



## Country report

## Producing organic amendments: Physicochemical changes in biowaste used in anaerobic digestion, composting, and fermentation



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## ABSTRACT

Organic amendments (OAs) produced via composting, anaerobic digestion, or lactic acid fermentation, can be used to replenish soil carbon. Not all OAs production technologies preserve C and nutrients in the same way. In this study, we compared the influence of these technologies (i.e., treatments) on C and nutrient preservation and OAs chemical composition after production. We produced compost, digestate, and lactic-acid fermentation product using the same biowaste-resembling model substrate using three reactors under laboratory conditions. We compared the chemical conversions and end-products using mass balances over C, N, and P. Overall results show that losses are minimal under reducing production conditions. Fermentation and digestion conserved 99% and 64% of C; and 93% and 100% of N, respectively. While compost conservation of nutrients was limited to 25% of C and 38% of N. Digestate had the highest concentrations of C, N, and P in the water-soluble phase, enabling their accessibility for soil microbes. Concentrations in the fermentation product were one order of magnitude lower but still higher than in compost. The treatments also influence the final availability of C, N, and P, which could potentially improve the fertilising and soil-improving properties of produced OAs. Our results show that under reducing conditions, losses of C, N, and P can be decreased while increasing OAs applications in terms of sources for soil-microbial development.

## 1. Introduction

Soil organic carbon loss is the second-largest global threat to soil functions that hampers the achievement of worldwide sustainable soil management (FAO, 2019). The use of organic amendments (OAs) has been proposed as a management practice to increase soil carbon (FAO, 2017) but also as a measure to mitigate climate change (Amelung et al., 2020). However, OAs definition is broad; it includes applying raw organic material, green manures, and adding pre-treated organic substrates, such as compost, digestate, and products of fermentation (silage and Bokashi-like products). With this variety of products, it is difficult to decide which is the best alternative to increase organic matter while maximising the value of the organic residues. On one side, raw materials can be directly used, but by pre-treating organic substrates, there are opportunities to get additional value, such as biogas production during

anaerobic digestion. On the other side, composting, fermentation, and anaerobic digestion show differences in the physicochemical properties of the end-products (Fernandez-bayo et al., 2018; Voelkner et al., 2019), which could thus have different effects on soil properties.

During anaerobic digestion, for example, the retention times are optimised to keep high methane production rates (Wellinger et al., 2013). As a result, the substrate degradation has an asymptotic limit resulting in effluents that still contain easy-degradable-organic fermentation intermediates (Braun, 2007; Rosato, 2018). Therefore, the product is less stable than compost. Easy-degradable organic compounds, such as organic acids produced during fermentation, can increase the proportion of active microorganisms in soils (Macias-Benitez et al., 2020; Odlare et al., 2008). For instance, Odlare et al. (2008) reported an increase in microbial activity in the soil after digestate application compared to compost-treated soil. Another difference with

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digestate is that mature compost generally contains less mineral nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) (Tambone et al., 2010). This occurs because during composting, most of the  $\text{NH}_4^+$  produced during ammonification volatilises as  $\text{NH}_3$  before it is nitrified into  $\text{NO}_2^-$  which is a precursor to  $\text{NO}_3^-$  (Tiquia and Tam, 2000). In the case of fermentation, the end-products are semi-stabilised, as in digestate. Consequently, more easily-degradable material can further break down in the soil after application (Aulinas Masó and Bonmatí Blasi, 2008).

Digestate, fermented products, and compost show not only differences in the nature of their organic matter and nutrient compounds but also differences in the C and N content that remains in the final product. In a well-managed composting process, about 50% of the biodegradable organic matter is converted into  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , mineral salts, and heat (Eghball et al., 1997; Insam et al., 2010a; Martins and Dewes, 1992; Rao Bhamidimarri and Pandey, 1996; Tam and Tiquia, 1999; Tiquia et al., 2002; Tiquia and Tam, 2000). The losses in N and P are also noteworthy; from 20 to 77% of the total initial N of the substrate is lost to the environment, mainly as  $\text{NH}_3$  (Martins and Dewes, 1992; Rao Bhamidimarri and Pandey, 1996; Tiquia et al., 2002; Tiquia and Tam, 2000), and up to 39% of initial P leaves the system as leachate mainly after raining events (Tiquia et al., 2002). In contrast, in the case of fermentation, OA production targets minimal  $\text{CO}_2$  formation to maximise the production of organic acids, such as lactic acid, by inoculating the initial substrate with lactic acid bacteria (Higa and Parr, 1994; Ikeda et al., 2013). During solid-state anaerobic digestion, between 20 and 80% of the organic carbon is converted into methane and carbon dioxide (Braun, 2007; Möller and Müller, 2012), but in exchange for this loss, biogas can be recovered for energy production. Moreover, nitrogen is mostly preserved, and under well-managed settings, only traces of  $\text{NH}_3$  should leave the system in the gas phase (Möller and Müller, 2012). There are no phosphorus losses in AD, but a reduction of the water-extractable P among Ca and Mg due to crystallisation processes (Möller and Müller, 2012).

Although much is known about the physicochemical characteristics of the end-products of digestion and composting (Haug, 2017; Möller and Müller, 2012; Rosato, 2018; Wellinger et al., 2013), only a few studies have compared the characteristics of the organic amendments produced by using the same initial substrate (e.g., Fernandez-bayo et al., 2018; Lin et al., 2014; Younesian et al., 2021). Moreover, a mass balance of carbon and nutrients over the entire process is lacking mainly for fermentation processes that have been neglected in this matter until now. Therefore, after decades of studies, it is still difficult to compare the fate of organic carbon during the treatment of bio-waste and determine which technology can ensure a more efficient conversion of organic streams into OAs with high carbon content and nutrients for the soil.

Assessing the mass balances during OAs production and comparing the effect of bio-waste treatment technologies on the fertilising and soil-improving properties of the end-products is the first step to developing sustainable soil management practices, refining organic-residue-resource management, and tackling climate change by enhancing the carbon-sequestration efficiency in soils.

