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Nitrogen Isotope Fractionation during Composting of Sewage and Agri-Food Sludge with Pruning Waste

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Abstract: This work studies the changes in δ^{15} N signature during the composting of sewage sludge (MS) and agri-food sludge (AS) with different bulking agents and the potential relationships between the changes in this parameter with both the source of the organic matter used as a raw material and the stability of the end-materials obtained. For this, eleven mixtures were prepared in commercial composting conditions using sewage sludge from municipal wastewater in a range of 60–85% (on a fresh weight basis) or agri-food sludge in a range of 65–75%, mixed with seven different pruning wastes as bulking agents. The thermal profile was monitored throughout the composting processes, and the main physico-chemical and chemical parameters were determined. The results obtained confirmed a correct development of the composting processes, observing slight differences in process evolution depending on the type of sludge used. The composts obtained showed adequate contents of nitrogen, phosphorus and potassium (NPK) and reached a good degree of maturity. Significant differences in the specific nitrogen isotopic composition were found in the initial materials. Moreover, the results suggest that the type of sludge had a main contribution in the δ^{15} N value of the initial composting mixtures. The use of δ^{15} N is recommended as an indicator of the composting process, especially to evaluate N dynamics, and the quality of the resultant compost.

Keywords: N isotopic composition; compost; biosolid; agri-food waste; organic matter

1. Introduction

Composting constitutes one of the main treatments for the management and valorisation of a wide typology of organic wastes. Typical organic wastes used as raw materials in composting are animal manures and crop wastes [1–3], agro-industrial wastes [4,5], municipal solid wastes [6,7], sewage sludge [8,9] and agri-food sludge [10,11]. Moreover, there is extensive literature on the benefits of the use of compost as soil amendment, maintaining soil organic matter contents, sustaining crop productivity and improving soil quality, especially in the long-term, due to the slow release of nutrients [12,13]. Compost is one of the main sources of nitrogen, especially in agricultural systems, such as organic farming, where only organic amendments and specific fertilisers of natural origin are allowed [14]. However, currently there are no standard analytical techniques to identify the type of fertilisers used, which makes it difficult to detect the use of prohibited synthetic fertilisers in organic farming systems [14,15]. In this context, in the last decade, the use of the ¹⁵N natural



abundance ($\delta^{15}N$), based on the use of the N isotope ratio ($^{15}N/^{14}N$) has emerged as a promising technique for discriminating between organically and conventionally grown crops [14,16]. The method is based on the higher $\delta^{15}N$ values of composted materials with respect to synthetic fertilisers, because of the physical and biological processes that take place during composting, which leads to ¹⁵N isotope enrichment in favour to lighter 14 N isotope [2]. Thus, the crops obtained using compost as organic fertiliser likely contain higher δ^{15} N values in their tissues than those obtained with mineral fertilisers [16,17]. Accordingly, the ¹⁵N signature will be different in the soil and crops of a farming system, depending on the type of N source, reflecting the inputs of N fertilisers with different δ^{15} N values [15,18]. Nevertheless, very few studies have dealt with δ^{15} N dynamics during the composting and especially regarding the influence of the feedstock. Lynch et al. [19] studied the changes in $\delta^{15}N$ during the composting of corn silage, reporting an increase in δ^{15} N values and a reduction in the heterogeneity of the bulk organic material ¹⁵N signature. Kim et al. [2] evaluated the temporal changes during composting of livestock manure and the effect of the bedding material used, observing a clear effect of the bedding material on the δ^{15} N of the composts obtained. Therefore, the aims of this work were twofold: (i) to examine the dynamics of the $\delta^{15}N$ signature during composting of municipal sewage sludge (MS) and agri-food sludge (AS), mixed with different bulking agents; (ii) to establish potential relationships between δ^{15} N signature and the properties of both the feedstock and the corresponding compost.

