposting process, reaching a density of 1265 ± 20 individuals m⁻² and a biomass value of 400.6 ± 1.9 g m⁻² on day 42 (Figure 2). This indicates that the input and availability of organic matter from the Scotch broom plant material led to optimum environmental conditions for earthworm growth and reproduction during the process.

Table 1. Changes in the physico-chemical and biological properties of the plant material from Scotch broom (*Cytisus scoparius* (L.) Link) throughout the vermicomposting process.

	Scotch broom Day 0	Vermicompost		
		Day 14	Day 28	Day 42
Moisture content	84.78 ± 0.60 ^a	83.10 ± 2.52 ^a	80.27 ± 0.55 ^b	81.58 ± 1.01ab
pH	7.43 ± 0.09^{a}	7.25 ± 0.04^{b}	6.99 ± 0.04°	6.6 ± 0.02^{d}
Electrical conductivity (mS cm ⁻²)	0.46 ± 0.00^{a}	0.40 ± 0.00^{b}	$0.32 \pm 0.00^{\circ}$	0.20 ± 0.00^{d}
Organic matter (%)	95.36 ± 0.46a	88.73 ± 1.06 ^b	86.42 ± 0.68°	79.52 ± 0.51^{d}
Total C (g kg ⁻¹)	489.40 ± 2.96^{a}	485.80 ± 1.55 ^b	476.88 ± 0.68°	471.44 ± 0.45^{d}
Total N (g kg ⁻¹)	42.40 ± 0.52^{a}	42.12 ± 0.24^{a}	38.74 ± 0.40^{b}	36.42 ±0.22 ^c
C to N ratio	11.54 ± 0.17a	11.53 ± 0.06^{a}	12.31 ± 0.11 ^b	12.94 ± 0.08 ^c
Total P (g kg ⁻¹)	2.93 ± 0.32^{a}	3.02 ± 0.06^{a}	3.19 ± 0.07^{a}	3.07 ± 0.03^{a}
Total K (g kg ⁻¹)	10.33 ± 1.19 ^a	9.01 ± 0.30^{a}	7.32 ± 0.16^{b}	5.99 ± 0.14 ^c
Total Ca (g kg ⁻¹)	3.91 ± 0.42^{a}	5.48 ± 0.36^{b}	7.19 ± 0.06°	8.99 ± 0.18^{d}
Total Mg (g kg ⁻¹)	2.62 ± 0.28^{a}	2.88 ± 0.05^{b}	$3.05 \pm 0.03^{\circ}$	2.90 ± 0.05^{b}
Total S (g kg ⁻¹)	2.38 ± 0.27^{a}	2.88 ± 0.07^{b}	$3.12 \pm 0.03^{\circ}$	3.20 ± 0.06^{d}
Total Fe (g kg ⁻¹)	0.53 ± 0.07^{a}	0.79 ± 0.03^{b}	0.97 ± 0.02^{c}	1.89 ± 0.02^{d}
Total Mn (g kg ⁻¹)	0.35 ± 0.03^{a}	0.46 ± 0.02^{b}	$0.59 \pm 0.00^{\circ}$	0.64 ± 0.01 ^d
Total B (mg kg ⁻¹)	34.2 ± 3.56^{a}	39.8 ± 1.53ab	42.6 ± 1.60 ^b	40.31 ± 0.67^{b}
Total Mo (mg kg ⁻¹)	1.98 ± 0.16^{a}	3.69 ± 0.18^{b}	5.67 ± 0.16°	7.65 ± 0.26^{d}
Basal respiration (mg O_2 kg OM^{-1} h ⁻¹)	779.89 ± 9.43^{a}	670.89 ± 8.36 ^b	522.81 ± 9.91 ^c	323.59 ± 4.14^{d}
Microbial biomass C (mg kg ⁻¹)	89,395 ± 17074 ^a	23,475 ± 10,877b	na	15,599 ± 8203°
Lignin (g kg ⁻¹)	206.04 ± 4.72^{a}	210.45 ± 5.0 ^a	241.94 ± 5.15 ^b	410.06 ± 5.86°
Cellulose (g kg ⁻¹)	294.88 ± 13.89 ^a	296.7 ± 10.20a	292.66 ± 7.49^{a}	201.54 ± 5.44 ^b
Hemicellulose (g kg ⁻¹)	258.6 ± 0.40^{a}	252.4 ± 6.2^{a}	257.24 ± 9.23^{a}	84.38 ± 1.58 ^b