In this study, we assess the influence of anaerobic digestion (AD), composting (COM), and lactic-acid fermentation (LAF) on the fate of carbon, nitrogen and phosphorus in a model-biowaste substrate when it is converted into an OA and how this is reflected in the concentration and availability of the organic carbon and nutrients in the OAs. We hypothesise that different technologies that implicitly represent different redox conditions and active microbial communities would cause important differences between treatments in C and nutrient preservation, end-product properties, and their possible applications as fertilisers and/or soil improvers. Therefore, we do mass balances on C, N and P and compare the composition of all end-products and initial substrate in laboratory tests (as opposed to full-scale reactor tests) to allow for optimal control and characterisation of all the effluents of the processes. Also, we discuss the suitability of these products for fertilising and soil improvement based on C, N, P and K content, using the

Regulation (EC) No 2019/1009 of the European Parliament about the conditions for making fertilisers available on the internal market. In a follow-up study, we will report the effects of these OAs as organic soil improvers on the development of soil properties using a mechanistic approach.

## 2. Materials and methods

### 2.1. Preparation of the model organic residue MOR

The design of the model biowaste (MOR) considered the following criteria: 1) suitability for all the selected technologies, 2) resemblance to high-quality bio-waste, 3) composition of a wide variety of compounds: from recalcitrant biochemical molecules (lignin, hemicellulose) to more easily degradable compounds (carbohydrates, proteins, and lipids), 4) homogeneous composition, and 5) all year long availability with a reproducible composition. To comply with criteria 2, 3, 4, and 5, we selected a combination of premium-meadow hay and dog food to resemble bio-waste.

Each kilogram of MOR was prepared by mixing 680 ml of water with 69 g of premium meadow hay (Pet's own choice, Netherlands) and 272 g of Oral Care Adult Dog Food (Hill's Science Diet, USA). To improve homogeneity, both dry materials were knife milled separately for 20 s at 4000 rpm with intervals of 5 s between cycles in a Retsch mill grindomix gm300 (Haan, Germany). The average particle size was 2.5 cm, ranging between < 20  $\mu\text{m}$  to 7 cm. The alkalinity was adjusted to 6000 mg  $\text{CaCO}_3/\text{L}$  by using  $\text{CaCO}_3$ . The mix was left covered for equilibration overnight at 4 °C.

### 2.2. Production of the organic amendments

Three identical glass-jacketed reactors (12-L working volume) were used to produce: digestate, fermented product and compost (Fig. 1). For all these reactors, the temperature inside the jacketed reactor was controlled with a Circulating Chiller (Thermo Haake K10). Due to the differences in the processes, the amount of initial substrate used in each reactor had to be adjusted to produce at least 1 kg of dry end-product.

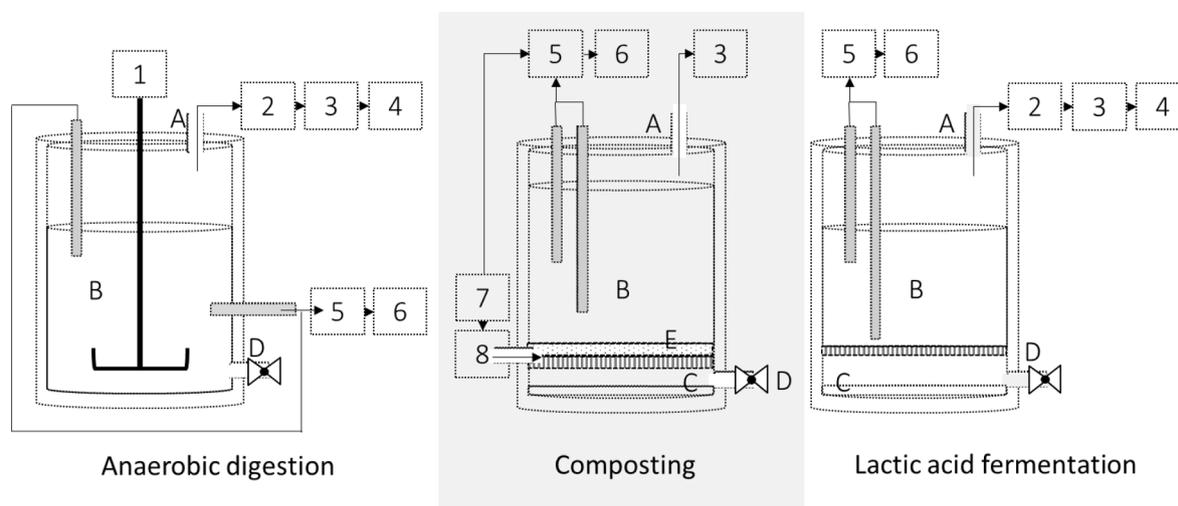
#### 2.2.1. Anaerobic digestion

The solid-state anaerobic digestion was carried out in mesophilic conditions (30 °C) in a fed-batch bioreactor (Fig. 1). The reactor was operated for 165 days, of which 30 were used for acclimation of the inoculum to MOR feed. The reactor was inoculated with digestate taken from another reactor fed with sugar beet residue (Table 1). The inoculum was added three times during the entire experiment (1.6, 1, and 0.5 kg, respectively). Twice at the beginning of the acclimation stage and a third time (on day 150). The third feeding was used to reduce the concentration of  $\text{NH}_4$  and  $\text{C}_3$  as the nitrogen concentration, as  $\text{NH}_4$ , increased with each feeding ( $\text{C}_3 = 20 \text{ g/l}$ ,  $\text{NH}_4 = 58 \text{ mg/l}$ ). By the end of the experiment, the inoculum-to-MOR ratio was 1:3 (3 kg of inoculum: 10 kg MOR).

The reactor was fed with MOR when the normalised-accumulated methane production was > 75–80 % of the expected methane production ( $\text{COD}_{\text{CH}_4}/\text{COD}_{\text{total feed}}$ ). The expected methane production was calculated using a conversion factor of 0.35 L  $\text{CH}_4/\text{g COD}_{\text{total-feed}}$  (Wellinger et al., 2013). The biogas composition was determined daily after substrate addition, but the sampling frequency was reduced once it became constant. To monitor the process and before each feeding, digestate samples were regularly taken to analyse pH, total solids (TS) and volatile solids (VS),  $\text{C}_2$ ,  $\text{C}_3$ ,  $\text{nC}_4$  and  $\text{NH}_4$ . After feeding the reactor, the headspace was flushed with  $\text{N}_2$  gas for 3 min to ensure anaerobic conditions. A gas sample was taken hereafter to verify oxygen absence. This procedure was followed every time the reactor was opened.