2. Materials and Methods

2.1. Composting Procedure

Eleven different commercial-scaled piles in trapezoidal form (25 m^3 each pile) were prepared and composted using the passive windrow composting system with periodical mechanical turning. The main raw materials used in the elaboration of these composting mixtures were sewage sludge (MS) and agri-food sludge (AS), respectively. Four different MS were collected at different municipal wastewater treatment plants of Alicante (Spain). In all cases, the different MS samples were produced by aerobic treatment of wastewater, followed by a step of stabilization in anaerobic conditions. The four AS samples used come from the treatment of the wastewaters generated in two agri-food industries-i.e., pear and strawberry processing plant and dairy desserts-both located in Murcia (Spain). The treatment for these wastes consisted of three stages: a first stage of flotation by cavitation air supply (CAF), a second stage of denitrification in an anoxic reactor, and a later treatment in USBF reactor under aerobic conditions by forced aeration. The bulking agents used were different types of pruning wastes produced after park maintenance activities in the urban area of the municipality of Orihuela (Spain). These wastes were the following: tipa (*Tipuana tipu*) waste (TW); palm (*Phoenix dactylifera* L.) leaves (LP) and trunk (TP1, TP2, TP3 and TP4); giant reed (Arundo donax L.) waste (AD); cotton gin (CG); mulberry (Morus alba L.) waste (MA) and mixed garden pruning (GP). All these wastes were homogenised and crushed to <4 cm particle size, prior to the preparation of the composting mixtures in order to improve the homogeneity and aeration into the heaps. Table 1 summarises the mixtures of feedstocks and bulking agents on a fresh weight basis:

Moisture and temperature (piles and ambient) were periodically monitored throughout the composting process. Pile moisture was maintained at levels not less than 40%, while the temperature was registered using multiple probes connected to data loggers (HOBO-Data Logger). Four samples were conducted during the process (initial stage and maturity) obtaining the samples after mixing seven sub-samples collected the whole profile of each mixture and from seven sites [20]. Each pooled sample was divided into two fractions: the first fraction was dried at 105 °C for 24 h to determine the moisture contents, and the second fraction was dried at 45 °C, and ground using an agate ball mill (Frischt Pulverisette 3 SPARTAN) to obtain a sample of particle size < 0.5 mm, which was stored for later analyses.

Composting Pile	Raw Material	Bulking Agent	Ratio (Dry Weight)
1	68% (MS2)	32% (LP)	36:64
2	60% (MS2)	40% (AD)	30:70
3	70% (MS2)	30% (CG)	38:62
4	75% (MS2)	25% (GP)	54:46
5	75% (MS3)	25% (TW)	41:59
6	85% (MS3)	15% (MA)	55:45
7	68% (MS4)	32% (TP1)	30:70
8	66% (AS1)	34% (TP2)	28:72
9	68% (AS2)	32% (TP3)	24:76
10	75% (AS3)	25% (TP4)	35:65
11	75% (AS4)	25% (TP4)	34:66

Table 1. Proportions of feedstocks and bulking agents in the composting treatments.

Raw material: municipal sewage sludge (MS1, MS2, MS3 and MS4), and agri-food sludge (AS1 and AS2 from fruit-processing plants, AS2 and AS4 from dairy desserts). LP: palm (*Phoenix dactylifera* L.) leaves; AD: giant reed (*Arundo donax* L.) waste; GP: mixed garden pruning; CG: cotton gin; TW: tipa (*Tipuana tipu*) waste; MA: mulberry (*Morus alba* L.) waste; TP: palm (*Phoenix dactylifera* L.) trunk.

