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Short communication

Using FAME profiles for the characterization of animal wastes and vermicomposts

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Abstract

This study investigated the possibility of fingerprinting different organic wastes (cow, pig and horse manure) and the vermicomposts produced by different earthworm species (*Eisenia andrei*, *Eudrilus eugeniae* and *Lumbricus rubellus*) analyzing the profiles of fatty acid methyl esters (FAMEs). We found clear differences between their microbial communities, demonstrating the power and sensitivity of the total FAME analysis. In addition, qualitative and quantitative analyses of specific biomarkers permitted to determine differences between samples and to evaluate the effect of earthworms in the decomposition of organic matter. Fatty acid profiles were largely determined by the different vermicomposting earthworm species. Fatty acid 18:2ω6 increased significantly in horse manure vermicomposted by *L. rubellus* and in cow manure vermicomposted by the three earthworm species, whereas it decreased significantly in pig manure vermicomposted by *L. rubellus* and *E. eugeniae*. Fatty acid 20:4ω6 increased significantly in all vermicomposts obtained with the three earthworm species.

Keywords: Earthworms; Vermicompost; Animal wastes; FAME profiles; Biomarkers

Vermicomposting is the biooxidation and stabilization of organic matter through the joint action of earthworms and microorganisms. Although earthworms are the main drivers of the process by conditioning the substrate and altering its biological activity, the composition and activity of soil microbial communities are responsible for the biochemical degradation of the organic matter, largely determining the turnover processes (Domínguez, 2004).

All cells contain fatty acids that can be extracted and esterified to form fatty acid methyl esters (FAMEs) (Klug and Tiedje, 1993). When the FAMEs extracted from an organic matter sample are analyzed using gas chromatography–mass spectrometry, the resulting profile of fatty acids constitutes a 'fingerprint' of the microorganisms in the sample. Microbial communities can be compared by extracting FAMEs from different samples and using multivariate statistical techniques to analyze the differences.

In this study, FAME profiles were analyzed to assess the diversity and quantity of fatty acids and to compare the biological fingerprints of three animal manures and their corresponding vermicomposts with three earthworm species.

Animal manures were collected in farms in Tomiño (Galicia, Spain). For vermicomposting, we performed a two-factorial experiment in plastic containers (2 L) with each combination of manure (pig slurry, horse and cow manure) and earthworm species (*Eisenia andrei*, *Eudrilus eugeniae* and *Lumbricus rubellus*) in triplicate ($n = 27$). Each animal manure (ca. 500 g, fresh weight) was placed into the container and inoculated with 50 mature earthworms for *E. andrei* and *L. rubellus*, and with 20 mature earthworms for *E. eugeniae*. We selected different number of *E. eugeniae* due to their greater biomass in comparison with the other earthworm species. The containers were maintained at 20 °C and 90% relative humidity in a

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scientific incubator and after 1 month, earthworms were removed and vermicomposts were sampled for FAME analysis. Since earthworm species used in this study are epigeic, with rapid gut transit times, all the manures were completely processed at the end of the vermicomposting process.

Total fatty acids were extracted from 500 mg (f.w.) samples from each fresh animal manure ($n = 9$) and vermicompost ($n = 27$) using microwave-assisted extraction. After cooling, each sample was allowed to settle and the supernatant was separated, filtrated through Na_2SO_4 (drying) and collected in a glass test tube. The residue from washing (2 times × 5 mL *n*-hexane:acetone (1:1, v/v)) was also dehydrated through Na_2SO_4. The combined solutions were evaporated to dryness under a stream of N_2 gas and redissolved in 500 μL of methyl-*tert*-butyl ether. One hundred microliter of this solution were placed in a 1.5 mL vial with 50 μL of the derivatizating agent (trimethylsulfonium hydroxide, TMSH), vortexed for 30 s and allowed to react for 30 min; then 10 μL of nonadecanoic acid methyl ester were added as internal standard.