#### 2.2.2. Composting

Compost was produced in a packed-bed reactor (Fig. 1) with bottom-



**Fig. 1.** From left to right: Diagram of set-up for the anaerobic digestion (AD), composting (COM), and lactic acid fermentation (LAF). The letters indicate different parts of the 12-L glass jacketed reactors: A) gas outlet of the reactor, B) initial substrate (MOR), C) leachate collector, D) liquid/slurry sampling point, and E) air diffuser. The numbers indicate other devices connected to the reactors: 1) paddle stirrer, 2) water lock, 3) gas sampling port, 4) gas-meter, 5) transmitter that collects the signal of sensors of pH and temperature, 6) data logger, 7) flowmeter, and 8) gas humidifier.

**Table 1**

Main characteristics of the inoculum for compost and anaerobic digestion. SE: standard error (n = 4). Symbol (-) means “not applicable”.

Parameters	Units	Digestate		Compost	
		Inoculum		Inoculum	
		Value	SE	Value	SE
pH		8.7	± 0.04	8.7	± 0.01
Total solids	(%)	8.9	± 0.16	45.8	± 1.78
Volatile solids	(%)	4.5	± 0.06	14.3	± 0.46
COD	g/kg dry	385.3	± 3.64	-	
Soluble COD	g/kg dry	57.1	± 2.05	-	
Total elements					
C	g/kg dry	145.2	± 10.07	176.5	± 1.85
N	g/kg dry	16.5	± 0.88	14.8	± 0.24
P	g/kg dry	4.1	± 0.05	2.25	± 0.30

top forced aeration. The in-flowing air was controlled and measured by a controller/flowmeter (Bronkhorst Ruurlo, The Netherlands) connected to the same transmitter and data logger used for AD. The moisture in the vessel was kept relatively constant by bubbling the in-flowing air in water before entering the system. The leachate was collected four times from the bottom of the reactor and evacuated to avoid blocking the air diffuser. The total weight of the leachates was registered and individually analysed to determine their composition for the mass balance.

A mature commercial compost (Attero, The Netherlands) was used as inoculum (Table 1). The inoculum (1.7 kg) was mixed with 3.3 kg of MOR (ratio of 1:2). Additionally, inert polyethene packaging peanuts were used to increase the porosity of the mix and allow a better oxygen transfer along the reactor.

The reactor was operated for 60 days in a two-stage process: the first stage lasted 30 days at 50 °C, whereas during the second curing/maturation stage, the temperature was set at 30 °C. Temperature, pH, and gas composition were followed to monitor the process. The in-flowing air volume and the CO<sub>2</sub> % measurements were used to calculate C mineralised. The difference between the oxygen in the in-flow and out-flow was used to determine the oxygen uptake (OUR) using the equation reported in Veeken et al. (2004).

### 2.2.3. Lactic acid fermentation

LAF was done under anoxic conditions at 25 °C for 60 days (Fig. 1). Ten kilograms of MOR were mixed with 29 g of montmorillonite clay

(Edasil®, Agriton, The Netherlands); 29 g of a commercially available carbonate source (Aegir, Agriton, Netherlands) and inoculated with 5 ml of Microferm (Agriton, The Netherlands). The total dry weight of this inoculum was negligible (<0.4 mg), and it was not considered for the mass balance. The mix was compressed inside the reactor to remove the air entrapped in the matrix. Before closing the reactor, the headspace was flushed with N<sub>2</sub> gas in the same way as in the AD reactor. The leachate was collected and weighted for further analysis. The gas production and composition were determined the same way as in AD.

### 2.3. Analytical methods

All process parameters were analysed in four independent samples (n = 4). The pH and electrical conductivity (EC) were measured directly in the liquid samples. For solid samples, a 1:5 cold-water extract was prepared using the same method described in Ghani et al. (2003). TS and VS were determined gravimetrically following Federal Compost Quality & Organisation, (2003).

To determine the total C and N, oven-dry samples were ground and transferred to a 2 ml plastic tube with a glass beat. The samples were further ground in a tissue homogeniser (Precellys Evolution, Bertin, France) at 8000 rpm with four cycles of 40 s and 30 s pause. Three milligrams of the powdered sample were weighted in tin cups to be analysed in an Elemental Analyser (Thermo Scientific™ FlashSmart™, Courtaboeuf, France). The C and N loss during the oven-drying process was corrected by analysing volatile fatty acids (VFA) and NH<sub>4</sub><sup>+</sup> before and after the drying process and estimating the percentage of loss of these compounds. In the case of NH<sub>4</sub><sup>+</sup>, only 0.1–0.4% of the initial value remained in the oven-dried sample. There was more variability in VFA that remained after the oven-drying process, with values between 17 and 83% (non-reported results). For practical purposes, in the mass balance calculation, the C and N present in VFA and NH<sub>4</sub> were assumed to be removed entirely from the oven-dried samples and then added to the value obtained from the elemental analysis. The overall VFA content was 197 mg/kg on a dried basis. For the determination of other elements (P, Na, K, Ca, and Mg and trace elements), 100 mg of ground dry sample were digested using 12 ml of aqua regia in a High-Performance Microwave Digestion System ETOS EASY (Italy) at 220 °C during 35 min. The digested sample was diluted in water and analysed using inductively coupled plasma optical emission spectrometry (ICP-OES; type Optima 5300 DV, Perkin Elmer).