2.2. Chemical Determinations

The physico-chemical and chemical parameters studied in the initial materials and composting samples were determined as follows: electrical conductivity (EC) and pH were measured in a 1:10 water-soluble extract (w/v), whereas organic matter (OM) content was determined by mass loss on ignition at 430 °C for 24 h. Total organic carbon (TOC) and total nitrogen (TN) were quantified using an automatic elemental microanalyser (EuroVector Elemental Analyser, Milano, Italy). The cation exchange capacity (CEC) was determined according to the method used by Bustamante et al. [20]. Na and K were measured by flame photometry (Jenway PFP7 Flame Photometer) after acid digestion (HNO₃/HClO₄, 1:4 v/v) of the sample, whereas P concentration was colorimetrically determined according to the method described in Bustamante et al. [20]. The phytotoxicity of composts was also determined by the germination index (GI) according to the method described by Zucconi et al. [21]. All the determinations were conducted in triplicate. TN losses were determined following Equation (1), where N₁ and N₂ are the initial and final TN contents, respectively, and X₁ and X₂ represent the initial and final ash contents [22]:

$$\Gamma N - \log \left(\frac{1}{2} \right) = 100 - 100 \left[\frac{(X_1 N_2)}{(X_2 N_1)} \right], \tag{1}$$

2.3. Determination of $\delta^{15}N$ Signature in the Initial and Composting Materials

The samples were weighed (1–5 mg) with an accuracy of 0.001 mg (Mettler Toledo MX5), placed in tin capsules and measured using an EA1108 analyser (Carlo Erba Instruments) coupled to a MAT253 isotopic mass spectrometer (ThermoFinnigan) through a ConfloII interface. The molecules produced during the sample combustion are ionized and separated according of the masses of the constituent isotopes under the action of a magnetic field. The results of δ^{15} N values are expressed in ‰ relative to atmospheric air. The accuracy of the method, evaluated by using the external standard acetanilide, resulted in ±0.15‰ (n = 10).

2.4. Statistical Analyses

The viability of the composting process was evaluated through the Quadratic Exothermic Index (EXI²), which was calculated as the quadratic sum of the daily difference between the temperature inside the pile and that in the surrounding environment during the bio-oxidative phase of composting [9]. The statistical analysis of the data was conducted using ANOVA followed by the least significant difference (LSD) test at p < 0.05. The Tukey-b test was used to check the statistically significant differences among feedstocks and composts in relation to the physico-chemical and chemical properties. The normality and homogeneity of the variances were checked using the Shapiro–Wilk and Levene

test, respectively. In addition, factorial analysis (FA) was carried out to interpret the data set of initial mixtures and composted materials, initially considering all the parameters studied. However, the best results were obtained considering the following parameters: EC, OM, CEC, P, δ^{15} N, TN and TOC. All statistical tests were conducted with the IBM SPSS 25 software package.

3. Results and Discussion

3.1. Chemical Characteristics and Isotopic Composition ($\delta^{15}N$) of the Initial Materials

Tables 2 and 3 summarise the main chemical characteristics of the raw materials and bulking agents used in the composting mixtures. The different samples of MS and AS showed similar physico-chemical characteristics, with an acidic pH and electrical conductivity values varying between 3.4–5.8 dS/m (Table 2). However, AS samples showed higher contents of the organic matter fraction (69.4%) than MS samples (55.8%). Conversely, agri-food sludge samples had lower concentrations of N and K than MS samples and thus, lower δ^{15} N mean values than MS samples (7.6 and 4.9‰, respectively). Moreover, the mean values of total nitrogen (TN) found in all the sludge samples were lower than those reported by Rigby et al. [23] in a review about biosolids from mesophilic anaerobic digestion treatment (7.5% TN). This discrepancy could be due to several sources of variation: (1) the source of N, (2) the proportion of N derived from the degradation of organic matter [24], and (3) the techniques used for producing and stabilizing each sludge. In our study, the higher $\delta^{15}N$ mean values observed in the MS samples compared to AS samples could be explained by the stabilization technique used in each type of sludge. All the AS samples used in this study were stabilized in aerobic treatments. However, the stabilization of the MS samples was performed by anaerobic treatment, which could favour reduction conditions and, thus, the biological denitrification process. Consequently, the nitrogen is released as atmospheric N₂ enriched in ¹⁴N and the values of δ^{15} N in the remaining material increase [25]. Aside from AS3 (2.7‰), the range of the δ^{15} N values determined in our sludge samples (6.1-8.5‰) was close to that found in other composting studies with N-rich organic wastes, such as livestock manures ($\delta^{15}N = 5.3$ and 7.2%) [26]. Similarly, Choi et al. [27] and Kim et al. [2] reported close values of δ^{15} N for cattle manure (7.5 and 7.9‰, respectively).