GC–MS analysis of the extracts containing FAMEs was performed on a Varian system: GC 3800C, equipped with a CP-SIL 88 Varian Select FAME FS 50 m × 0.25 mm × 0.2 μm capillary column and a MS detector Saturn 2000. One microliter aliquots were injected using splitless mode (2 min split closed and 1:50 split ratio). The oven temperature was programmed from 50 °C (2 min) to 140 °C at 20 °C/min and then to 250 °C at 3 °C/min. Helium (1 mL/min) was used as carrier gas. To identify the fatty acids, retention times were compared to those obtained for standard FAMEs (Larodan, Sweden). For FAMEs with no available standards, NIST library was used. The FAMEs were quantified by comparing the peak areas with those of the internal standard peak (19:0). Fatty acids 18:2ω6 and 20:4ω6 were quantified to study the effect of the different earthworm species during the vermicomposting process of different animal wastes. Discriminant analysis was used to identify the most sensitive FAMEs to differentiate manure and vermicompost samples. Two-way analysis of variance (ANOVA) with animal waste and earthworm species as factors allowed determination of significant differences in the concentration of 18:2ω6 and 20:4ω6 in the samples.

The discriminant analysis of the 44 identified FAMEs clearly differentiated between animal manures, independently if they were raw manures or vermicompost obtained with the different earthworm species (98.2% with 6 FAMEs) (Fig. 1, Table 1) and between the three vermicomposts obtained with the three different earthworm species (100% with 9 FAMEs) (Fig. 2, Table 2). Moreover, the separation between vermicomposts and the initial fresh manures was also very clear (Fig. 2).

Fatty acid 18:2ω6 was significantly different between manures (Fig. 3, $F = 44.51$, $P = 0.0001$). Earthworm species affected differently this fatty acid (Fig. 3, $F = 3.36$, $P = 0.03$) although this effect was dependent on the type of manure (animal waste × earthworm sp., $F = 4.26$, $P = 0.0001$).

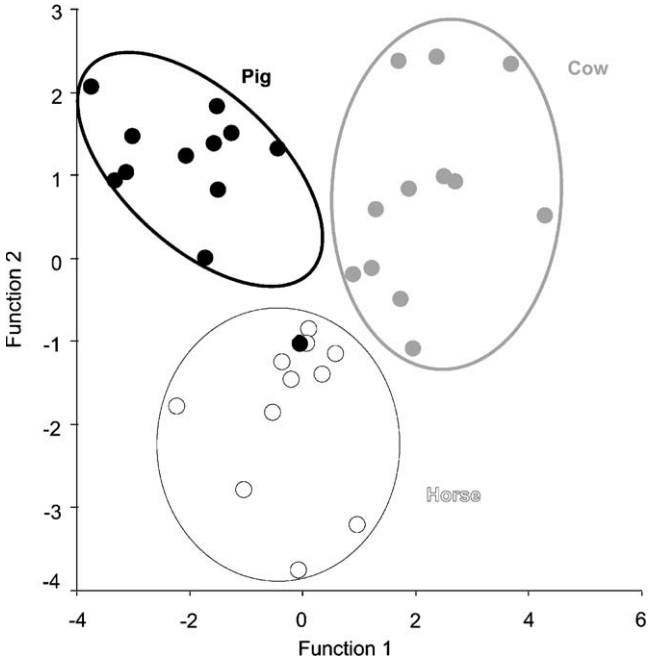

Fig. 1. Discriminant analysis for the samples irrespective whether the manure is raw material ($n = 9$) or vermicompost obtained with the three earthworm species (*E. andrei*, *E. eugeniae* and *L. rubellus*) ($n = 27$).