The water-soluble C, organic carbon (OC), N, P, cation, anions, and

organic acids were determined in the liquid samples and in the 1:5 cold-water extracts (prepared similarly as for pH and EC in the case of solid samples). The total water-soluble carbon (TC) and total organic carbon (TOC) were measured by oxidation through heating and combustion in a Shimadzu TOC Analyzer. In unfiltered samples, water-soluble N and P content was measured using Hach Lange kits LCK 338 and LCK 348. The sample for quantification of cations, anions, and organic acids was filtered using a 0.45  $\mu\text{m}$ -membrane. Dissolved anions were measured by injecting 20  $\mu\text{l}$  of filtered sample into an Ion chromatograph, Metrohm Compact IC Flex 930 equipped with an inline degasser, pre-column, Metrohm Metrosep A Supp 4/5 Guard, a chemical and  $\text{CO}_2$  suppressor, and a conductivity detector. Cations were separated on a column, Metrohm Metrosep C 4 – 150/4.0 mm. The mobile phase was a solution of 3.2 mM sodium carbonate, 1 mM sodium bicarbonate, and 1% acetone. The suppressor solution had 0.5 mM orthophosphoric acid eluent and 1% acetone. The flow rate was (0.7 ml/min for the mobile phase and 0.3 ml/min for suppressor solutions). The run time was 18 min. The data processing unit was Metrohm, type IC Net, version 2.3. Dissolved cations were measured by injecting 100  $\mu\text{l}$  of filtered sample into an Ion chromatograph, Metrohm Compact IC Flex 881 and Metrohm Compact IC Flex 930 equipped with an inline degasser pre-column, Metrohm Metrosep RP 2 Guard/3.6 and conductivity detector. Cations were separated on a column, Metrohm Metrosep C 4 – 150/4.0 mm. The mobile phase was 3 mM nitric acid. The flow rate was 0.9 ml/min, and the run time was 18 min. The data processing unit was Metrohm, MagIC Net, version 2.3 and version 3.2. Organic acids were measured by injecting 50  $\mu\text{l}$  of filtered sample into an HPLC Dionex ultimate 3000RS (Thermo Scientific™) equipped with an inline degasser, quaternary pump, autosampler and UV-detector. Organic acids were separated on a column, Phenomenex Rezex Organic Acid H+, 300  $\times$  7.8 mm. The mobile phase was 2.5 mM sulphuric acid. The flow rate was 0.50 ml/min, and the run time was 30 min. The column was kept at 80 °C, and the detection was done at 210 nm. Data collection and processing of chromatograms were done using Chromeleon 7 software.

#### 2.4. Gas sampling

Five millilitres of gaseous samples were taken from the sampling ports using gas-tight syringes. Concentrations in volume % of  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$ ,  $\text{CH}_4$ ,  $\text{H}_2$  and  $\text{H}_2\text{S}$  were measured in a gas chromatograph (Varian CP4900 Micro GC, TCD detector and two separate column models Mol Sieve 5 Å PLOT (MS5) and Pora PLOT U (PPU)). Every time a gas sample was taken from AD and LAF, the accumulated gas volume was registered from the gas meters connected to the gas line (Typ MGC-1, MilliGascounter®, Ritter, Germany).

#### 2.5. Mass balances in the reactors

Mass balances were performed on fresh and dry masses, C, N, and P. The equations used for the mass balances and performance indicators are reported in the supplemental material. Since all the reactors were set to get around 1 kg of end-product, the C, N and P values in Table 2 indicate the final production yield (i.e., g C/g of dry-MOR).

#### 2.6. OA assessment as fertilisers and soil improvers

We explored the possible properties of OAs as fertilisers and organic improvers. To address the fertilising properties, we used the definitions of Regulation (EU) 2019/1009 to classify the end-products according to their carbon and nutrient content into solid organic fertiliser, liquid organic fertiliser, and soil organic improver. Values and definitions established by this regulation were preferred over peer-reviewed scientific papers because these references come from consensus between different scientific fields that include economic and environmental concerns. The characteristics of the mentioned OAs categories are described below:

**Table 2**

Mass balances over fresh and dry mass, C, N, and P. Symbols: (-) “Not applicable” given the type of technology, (-) “Non-detectable”, (\*) “Does not produce volatile forms”, and (\*\*) “No missing mass quantified” because the percentage of C and N in the gas phase was calculated from the other data measured.

	Start	Final	End-product	Leachate	Gas	Missing on the balance
	kg fresh	kg fresh	%	%	%	%
<b>Fresh</b>						
Fermentation	10.0	7.8	78.81	17.34	0.22	3.63
Composting	4.7	1.8	37.33	15.71	46.77 <sup>a</sup>	**
Anaerobic digestion	13.1	12.0	87.87	–	8.56	–0.19
<b>Dry basis</b>						
Fermentation	3.0	2.6	86.16	4.54	0.74	8.57
Composting	1.5	1.2	79.72	0.82	0.58	18.87 <sup>b</sup>
Anaerobic digestion	2.5	1.4	52.85	–	44.66	0.75
<b>C Mass</b>						
Fermentation	1.3	1.2	91.54	4.25	0.45	3.75
Composting	0.5	0.2	38.66	0.99	60.91 <sup>a</sup>	**
Anaerobic digestion	1.1	0.7	64.19	–	41.44	–5.63
<b>N Mass</b>						
Fermentation	10	9.3	92.13	2.24	–	5.63
Composting	3.6	1.9	51.54	1.24	47.22 <sup>a</sup>	**
Anaerobic digestion	8.9	9.1	103.02	–	–	–3.03
<b>P Mass</b>						
Fermentation	1.4	1.3	90.52	1.80	*	7.68
Composting	5.2	5.0	96.81	0.58	*	4.29
Anaerobic digestion	1.1	8.4	72.65	–	*	24.96 <sup>c</sup>

<sup>a</sup> Calculated values.

<sup>b</sup> This amount includes the weight loss due to water respiration.

<sup>c</sup> Phosphorus precipitated at the bottom of the reactor.

**Solid organic fertiliser:** It should be produced from solely biological origin containing  $\geq 15\%$  by mass of organic carbon and at least one of these primary nutrients: N, P or K. If only one primary nutrient is declared; It should contain at least: 2.5 % by mass of N or 0.9 % by mass of P, or 1.6 % by mass of K. When there is more than one primary nutrient, those nutrients should be at least: 1 % by mass of N, 0.4 % by mass of P, or 0.8 % by mass of K. The sum of the reported nutrients should be at least 4 % by mass.