Table 2. Main physico-chemical and chemical characteristics of the raw materials used in the composting mixtures (data on a dry weight basis).

Raw Material	pН	EC (dS/m)	OM (%)	TOC (%)	TN (%)	δ^{15} N (‰)	Ratio C/N	K (g/kg)	P (g/kg)	Na (g/kg)
MS1	6.1	4.4	66.8	43.6	5.5	6.9	7.9	26.2	2.43	3.73
MS2	6.8	4.8	47.5	31.1	4.2	7.6	7.4	36.0	2.73	3.03
MS3	5.1	5.0	50.8	36.0	5.2	7.4	6.9	30.0	2.58	2.58
MS4	4.9	5.8	58.1	40.1	7.0	8.5	5.8	25.1	4.72	2.28
Mean \pm SD	5.7 ± 0.8	5.0 ± 0.6	55.8 ± 8.5	37.7 ± 5.4	5.5 ± 1.1	7.6 ± 0.6	7.0 ± 0.9	29.3 ± 5.0	3.12 ± 1.0	2.90 ± 0.6
AS1	5.6	5.5	81.4	50.5	6.7	4.6	7.5	17.4	6.88	4.54
AS2	7.2	5.1	52.4	34.5	3.3	6.1	10.4	5.87	5.68	5.68
AS3	5.5	3.6	69.0	52.3	4.9	2.7	10.4	6.07	4.34	4.34
AS4	5.4	3.4	74.7	44.8	4.4	6.2	10.3	3.98	5.45	5.45
Mean \pm SD	5.9 ± 0.8	4.4 ± 1.0	69.4 ± 12.4	45.5 ± 8.0	4.8 ± 1.4	$4.9 \pm 1.6 *$	$9.7 \pm 1.4 *$	$8.3 \pm 6.1 *$	$5.59 \pm 1.0 *$	5.00 ± 0.6 *

MS: sewage sludge; AS: agri-food sludge. EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; TN: total nitrogen. * p < 0.05, *t*-Student test. SD: standard deviation.

Regarding the selected characteristics of the bulking agents (Table 3), all the materials showed pH values close to neutrality; ranging from 5.5 (mulberry waste) to 7.3 (cotton gin waste). The electrical conductivity showed a larger variability range, with values from 1.4 dS/m in MA to >7 dS/m in the palm trunk wastes (PT). However, the organic matter contents were similar in the bulking materials (70–92%). In general, the concentrations of the macroelements studied (N, P, K and Na) did not differ much among them, except for the low TN values found in MA (0.7%) and the low P concentrations of MA (2.3 g/kg) and TW (3.2 g/kg). The δ^{15} N mean value of pruning waste (8.0%) was close to the values reported in previous studies. For example, Santiago et al. [28] found a similar value in a study about C₃ metabolism bush plants (7.7%). However, Barbanti et al. [3] reported 2.05% δ^{15} N in C₄ and CAM

photosynthetic pathway metabolism plants, which was related to a more effective use of N, linked to mechanisms of C assimilation by tissues. In our study, the lowest value of δ^{15} N corresponded to TW (2.6‰), which is in agreement with the results found by Santiago et al. [28] in leguminous species trees. It is postulated that the lower ¹⁵N natural abundance in leguminous species is due to symbiotic associations (root nodules) with *Rhizobium* group bacteria, which are capable of fixing atmospherics N₂. Finally, it is interesting to remark the differences observed in the isotopic composition between the palm trunk samples (7.8, 10.5 and 8.5‰) and of the palm leaves (12.2‰). This tissue-specific difference in δ^{15} N has also been documented in previous studies [24], and it is explained by tissue N redistribution. In both evergreen and deciduous species (caducifolias), the translocation of N from senescent leaves to new tissues increases the δ^{15} N values during the assimilation of nitrate in leaf [29].