Values are mean \pm standard error values (n=5). Different letters within the same row indicate significant differences among the sampling times according to Tukey's HSD test. Data are expressed on a dry weight (dw) basis. na: not available.

Changes in physico-chemical and biological properties of Scotch broom plant material during vermicomposting

Earthworm activities largely affect the turnover rate of organic matter during vermicomposting by grazing directly on microorganisms and/or by increasing the surface area of organic matter available for microbial attack after comminution (Domínguez and Gómez-Brandón, 2012). We observed a significant reduction (83.7%) in the initial mass of the Scotch broom plant material (120 kg fw; 18.26 kg dw) as a result of earthworm activity, reaching a final value of 19.6 kg fw (3.77 kg dw) at the end of the trial (42 days). In line with this, Gómez-Brandón et al. (2012) also observed that earthworm activity (*E. andrei, E. fetida* and *Perionyx excavatus*) accelerated the loss of total C of three types of animal manure (cow, horse and pig) in the short term, i.e. after 30 days of vermicomposting.

The pH and electrical conductivity values of the initial Scotch broom plant material were 7.43 ± 0.09 and 0.46 mS cm⁻² (Table 1). Both parameters decreased significantly over time (pH: $F_{3,12} = 32.24$, p < 0.001; electrical conductivity: $F_{3,12} = 302.06$, p < 0.001) reaching final values of 6.6 ± 0.02 and 0.20 mS cm⁻², respectively, after 42 days of vermicomposting (Table 1). Such a reduction in electrical conductivity could be due to the decrease in soluble ion concentrations via leaching, immobilisation by microorganisms or earthworms, or precipitation in the form of

non-soluble salts (Fornes et al., 2012; Huang et al., 2014). In fact, the K concentration, which is considered to be a main contributor to the electrical conductivity in solution, also decreased throughout the vermicomposting process of Scotch broom. Previous studies dealing with vermicomposting showed in increase in electrical conductivity during the process, which was primarily attributed to the degradation of organic matter and release of mineral salts in available forms, such as K, P and NH₄ (Fernández-Gómez et al., 2010; Li et al., 2011; Yan et al., 2014).

The organic matter content in the initial Scotch broom plant material was $95.36 \pm 0.46\%$ and it was greatly reduced throughout the process $(F_{3,12} = 64.43, p < 0.001)$ to a final level of 79.52 $\pm\,0.51\%$ (Table 1). A similar trend over time was recorded for the total C and N contents (C_t: $F_{3,12} = 22.36$, p < 0.001; N_t: $F_{3,12} =$ 74.80, p < 0.001), while the C to N ratio gradually increased from 11.54 ± 0.17 (day 0) to 12.94 ± 0.02 (day 42; Table 1). Overall, there was a significant increase in the initial nutrient content of the Scotch broom plant material throughout the process (Ca: $F_{3,12}$ = 59.32, p < 0.001; Fe: $F_{3,12}$ = 245.02, p < 0.001; S: $F_{3,12}$ = 6.20, p = 0.009; Mn: $F_{3.12} = 46.92$, p < 0.001; Mo: $F_{3.12} = 241.74$, p < 0.0010.001) and the highest values for these nutrients were recorded at the end of the trial (Table 1). Higher values of P, Mg and B were detected after 28 days of vermicomposting (Table 1), even though, in general, these values did not differ significantly from those registered on day 42 (based on the Tukey HSD test; Table 1). However, the total K was significantly reduced over time

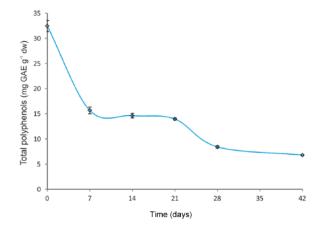


Figure 3. Changes in the total content of pholyphenols (mg GAE g^{-1} dw) during vermicomposting of the Scotch broom plant material (*Cytisus scoparius* (L.) Link). Values are mean \pm SE values (n = 5).