**Liquid organic fertiliser:** It should be a liquid that comes from solely biological origin containing  $\geq 5\%$  by mass of organic carbon and at least one of these primary nutrients: N, P or K. If only one primary nutrient is declared; It should contain at least: 2 % by mass of N or 0.4 % by mass of P, or 1.6 % by mass of K. When there is more than one primary nutrient, those nutrients should be at least: 1 % by mass of N, 0.4 % by mass of P, or 0.8 % by mass of K. The sum of the reported nutrients should be at least 3 % by mass.

**Organic soil improver:** It should consist of 95% solely biological origin, have  $\geq 20\%$  dry matter and  $\geq 7.5\%$  by mass of organic carbon.

Despite that these guidelines are specific, they do not mention parameters to describe the quality of organic-carbon sources, which is particularly important for the effectiveness of soil improvers. In scientific literature, the quality of organic sources is defined according to new insights on soil carbon stabilisation mechanisms which emphasise the role of microbial biomass building-up as the primary contributor of stable carbon pools in aggregates and mineral-associated organic matter (MAOM) (Cotrufo et al., 2013). Therefore, high quality will be referred to as appropriate nutrient sources for soil microbial biomass production,

which are linked to the quality of carbon sources, low carbon nutrient ratios, and low concentrations of biochemically recalcitrant compounds (Angst et al., 2021). Correspondingly, to describe the quality of carbon sources, water-soluble fractions of C-based compounds, organic acids and nutrients were measured in the water-extractable phase. This is relevant because nutrients in this phase can be more accessible for microbial biomass formation. In a follow-up study, we will report the effects of these OAs as soil-organic improvers on the development of soil properties using this same mechanistic approach.

## 2.7. Statistical analysis

We used R (R Core Team, 2021) with RStudio version 2021.09.1 + 372 “Ghost Orchid” for the descriptive statistical analysis and graphs.

## 3. Results

### 3.1. Carbon, nitrogen, and phosphorus mass balances in the reactors

After COM, only 37% of the initial-fresh-mass weight remained as an end-product (Table 2). Most of the lost mass was leachate (16%) and gas (47%), which in both cases had water as its main compound. LAF and AD kept 79% and 88% of the total fresh mass correspondingly. In LAF, this loss was related to leachate production (1.7 L), which had a water content of 92%. For AD digestion, these losses were not related to water but to the total gas emissions from the process.

When considering dry mass, LAF and COM preserved 86% and 80%, respectively. For AD, this was lower, namely 53%. In LAF, the missing solids were present in the leachate. While in COM, part of the dry mass was converted to CO<sub>2</sub>, H<sub>2</sub>O, and NH<sub>3</sub> due to respiration and protein

breakdown. It was calculated that C and N represent 81% and 5% of the total dry matter that was gone (0.35 kg). AD lost 1.1 kg of dry matter (45% carbon, 59% oxygen and 7% hydrogen) during the conversion into CH<sub>4</sub> and CO<sub>2</sub>.

LAF maintained more C in the end-product (92%), and only 0.4% of C was released into the atmosphere as CO<sub>2</sub>. During AD, 64% of the carbon remained digestate, and 41% was converted into CH<sub>4</sub> and CO<sub>2</sub>. Composting reported the highest C loss to the atmosphere (61%). Only 39% of the total carbon was kept in the end-product, the lowest among all the systems.

Concerning N content, 47% of the initial N was lost to the gaseous phase during composting. LAF lost 2% of N through the leachate while AD maintained all N inside the reactor. In the same way, AD kept all P, whereas COM and LAF reported losses of P in their leachates (1% and 2% in that order). AD reported 73% of P instead of 100% because a fraction of P was unavailable in the digestate that we considered as an end-product due to precipitation. The P-precipitate can be recovered as well by other means.

### 3.2. Comparison of the chemical composition of MOR and OAs as end-products

Differences in the means of the chemical composition of MOR and the end-products were compared. The most meaningful change in composition occurred in AD since only two parameters of 28 remained similar (water-extractable Ca and Mg). On the other hand, COM and LAF end-products had four (P, S, Na, and NO<sub>3</sub><sup>-</sup>) and six parameters (VS, N, P, S, NO<sub>3</sub><sup>-</sup>, and water-extractable P) similar to the initial substrate (Table 3).

All the treatments differed in pH value. The pH of the initial substrate was 6; after COM, it reached nine. Digestate had a value of 8. After LAF,

**Table 3**

Physicochemical characteristics and composition of the initial substrate, digestate, fermented product and compost. SE: standard error (n = 4). (\*) Calculated from the organic acid concentration.