	pН	EC (dS/m)	OM (%)	TOC (%)	TN (%)	δ ¹⁵ N (‰)	Ratio C/N	K (g/kg)	P (g/kg)	Na (g/kg)
LP	6.6	5.1	91.9	45.1	2.6	12.2	18.0	2.1	14.6	6.8
AD	6.6	3.7	74.8	34.8	2.4	6.9	14.3	1.4	9.1	2.4
CG	7.3	4.5	71.4	39.3	2.3	7.6	17.1	3.1	17.7	3.0
GP	6.9	5.8	69.3	30.6	2.4	8.0	13.1	7.0	17.2	4.7
TW	5.7	2.2	88.9	43.7	1.6	2.6	27.7	2.1	3.2	2.0
MA	5.5	1.4	94.0	43.9	0.7	6.1	61.1	1.9	2.3	1.5
TP1	6.7	6.6	83.2	40.4	2.0	7.8	20.9	1.1	14.2	8.5
TP2	5.7	7.8	80.2	37.7	1.3	9.4	28.9	1.6	6.4	8.3
TP3	6.5	7.4	88.0	41.4	1.2	10.5	33.7	2.7	16.2	4.5
TP4	6.1	13.1	85.5	38.9	1.3	8.5	31.7	1.3	28.5	12.3
Mean \pm SD	6.4 ± 0.6	5.8 ± 3.3	82.7 ± 8.5	39.6 ± 4.4	1.8 ± 0.6	8.0 ± 2.6	26.7 ± 14.1	2.4 ± 1.7	12.9 ± 8.0	5.4 ± 3.5

Table 3. Main physico-chemical and chemical characteristics of the bulking agents used in the composting mixtures (dry weight basis).

LP: palm leaves (*Phoenix dactylifera* L.); AD: giant reed (*Arundo donax* L.) waste; CG: cotton gin; GP: mixed garden pruning; TW: tipa (*Tipuana tipu*) waste; MA: mulberry (*Morus alba* L.) waste; TP: palm trunk (*Phoenix dactylifera* L.) waste. EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; TN: total nitrogen. SD: standard deviation.

3.2. Thermal Development of the Composting Mixtures

The temperature in all the composting mixtures increased quickly, reaching thermophilic values (>40 °C) in the first week, these values lasting more than 30 days in all piles (Figure 1a,b). In general, the mixtures with MS showed temperature values >65 °C during a longer period than those with AS, although some of these piles showed higher temperature values. In addition, pile 5, containing MS, and pile 11, containing AS, remained more than 100 days with thermophilic temperature values. With this temperature dynamics, the Quadratic Exothermic Index (EXI²) (Vico et al., 2018) was used to describe, in detail, the exothermic behaviour of the composting mixtures. This parameter revealed marked differences in the exothermic behaviour between piles (Figure 2a,b). Pile 3 (MS and CG) and pile 8 (AS and PT) showed a faster increase in the temperature at the beginning of the process (Figure 2a,b), thus defining a short bio-oxidative phase with higher accumulated values of the index EXI² at the end of this composting phase (Table 4). Some studies have reported the beneficial effects of adding bulking agents, such as cotton gin and palm tree wastes to the composting heap [1,9]. These materials generally provide an adequate physical structure in the mixture, thereby affecting the temperature reached during composting, requiring less energy to achieve maximum temperature values into the windrow. In general, the temperature of all the composting piles dropped to values close to ambient values after 45–50 days of the process, which corresponds to unaltered, high values of EXI² until to the end of the process (Figure 2a,b). The mean EXI² values were close for MS and AS piles, but the former was kept at temperature values >60 °C for a longer period than the latter, indicating that the thermophilic stage was more intensive and shorter for MS composts, and was most progressive in AS composts. In addition, the results obtained regarding the thermal behaviour in all the piles suggest that the maximum pathogen reduction is ensured in all the composting mixtures [30].