Table 1
Standardized canonical discriminant function coefficients to differentiate between the different types of animal wastes (pig slurry and cow and horse manure)

	Function	
	1	2
15:0	−0.256	−0.770
18:2ω6	−2.684	1.611
18:3ω6	5.158	2.738
21:0	11.321	24.184
20:3ω6c	0.514	−24.612
$C_{20}H_{38}O_2$[a]	31.426	12.732
(Constant)	1.039	−1.387

No typified coefficients.
[a]Cyclopropaneoctanoic acid-2octyl (tentatively identified by MS).

Fatty acid 20:4ω6 was undetectable in the animal manures (Fig. 4). Earthworm species increased this fatty acid within varying degrees (Fig. 4, $F = 9.56$, $P = 0.0001$) independently of the type of manure (animal waste × earthworm sp., $F = 1.49$, $P = 0.22$).

This study demonstrated the power and sensitivity of the total FAME analysis to fingerprint different animal manures and their vermicomposts obtained with different earthworm species.

The different profiles associated with the animal manures may be due to the type of food eaten by the farm animals and/or their symbiotic microflora, irrespective of

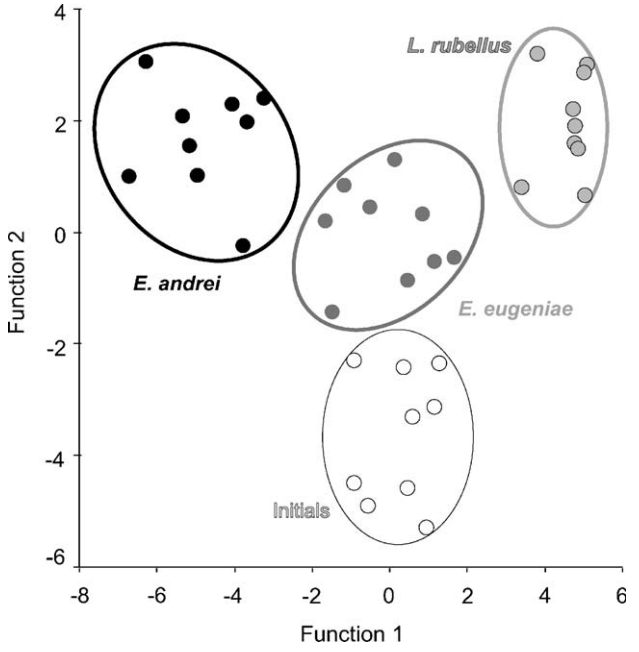

Fig. 2. Discriminant analysis for the initial animal wastes (pig slurry and cow and horse manure) ($n = 9$) and their corresponding vermicomposts obtained with the three earthworm species (*E. andrei*, *E. eugeniae* and *L. rubellus*) ($n = 27$).

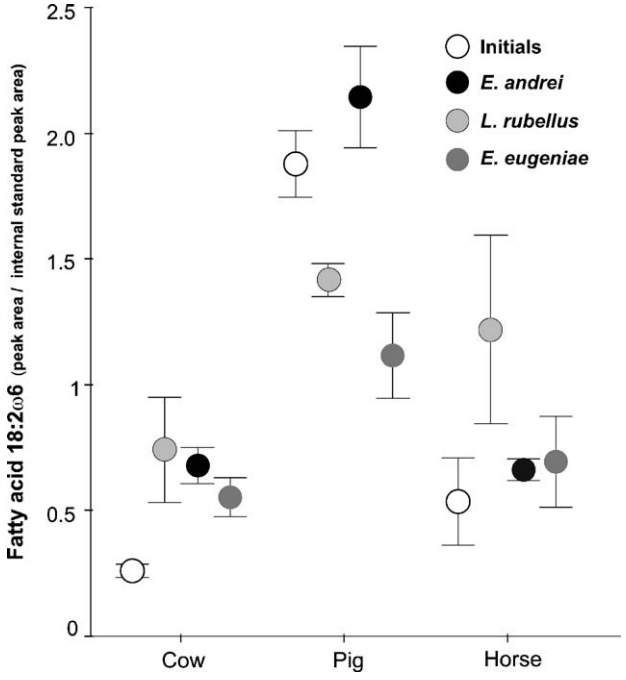

Fig. 3. Changes in the concentration of the fatty acid 18:2ω6 during the vermicomposting with the three earthworm species (*E. andrei*, *E. eugeniae* and *L. rubellus*).