Parameter	Units	Substrate - Biowaste				Digestate				Compost				Fermented product			
		MOR				DOA				COA				FOA			
		Value	SE	Value	SE	Value	SE	Value	SE	Value	SE	Value	SE				
pH		8.9	± 0.01	8.2	± 0.01	3.8	± 0.09	6.1	± 0.05								
Electrical conductivity	mS/cm	19.2	± 0.02	29.4	± 0.56	12.0	± 0.92	2.2	± 0.00								
Total solids	(%)	69.7	± 0.19	11.5	± 0.08	33.2	± 0.48	30.7	± 0.67								
Volatile solids	(%)	19.3	± 0.18	8.6	± 0.08	29.2	± 0.15	28.7	± 0.55								
Total elements																	
C	g/kg dry	145.2	± 8.9	529.0	± 6.9	507.9	± 10.1	443.5	± 1.5								
N	g/kg dry	16.5	± 2.02	80.7	± 3.17	36.0	± 0.88	34.3	± 0.69								
P	g/kg dry	4.1	± 0.27	6.1	± 0.25	4.9	± 0.05	4.6	± 0.12								
S	g/kg dry	3.1	± 0.08	7.8	± 0.16	4.7	± 0.03	3.0	± 0.25								
Ca	g/kg dry	19.5	± 0.62	28.4	± 0.61	7.1	± 0.25	10.6	± 0.23								
K	g/kg dry	7.6	± 0.08	32.6	± 0.85	8.4	± 0.05	5.5	± 0.10								
Mg	g/kg dry	3.0	± 0.01	2.0	± 0.15	1.1	± 0.03	0.8	± 0.02								
Na	g/kg dry	1.5	± 0.04	6.2	± 0.16	2.0	± 0.04	1.4	± 0.02								
C/N		8.8		6.6		14.1		12.9									
C:N:P:S		47:5:1:1		68:10:1:1		107:8:1:1		147:11:2:1									
Available compounds in the water extract																	
Soluble Total C	g/kg dry	5.43	± 0.99	325.08	± 2.84	83.75	± 0.79	33.97	± 1.87								
Total organic C	g/kg dry	4.79	± 1.13	310.85	± 2.43	83.61	± 0.73	33.66	± 1.91								
C - Organic acids	g/kg dry	0.04*		90.41*		45.33*		6.42*									
Soluble Total N	g/kg dry	1.85	± 1.18	68.42	± 0.20	6.84	± 0.11	13.23	± 0.59								
NO <sub>2</sub> <sup>-</sup>	g/kg dry	0.00	± 0.00	0.05	± 0.00	0.03	± 0.00	0.01	± 0.01								
NO <sub>3</sub> <sup>-</sup>	g/kg dry	0.01	± 0.00	0.14	± 0.02	0.04	± 0.00	0.05	± 0.01								
NH <sub>4</sub> <sup>+</sup>	g/kg dry	0.93	± 0.01	63.12	± 6.06	1.03	± 0.01	0.73	± 0.07								
Soluble Total P	g/kg dry	0.38	± 0.06	5.21	± 0.20	3.77	± 0.05	3.94	± 0.28								
PO <sub>4</sub> <sup>3-</sup>	g/kg dry	0.17	± 0.05	3.01	± 0.34	11.77	± 0.07	4.21	± 0.11								
SO <sub>4</sub> <sup>2-</sup>	g/kg dry	1.28	± 0.06	0.96	± 0.14	1.49	± 0.03	2.62	± 0.07								
Ca	g/kg dry	0.04	± 0.05	2.83	± 0.36	5.15	± 0.01	3.26	± 0.30								
Na	g/kg dry	0.67	± 0.02	6.17	± 0.36	1.75	± 0.04	2.35	± 0.09								
K	g/kg dry	5.84	± 0.04	53.11	± 1.74	7.47	± 0.05	4.72	± 0.42								
Mg	g/kg dry	0.01	± 0.06	0.64	± 0.09	0.99	± 0.00	0.73	± 0.06								

the pH dropped to 3.8. TS and EC were different in all the treatments as well. COA had the highest value of TS, while AD reported the lowest (70% and 12%, respectively). FOA showed a value slightly higher (33%) than the initial substrate (31%). VS in FOA was closer to the value of the initial substrate. On the contrary, COA and DOA indicated a decrease in VS, from 29% in the initial substrate to 19% and 9%, correspondingly.

FOA and DOA had 15% and 19% more C than MOR. On the contrary, COA had 67% less C. Respect MOR, the N content was 135% higher in DOA and 51% lower in COA, while FOA did not seem to report a difference. For P and S, only DOA showed an increase in the concentration of both elements; 32% for P and 159% for S.

For the major cations in soil, DOA reported higher concentrations of Ca and Na in comparison to the initial substrate, 168% higher for Ca and 329% for Na. Potassium concentrations in DOA and FOA were greater by 487% and 51%, correspondingly. COA and DOA reported Mg concentrations 280% and 157% higher than MOR. Among OAs, COA had the highest concentrations of Mg and Na, while DOA had the highest contents of Ca and K.

### 3.3. Differences in water extractability and forms

AD, COM, and LAF influenced the forms of C, N and other nutrients and how easily they can become available in solution. DOA and FOA had similar total C concentrations (Table 3) but different water-extractable C fractions. In FOA, this meant that 148% more C was available in the water phase compared to MOR. In DOA, this value increased to 824% representing 62% of the total C in this end-product (Fig. 2). The composition of the organic C fraction displayed diverse types of organic acids for DOA, FOA, and MOR that represented 28%, 54%, and 19% of C in the water-extractable fraction, respectively (Table 4). Conversely, COA had only 3% water-extractable C, and organic-acid content fell under detection limits.

DOA reported the highest amount of water-extractable N (417% more than MOR), while COA and FOA showed less N in this fraction (86% and 48% less than MOR). Most of the total nitrogen in DOA (84%) was present in this water-extractable phase, 72% as  $\text{NH}_4^+$ . In contrast, ammonium content was less important in COA, FOA and MOR, being

other N-organic forms dominant in the water-extractable phase.

In the same way, as reported for C and N, DOA had the highest amount of water-extractable P (32% more than MOR). While for FOA, there was no major difference; COA had 90% less water-extractable P than MOR (Table 3). Despite that FOA and MOR had similar water-extractable P, the P-forms demonstrated to be different: FOA had most of its P as phosphate while MOR P was present in other forms that were not further identified (Table 3). Finally, DOA, COA, and FOA showed smaller concentrations of  $\text{S-SO}_4^{2-}$  than MOR.

In all treatments, Ca and Mg were present primarily in the non-water extractable phase, contrary to K and Na mainly in solution (Table 3). The smallest concentrations of cations in solution were observed in COA. For K and Na, DOA showed the highest cation concentration in the water extract compared to other treatments. In contrast, only 10% of Ca and 32% of Mg were free in the solution. In FOA, all cations were present mainly in the water extract; 72% of all Ca, 89% of K, 90% of Mg, and 87% of Na.

## 4. Discussion

The changes in the initial substrate (Tables 3 and 4) are likely related to the differences in redox environments and pH conditions, which dictate what thermodynamic reactions can occur and determine the outcome of microbe competition for the most energetically favourable pathways (Burgin et al., 2011). These pathways dominate as long as the supply of electron acceptors for those processes is not depleted (Burgin et al., 2011). During composting, bacteria and fungi will use oxygen as electron acceptor since it gives the highest energy yield (Haug, 2017), resulting in up to 67% of C losses (to  $\text{CO}_2$ ); during respiration, depending on the system of composting, retention time, aeration system, substrate, particle size, C:N ratio among other conditions (Eghball et al., 1997; Insam et al., 2010b; Larney and Hao, 2007; Tiquia et al., 2002).

AD and LAF occur in a reducing environment and under contrasting pH conditions (8 and 3.8, respectively). These conditions stimulate anaerobic metabolism conversions, usually mediated by a symbiotic network of microorganisms that operate in series feeding themselves from the sub-products of their antecessors in the decomposition process.