 $(F_{3,12}=11.30, p=0.025)$ reaching a final value of 5.99 ± 0.14 g kg⁻¹ dw (Table 1).

Earthworms are known to accelerate the decomposition of organic matter, as indicated by the lowest hemicellulose and cellulose contents registered at the end of this vermicomposting trial (hemicellulose: $F_{3,12}=15.44$, p=0.015; cellulose: $F_{3,12}=29.71$, p<0.001; Table 1). By contrast, and due to its slower degradation, an increase in the lignin content of the Scotch broom plant material occurred during the course of vermicomposting ($F_{3,12}=320.18$, p<0.001), reaching a final value of 410.06 ± 5.86 g kg⁻¹ dw on day 42 (Table 1). The accelerated mineralisation of organic matter, together with the breakdown of polysaccharides and the higher rates of humification achieved during vermicomposting, can affect the quantity and quality of the nutrients in vermicompost differently (Domínguez and Gómez-Brandón, 2013).

We observed a significant decrease in microbial biomass throughout the process ($F_{2,8} = 45.23$, p < 0.001; Table 1). A plausible explanation for such a reduction could be the depletion of the resources used by microbes due to losses of carbon as a result of earthworm activity (Domínguez et al., 2010). Accordingly, the lowest contents of total C, cellulose and hemicellulose were recorded at the end of the trial (Table 1). Previous studies have also attributed the decrease in microbial biomass to the digestion of bacteria, protozoa and fungi via the digestive enzymes present in the gut of earthworms (Aira et al., 2006; Gómez-Brandón et al., 2010, 2011a, 2012). This must nonetheless be weighed against the fact that some bacterial groups may be digested and others may survive and even flourish during transit through the earthworm gut (Pedersen and Hedriksen, 1993; Drake and Horn, 2007; Gómez-Brandón et al., 2011a).

Such a decrease in microbial biomass was accompanied by a significant reduction in microbial activity over the course of vermicomposting ($F_{3,12} = 451.73$, p < 0.001; Table 1). This is in accordance with previous studies dealing with the impact of epigeic earthworms in microbial communities in the short and long term (Aira et al., 2006; Lazcano et al., 2008; Gómez-Brandón

et al., 2011b, c). In our trial, the increase in the density of earthworms during vermicomposting and the relatively rapid gut transit time of the earthworm *E. andrei* of about 2.5–7 h (Domínguez and Edwards, 2011b) might have played a key part in the time required (42 days) for the stabilisation of the Scotch broom plant material in terms of microbial activity. Nonetheless, it should be noted that lower levels of microbial activity is not always an indicator of a non-phytotoxic vermicompost (Gómez-Brandón et al., 2013).

Changes in polyphenol contents of Scotch broom plant material during vermicomposting

The total polyphenol content of the Scotch broom plant material was 33.44 \pm 1.11 mg GAE g^{-1} dw and decreased significantly throughout the vermicomposting process; the amount of polyphenols was reduced by almost one-half in a period of only 7 days (Figure 3). From day 7, there was a progressive, albeit slower, reduction in the polyphenol content until the end of the trial, reaching a final value of 6.67 ± 1.11 mg GAE g^{-1} dw on day 42 (Figure 3). The amount of polyphenols in the final vermicompost was five times lower than in the initial plant material, which indicates that, in a relatively short period (42 days), earthworm activity contributed to the elimination of the polyphenol-associated phytotoxicity from the vermicompost. Rajiv et al. (2013) also reported a pronounced decrease (32-48%) in the phenol content after 45 days of vermicomposting of cow dung mixed with Parthenium weeds in the presence of the earthworm species Eudrilus eugeniae. Similarly, Domínguez et al. (2014) showed that the polyphenol content of grape marc (58 \pm 10 mg GAE g⁻¹ dw) was reduced by almost one-half in a period of only 14 days after vermicomposting on an industrial scale. Yahaya et al. (2017) found a pronounced decrease in the total concentration of phenolic compounds in oil palm empty fruit bunches, ranging from 31.1 GAE 100 g^{-1} extract to <1.5 g GAE 100 g^{-1} extract at the end of the vermicomposting process. Masciandaro et al. (2010) observed a significant reduction in phenolic compounds when pre-composted olive mill waste water was treated in the presence of the earthworm species E. fetida. Such a reduction was accompanied by a decrease in phytotoxicity of about 50%, leading to an increase in the germination index as a result of earthworm activity. The lack of these phytotoxic compounds is an indication of maturity in organic amendments (Wu et al., 2000; Domínguez and Edwards, 2011a), which is crucial for their application into soil and crop development.