Table 2
Standardized canonical discriminant function coefficients to differentiate the initial animal wastes from the vermicomposts and also between the vermicomposts obtained with the different earthworm species (*E. andrei*, *E. eugeniae* and *L. rubellus*)

	Function		
	1	2	3
14:0	−0.423	−0.477	−1.587
15:0	−0.165	−1.412	0.445
15:1ω5	11.589	3.866	6.520
17:0	−7.795	−0.436	1.167
17:1ω7	−11.957	6.079	−5.602
18:2ω6	2.406	0.167	1.312
18:3ω6	−17.162	−3.624	10.285
$C_{10}H_{18}O_4$[a]	9.045	5.294	0.682
16:1ω11[b]	1.391	2.108	1.118
(Constant)	2.001	−2.800	−1.503

No typified coefficients.
[a]Octanedioic acid dimethylester (tentatively identified by MS).
[b]11-Hexadecenoic acid methyl ester (tentatively identified by MS).

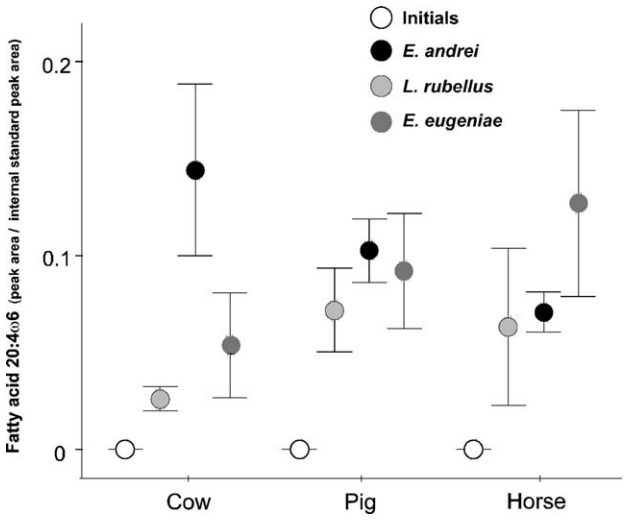

Fig. 4. Changes in the concentration of the fatty acid 20:4ω6 during the vermicomposting with the three earthworm species (*E. andrei*, *E. eugeniae* and *L. rubellus*).

whether the manure is raw material or material processed through the earthworms gut. Moreover, the vermicompost obtained from the different earthworm species could be differentiated between each other in line with the type of manure used. Also, using a different set of FAMEs this technique was able to differentiate between the resulting vermicompost that was produced by each specie of earthworm, even when using animal manure from cows, pigs or horses. Thus, in this case, the different vermicompost fingerprints cannot be due to the type of animal manure used, but due to the earthworm species and/or their associated gut microflora.

Specific fatty acids permitted us to also determine differences between samples and to evaluate the effect of earthworms in the decomposition of organic matter. Fatty acids 18:2ω6 and 20:4ω6 in the vermicomposts were largely determined by the earthworm species. Fatty acid 20:4ω6 is

a characteristic protozoan biomarker (Hill et al., 2000) and its increase in all vermicomposts may be due to an activation and proliferation of protozoa caused by the oxygenation of the manures by the action of the earthworms. In contrast, fatty acid 18:2ω6 increased or decreased depending on the animal waste and the earthworm species. Although sometimes this fatty acid has been used as a biomarker for fungi (Zelles, 1999), since phospholipids fatty acids were not extracted in this study and we used a non-specific extraction procedure, we cannot draw any conclusion about increases or decreases in fungal biomass. Fatty acid 18:2ω6 might represent fungi or other eukaryotes in the manure, or be a legacy of unprocessed feed that passaged through the animals.

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