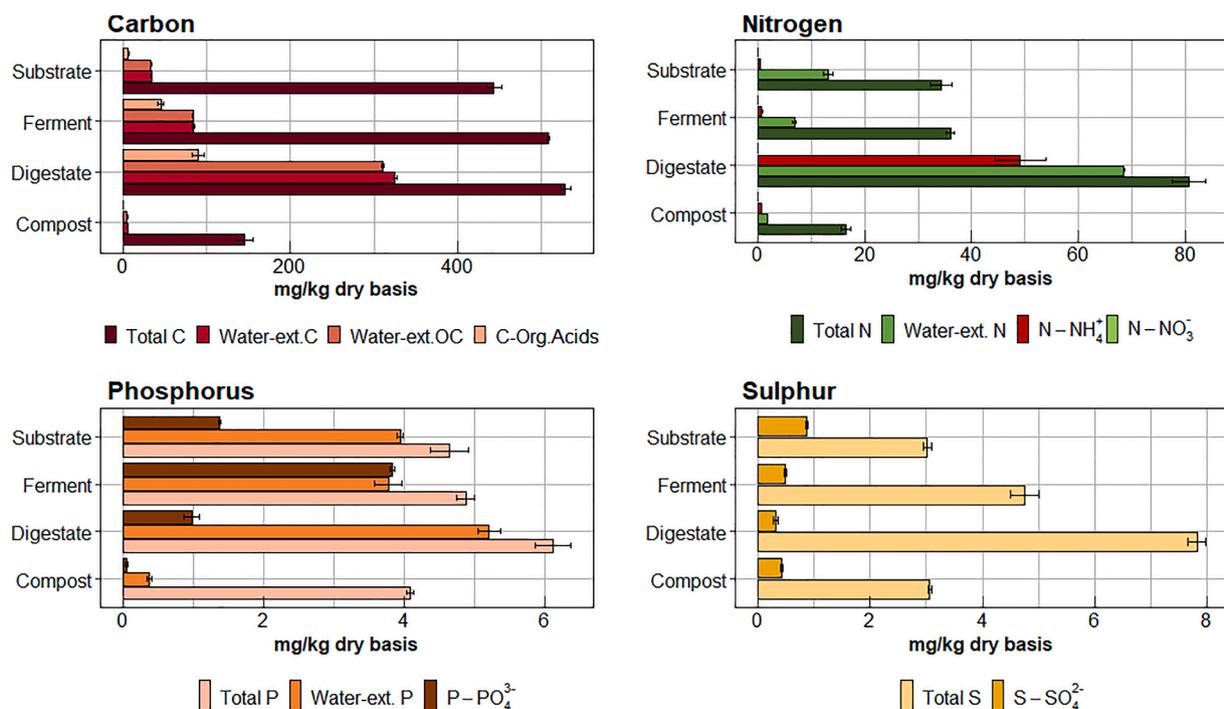


Fig. 2. Total concentrations of C, N, P and S including different fractions of the initial substrate, compost, digestate and fermented product (n = 4). Error bars represent ± one standard error.

**Table 4**

Organic acids concentrations in the organic amendments and the initial raw substrate (Biowaste). SE: standard error. BD = Below detection limit, this means values < 5 mg/kg dry. (n = 4).

Parameter	Units	Substrate - Biowaste		Digestate		Compost	Fermented product	
		MOR		DOA		COA	FOA	
		Value	SE	Value	SE	Value	Value	SE
Citric Acid	g/kg dry	1.5	± 0.08	BD		BD	BD	
Pyruvic Acid	g/kg dry	0.2	± 0.11	BD		BD	BD	
Malic Acid	g/kg dry	BD		BD		BD	BD	
Succinic Acid	g/kg dry	BD		BD		BD	6.1	± 3.52
Fumaric Acid	g/kg dry	0.1	± 0.00	BD		BD	BD	
Lactic Acid	g/kg dry	7.6	± 0.54	BD		BD	43.3	± 1.69
Formic Acid	g/kg dry	BD		BD		BD	BD	
Acetic Acid	g/kg dry	1.1	± 1.02	82.4	± 5.11	BD	16.8	± 3.72
Propionic Acid	g/kg dry	4.6	± 1.47	100.6	± 2.72	BD	38.5	± 2.63
Butyric Acid	g/kg dry	BD		BD		BD	BD	

Several intermediate products can be generated in this manner and if this chain of reactions is allowed to go to completion and methanogens are present, which is the case in AD, the end products will be CH<sub>4</sub> and CO<sub>2</sub> (Haug, 2017). The low pH in lactic-acid fermentation may inhibit the chain of reactions by restricting the development of other microbial communities that could have otherwise caused a further degradation of lactic acid. Nitrogen is emitted during COM in gaseous forms such as NH<sub>3</sub>, N<sub>2</sub>O, N<sub>2</sub>, and other NO<sub>x</sub> compounds (Martins and Dewes, 1992; Tiquia, 2002). AD resulted in higher ammonium concentrations (as compared to COM). However, this ammonium was preserved in the digestate because the pH was below the pKa of the ammonium – ammonia equilibrium (around pH 9) (Wellinger et al., 2013).

OAs production under reducing environment (AD and LAF) favoured the preservation of C, N and P better than under oxidising conditions (COM). Moreover, AD and LAF made C, N, P, and other nutrients more readily available for microbes by enabling their accessibility in the water-extractable fraction. As OAs, DOA and FOA provide more C/Kg of dry end-product and ensure a high-quality C surplus for soil biomass development. Changes in the initial substrate were anticipated at the elemental composition level. Nevertheless, the most important changes were present in nutrient forms and their proportion in the water available extract.

#### 4.1. Overall mass balance over carbon, nitrogen and phosphorus

Changes in the proportion of water-extractable C, N and P, and their chemical forms were the most critical changes in MOR under different process conditions. AD had the highest C, N, and P concentrations in the water extract. Even though the concentration of ammonium was particularly high during the entire process (60% of total N), the values were in the range reported in previous studies where the threshold limits fluctuate between 1.5 and 14 gNH<sub>4</sub><sup>+</sup>-N/l (Drosg et al., 2013; Möller and Müller, 2012). The dissolved P mineralised during AD is associated with suspended solids in the digestate (Möller and Müller, 2012) which could explain why most of the P in DOA was present in the water-extractable phase.