The total polyphenol content of the earthworm bedding was initially 3.83 ± 0.25 mg GAE g^{-1} dw and it barely changed at the end of trial, having a value of 3.15 ± 0.18 mg GAE g^{-1} dw on day 42, which confirms that the reduction in polyphenols shown in Figure 3 was not due to their transfer to the earthworm bedding.

The polyphenolic extracts of this leguminous plant were rich in flavones, mainly apigenin and chrysin, which are characterised by their antioxidant and anti-inflammatory properties. The initial concentration of apigenin in the Scotch broom plant material was

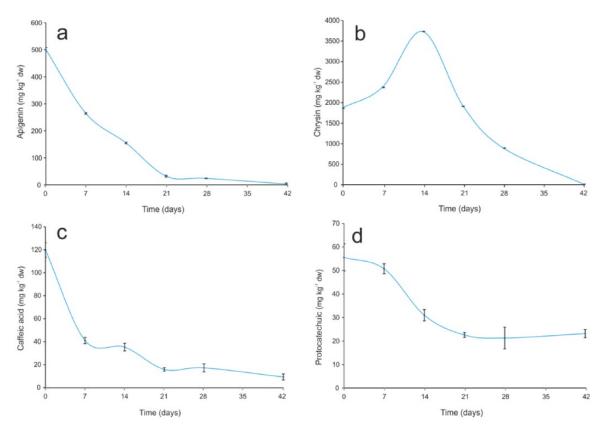


Figure 4. Changes in the concentration of (a) apigenin, (b) chrysin, (c) caffeic acid and (d) protocatechuic acid during vermicomposting of the Scotch broom ($Cytisus\ scoparius\ (L.)\ Link)$ plant material. Values are mean $\pm\ SE\ values\ (n=5)$.

 $502 \pm 6.9 \text{ mg kg}^{-1}$ dw. This concentration decreased quickly during the first three weeks until it reached a value of 34 ± 3.3 mg kg⁻¹ dw after 21 days (Figure 4(a)). At the end of the trial, only 1% of the initial apigenin content remained on day 42 (Figure 4(a)). Apigenin is of crucial interest for nutraceutical purposes due to its low toxicity and potential health-promoting effects in reducing the risk of cancer relative to other structurally related flavonoids (Shukla and Gupta, 2010). Chrysin is another flavone with well-known antioxidant, antimicrobial and ansyolitic properties (Gowthamarajan et al., 2002; Liu et al., 2010). It appeared to have the highest content in the Scotch broom plant material, with an initial content of $1867 \pm 10.2 \text{ mg kg}^{-1} \text{ dw (Figure 4(b))}$. In contrast with apigenin, the concentration of chrysin significantly increased during the first two weeks of vermicomposting, reaching double the initial concentration on day 14 (3732 \pm 4.5 mg kg⁻¹ dw; Figure 4(b)), followed by a decrease in the following weeks until reaching a residual value of 0.6% with respect to the initial chrysin content (Figure 4(b)). The labile nature of the bonds present in O-glucosides, C-glucosides or C-hexosyl derivatives makes these derivatives easily degradable in plants of the genus Cytisus (Barros et al., 2012). This can result in a release of chrysin as a degradation product, leading to an increase of this flavone at the beginning of the vermicomposting process. This opens up another avenue of exploration for vermicomposting focused on the recovery of chrysin at the beginning of the process for its further use for nutraceutical, pharmaceutical and cosmetic purposes.

The polyphenolic extracts of the Scotch broom plant material were also characterised by the presence of non-flavonoids, particularly phenolic acids such as protocatechuic acid and caffeic acid (Figure 4(c, d)), even though their concentration was lower than the flavones apigenin and chrysin. The concentration of caffeic acid decreased from 120 ± 6 mg kg⁻¹ dw (day 0) to 41 ± 3 mg kg⁻¹ dw (day 7), reaching a final value of 9 ± 3 mg kg⁻¹ dw on day 42 (Figure 4(c)). Protocatechuic acid showed a slower degradation over time (Figure 4(d)), with a concentration ranging from 56 ± 6 mg kg⁻¹ dw (day 0) to 23 ± 2 mg kg⁻¹ dw (day 42).

Although the different polyphenols detected in the Scotch broom plant material showed a specific biodegradation profile during the vermicomposting process, there was a general decrease in their concentrations (except for chrysin) in the first stages of the process (Figure 4), which is in line with the rapid reduction observed for the total polyphenol content after only 7 days of vermicomposting (Figure 3).

Conclusions

This study provides evidence that Scotch broom appears to be an optimum substrate for feeding earthworms, providing sufficient energy to sustain large populations on an industrial scale vermicomposting system. Earthworm activity resulted in a higher concentration of nutrients in the final vermicompost and certain polyphenolic compounds, which are responsible for the toxicity of *C. scoparius*, were significantly degraded during the process.

The concentration of the main individual polyphenols identified in the Scotch broom plant material significantly decreased from the beginning of the trial until day 42, even though each compound followed a specific pattern during the course of vermicomposting. This study demonstrates the potential of *E. andrei* to process Scotch broom plant biomass in a large-scale vermicomposting system, turning it into an environmentally friendly organic fertiliser.

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References

- Aira M, Monroy F and Domínguez J (2006) Eisenia fetida (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microbial Ecology 52: 738–747.
- Aira M, Gómez-Brandón M, González-Porto P, et al. (2011) Selective reduction of the pathogenic load of cow manure in an industrial-scale continuous-feeding vermireactor. *Bioresource Technology* 102: 9633–9637.
- Ali U, Sajid N, Khalid A, et al. (2015) A review on vermicomposting of organic wastes. Environmental Progress and Sustainable Energy 34: 1050–1062.
- Álvarez-Casas M, García-Jares C, Llompart M, et al. (2014) Effect of experimental parameters in the pressurized solvent extraction of polyphenolic compounds from white grape marc. *Food Chemistry* 157: 524–532.
- Barros L, Dueñas M, Carvalho AM, et al. (2012) Characterization of phenolic compounds in flowers of wild medicinal plants from northeastern Portugal. *Food and Chemical Toxicology* 50: 1576–1582.
- Domínguez J and Edwards CA (2011a) Relationships between composting and vermicomposting: Relative values of the product. In: Edwards CA, Arancon NQ and Sherman RL (eds) *Vermiculture Technology: Earthworms, Organic Waste and Environmental Management.* Boca Raton, FL: CRC Press, pp. 11–26.
- Domínguez J and Edwards CA (2011b) Biology and ecology of earthworm species used for vermicomposting. In: Edwards CA, Arancon NQ and Sherman RL (eds) *Vermiculture Technology: Earthworms, Organic Waste and Environmental Management*. Boca Raton, FL: CRC Press, pp. 25–37.
- Domínguez J and Gómez-Brandón M (2012) Vermicomposting: Composting with earthworms to recycle organic wastes. In: Kumar S and Bharti A (eds) *Management of Organic Waste*. Rijeka: Intech Open Science, pp. 29–48.
- Domínguez J and Gómez-Brandón M (2013) The influence of earthworms on nutrient dynamics during the process of vermicomposting. *Waste Management and Research* 31: 859–868.
- Domínguez J, Aira M and Gómez-Brandón M (2010) Vermicomposting: Earthworms enhance the work of microbes. In: Insam H, Franke-Whittle I and Goberna M (eds) *Microbes at Work: From Wastes to Resources*. Berlin: Springer, pp. 93–114.
- Domínguez J, Martínez-Cordeiro H, Álvarez-Casas M, et al. (2014) Vermicomposting grape marc yields high quality organic biofertiliser and bioactive polyphenols. Waste Management and Research 32: 1235–1240.