DOA and FOA showed higher C, N, and P concentrations in the water-extractable phase than COA. In FOA, the water-extractable N was 48% lower than in MOR, losing soluble forms of N to the leachate, which concentrations were ~1.8 g N/l. COA had two orders of magnitude less nitrogen available in water than digestate and 86% less than in MOR.

During COM, nutrients (e.g., K, P, Na and Ca) are lost in the leachate. Eghball et al. (1997) reported losses higher than 6.5% of total K and Na from composting windrows as leachate during rainfall. Tiquia et al. (2002) registered higher losses ranging from 23 to 39% for P, 20–52% for K, 32 to 53% for Na, and up to 2% for Ca. The previous results agree with those obtained in COA.

In DOA, all nutrients were preserved, but just as P, other ions like Ca, Mg and K were not entirely present in the digestate. A possible

explanation could be the formation of struvite or calcium phosphate minerals commonly reported during anaerobic digestion (Möller & Müller, 2012). The presence of rocky-like materials was observed at the bottom of the reactor, confirming this possibility.

#### 4.2. Assessment of the OAs as fertiliser and soil improver

As a solid organic fertiliser, the concentrations of primary and secondary macronutrients and their chemical forms are the most relevant characteristic (Rowell et al., 2001). The total N content in COA was relatively low (1.6%). To be considered a solid organic fertiliser, nitrogen content should be at least 2.5 % by mass of total nitrogen (N), according to EU (2019). It is not uncommon for composts to have lower N content than digestates (van der Wurff et al., 2016). As a result, COA cannot be regarded as N fertiliser. FOA and DOA can be classified as solid organic fertilisers. They can even contain more than one primary nutrient, including not only N but also P and K, thereby meeting the concentrations required for such denomination (EU, 2019). Additionally, considering the N-forms and nutrients available, digestates are appropriate for short and medium-term fertilisation (Ehmann et al., 2018).

Digestates have different compositions in their solid and liquid fractions. For this reason, it is possible to divide the end product into these two fractions and give them different uses (Möller and Müller, 2012; Wellinger et al., 2013). We saw that 95% of the nitrogen of the end-product was water-extractable, with 71% of this as ammonium (Fig. 2). If we separate the liquid fraction of DOA, the N concentrations are high enough to be labelled as liquid organic fertiliser, according to EU(2019).

As a soil enhancer/improver, it is expected that the organic matrices contribute to SOM turnover, as this latter process influences biological, chemical, and physical soil characteristics (Cotrufo et al., 2013; Lal, 2001). The most recent conceptualisations about SOM stabilisation suggest that the most stable carbon in soil comes from the accumulation of microbial residues such as MAOM and aggregates (Angst et al., 2021; Cotrufo et al., 2013; Miltner et al., 2012). Therefore, a high-quality OA that targets to increase stable SOM should improve the microbial substrate use efficiency and the build-up of microbial biomass (Cotrufo et al., 2013). In the case of pre-treated OAs, this will be determined by: 1) the availability of C, N, P and other nutrients, 2) low carbon to nutrient ratios (i.e., C:N of 6–24 as suggested in section 2.4.), and 3) low concentrations of biochemically recalcitrant compounds.

According to the regulation (EU) 2019/1009, an organic soil improver should contain at least 7.5% OC and have 20 % or more dry matter. In this aspect, digestate should be dried/fractionated to be classified as such (Table 3). The fermented product and digestate had similar carbon contents (Table 3). However, it is expected that the C present in digestate is more readily available for microorganism consumption, given that 61% is present in the liquid phase with higher

concentrations of organic acids than LAF (Table 3).

Usually, most P and C-fibres remain in the solid fraction of digestate (Möller and Müller, 2012; Wellinger et al., 2013). The C present in the fibres provides short- and long-term C sources. The solid fraction of the digestate (11%) was the smallest among all the treatments (Table 3). Nevertheless, it could be separated from the liquid fraction and used alone for soil enhancement due to the relatively higher C content, even without the C present in the liquid phase (46% higher than total C in compost). The fermented product increased the amount of water-extractable C by 146% compared to the raw material. It is one order of magnitude higher than water-extractable C in compost. This fact positioned the fermented product as the second-best resource of readily available carbon, followed by raw material (initial substrate).

It is essential to remember that these results will differ when using different substrates, operational conditions, and types of reactors. Nonetheless, we may infer that for the same initial substrate and without any organic supplement, the technology effect probably will not change the general trends described here because of thermodynamic constraints related to metabolic pathways. Further research on how the characteristics of the readily available fractions change with different initial substrates could improve the development of OAs. Also, mass balances on the production of OAs from high-quality biowaste should be incorporated to improve resource management in a developing circular economy that targets preserving not only carbon but also nitrogen and phosphorus. In a follow-up study, we will report the effects of these OAs on the development of soil properties.

## 5. Conclusion

Our study showed that the initial substrate (model-biowaste) undergoes significant physicochemical transformations when processed using anaerobic digestion, composting, and lactic acid fermentation, resulting in distinct end-products (organic amendments). Technologies that use reducing conditions (anaerobic digestion and lactic-acid fermentation) preserved C, N and P better than technologies that use oxidising conditions until organic amendment stabilisation (composting). In addition, technologies that use reducing conditions also influence the availability of C, N, P, and other nutrients in their end-products by making them more accessible in the water-extractable fraction (1 to 2 orders of magnitude bigger than in composting). These higher concentrations of readily available carbon and nutrients could increase soil C stabilisation by stimulating soil microbial biomass growth. Our findings agree with our hypothesis, indicating that we can modify an initial substrate by selecting a pre-treatment technology to acquire specific characteristics.

A better understanding of how different technologies alter the nature of initial substrates during the production of OAs could be fundamental to establishing strategies to preserve C and other essential macronutrients like N and P in soil. Furthermore, selecting C-rich OAs may be pivotal to maximising stable organic matter in soil and reducing greenhouse-gas emissions.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2022.06.005>.

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