- Drake HL and Horn MA (2007) As the worm turns: The earthworm gut as a transient habitat for soil microbial biomes. Annual Review of Microbiology 61: 169–189.
- Fernández-Gómez MJ, Nogales R, Insam H, et al. (2010) Continuous-feeding vermicomposting as a recycling management method to revalue tomato-fruit wastes from greenhouse crops. *Waste Management* 30: 2461–2468.
- Fornes F, Mendoza-Hernández D, García-de-la-Fuente R, et al. (2012) Composting versus vermicomposting: A comparative study of organic matter evolution through straight and combined processes. *Bioresource Technology* 118: 296–305.
- García-Sánchez M, Taušnerová H, Hanč A, et al. (2017) Stabilization of different starting materials through vermicomposting in a continuousfeeding system: Changes in chemical and biological parameters. Waste Management 62: 33–42.
- Goering HK and Van Soest PJ (1970) Forage Fiber Analysis. Agricultural Handbook No. 379. Washington, DC: Agricultural Research Service, United States Department of Agriculture.
- Gómez-Brandón M and Domínguez J (2014) Changes of microbial communities during the vermicomposting process and after application to the soil. *Critical Reviews in Environmental Science and Technology* 44: 1289–1312.
- Gómez-Brandón M, Lazcano C, Lores M, et al. (2010) Detritivorous earthworms modify microbial community structure and accelerate plant residue decomposition. *Applied Soil Ecology* 44: 237–244.
- Gómez-Brandón M, Aira M, Lores M, et al. (2011a) Epigeic earthworms exert a bottleneck effect on microbial communities through gut associated processes. *PLoS One* 6: 1–9.
- Gómez-Brandón M, Aira M, Lores M, et al. (2011b) Changes in microbial community structure and function during vermicomposting of pig slurry. *Bioresource Technology* 102: 4171–4178.
- Gómez-Brandón M, Lazcano C, Lores M, et al. (2011c) Short-term stabilization of grape mare through earthworms. *Journal of Hazardous Materials* 187: 291–295.
- Gómez-Brandón M, Lores M and Domínguez J (2012) Species-specific effects of epigeic earthworms on microbial community structure during first stages of decomposition of organic matter. *PLoS One* 7: 1–8.
- Gómez-Brandón M, Fernández-Delgado Juárez M, Domínguez J, et al. (2013) Animal manures: Recycling and management technologies. In: Matovic MD (ed.) *Biomass Book 2*. Rijeka: Intech Open Science, pp. 237–272.
- Gowthamarajan K, Kulkarni TG, Mahadevan N, et al. (2002) Antimicrobial activity of selected herbal extracts. Ancient Science of Life 21: 188–190.
- Hättenschwiler S and Vitousek PM (2000) The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in Ecology and Evolution* 15: 238–243.
- Herrera-Reddy AM, Carruthers RI and Mills NJ (2012) Integrated management of Scotch broom (*Cytisus scoparius*) using biological control. *Invasive Plant Science and Management* 5: 69–82.
- Huang K, Li F, Wei Y, et al. (2014) Effects of earthworms on physicochemical properties and microbial profiles during vermicomposting of fresh fruit and vegetable wastes. *Bioresource Technology* 170: 45–52.
- Inderjit (1996) Plant phenolics in allelopathy. Botanical Reviews 62: 186–202.
 ISO 16072 (2002) Soil Quality Laboratory Methods for Determination of Microbial Soil Respiration. Geneva: International Organization for Standardization.
- Lazcano C and Domínguez J (2011) The use of vermicompost in sustainable agriculture: Impact on plant growth and soil fertility. In: Miransari M (ed.) *Soil Nutrients*. New York: Nova Science, pp. 230–254.
- Lazcano C, Gómez-Brandón M and Domínguez J (2008) Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 72: 1013–1019.
- Li X, Xing M, Yang J, et al. (2011) Compositional and functional features of humic acid-like fractions from vermicomposting of sewage sludge and cow dung. *Journal of Hazardous Materials* 185: 740–748.
- Liu H, Mou Y, Zhao J, et al. (2010) Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. *Molecules* 15: 7933–7945.
- Masciandaro G, Macci C, Doni S, et al. (2010) Use of earthworms (Eisenia fetida) to reduce phytotoxicity and promote humification of pre-composted olive oil mill wastewater. Journal of the Science of Food and Agriculture 90: 1879–1895.

- Pedersen JC and Hendriksen NB (1993) Effect of passage through the intestinal tract of detritivore earthworms (*Lumbricus* spp.) on the number of selected Gram-negative and total bacteria. *Biology and Fertility of Soils* 16: 227–232.
- Pérez-Fernández MA, Calvo-Magro E, Rodríguez-Sánchez J, et al. (2017) Differential growth costs and nitrogen fixation in *Cytisus multiflorus* (L' Hér.) Sweet and *Cytisus scoparius* (L.) Link are mediated by sources of inorganic N. *Plant Biology* 19: 742–748.
- Peterson DJ and Prasad R (1998) The biology of Canadian weeds. 109.
 Cytisus scoparius (L.) Link. Canadian Journal of Plant Science 78: 497–504
- Potter K, Kriticos DJ, Watt M, et al. (2009) The current and future potential distribution of *Cytisus scoparius*: A weed of pastoral systems, natural ecosystems and plantation forestry. Weed Research 49: 271–282.
- Potvin C, Lechowicz MJ and Tardif S (1990) The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecology* 71: 1389–1400.
- Prévosto B, Robert A and Coquillard P (2004) Development of Cytisus scoparius L. at stand and individual level in a mid-elevation mountain of the French Massif Central. Acta Oecologica 25: 73–81.
- Rajiv P, Rajeshwari S, Yadav RH, et al. (2013) Vermiremediation: Detoxification of parthenin toxin from *Parthenium* weeds. *Journal of Hazardous Materials* 262: 489–495.
- Shaben J and Myers JH (2010) Relationships between Scotch broom (*Cytisus scoparius*), soil nutrients, and plant diversity in the Garry oak savannah ecosystem. *Plant Ecology* 207: 81–91.

- Shukla S and Gupta S (2010) Apigenin: A promising molecule for cancer prevention. *Pharmaceutical Research* 27: 962–978.
- Singleton VL and Rossi JA Jr (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144–158.
- Slesak RA, Harrington TB and D'Amato AW (2016) Invasive Scotch broom alters soil chemical properties in Douglas-fir forests of the Pacific Northwest, USA. *Plant Soil* 398: 281–289.
- Suleiman H, Rorat A, Grobelak A, et al. (2017) Determination of the performance of vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses in three earthworm species: Eisenia fetida, Eisenia andrei and Dendrobaena veneta. Bioresource Technology 241: 103–112.
- Vance ED, Brooks PC and Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19: 703–707.
- Wu L, Ma LQ and Martinez GA (2000) Comparison of methods for evaluating stability and maturity of biosolids compost. *Journal of Environmental Quality* 29: 424–429.
- Yahaya ANA, Hossain MdS and Edyvean R (2017) Analysis of phenolic compounds in empty fruit bunches in oyster mushroom cultivation and in vermicomposting. *Bioresources* 12: 4594–4605.
- Yan J, Lv B, Zhang J, et al. (2014) Insight into the roles of earthworm in vermicomposting of sewage sludge by determining the water-extracts through chemical and spectroscopic methods. *Bioresource Technology* 154: 94–100.