Figure 1. (a) Temperature profiles in the composting mixtures derived from sewage sludge (MS); (b) Temperature profiles in the composting mixtures derived from agri-food sludge (AS).



Figure 2. (a) Evolution of the cumulative values of EXI² during the bio-oxidative phase in the sewage sludge-derived composting mixtures; (b) Evolution of the cumulative values of EXI² during the bio-oxidative phase in the agri-food sludge-derived composting mixtures.

	Index EXI ² (°C ²)	Ratio Days in Bio-Oxidative Phase/Days with Temperature >40 °C	Ratio EXI ² /Days in Bio-Oxidative Phase	Days > 60 °C
Pile 1	50,535	107/79	472	13
Pile 2	52,264	68/37	769	5
Pile 3	112,255	71/58	1581	40
Pile 4	79,126	71/46	1114	46
Pile 5	75,035	112/110	670	112
Pile 6	61,654	109/99	566	105
Pile 7	65,904	81/78	814	78
Pile 8	108,673	75/56	1449	56
Pile 9	54,746	69/34	793	34
Pile 10	43,856	100/68	439	68
Pile 11	59,408	134/109	443	109

Table 4. Indicators of the exothermic behaviour of the composting mixtures.

EXI²: quadratic exothermic index (quadratic sum of the daily difference between the average temperature of the pile and the ambient temperature). Piles 1–7, mainly constituted by sewage sludge; Piles 8–11, mainly constituted by agri-food sludge.

3.3. Composting Process: Effect on ¹⁵N Natural Abundance

Figure 3 shows δ^{15} N variations in the raw materials, the initial composting mixtures and the composts obtained. There was a clear differentiation among the different groups of materials, and especially, between the initial composting mixtures and the composting end-products, the mature composts having the highest median δ^{15} N values. This finding could be due to the N losses (preferentially ¹⁴N)—e.g., ammonia volatilization—during the composting process, which produces an enrichment in ¹⁵N in the composting end-products. Moreover, the highest degree of humification found in the final composts can also influence the δ^{15} N values. Kramer et al. [31] found that, when the humified carbon fraction increased in aliphacity (ratio of unsubstituted aliphatics to carbohydrates), the δ^{15} N value also increased. This relationship suggests that the ¹⁵N abundance is low when the carbon organic materials are mainly present in labile forms.



Figure 3. Natural δ^{15} N values (‰) in the initial materials, initial composting mixtures and mature composts. The vertical lines of rectangles represent the lower and upper quartiles of distributions. The middle lines inside the rectangles represent median values.

Total nitrogen losses and total nitrogen (TN) concentrations were also studied in the composting mixtures, trying to establish a relationship between these parameters and the values of δ^{15} N in the composts at the beginning and end of the process (Table 5 and Figure 4). In addition, factorial analysis (FA) of the data corresponding to the parameters selected (EC, OM, P, TOC, δ^{15} N, TN and CEC) of the initial mixtures and composted materials was carried out. According to the mass balance, the N losses showed a wide range of values. Low N losses (<10%) were accounted for compost 2 (2.5%), compost 4 (0.9%), compost 7 (0.9%) and compost 10 (4.8%) (Table 5). However, in the composts 1, 5, 6, 8, 9 and 11, N losses were similar to those found in previous studies. Guo et al. [32] reported N losses from 18 to 46% when composted pig manure and sawdust at different C/N ratio, and Sáez et al. [33] observed N losses of 44% during the composting of the solid phase of pig manure with cereal straw. Previous studies have documented the relationship between ¹⁵N abundance and the N loss during composting [2,19,34]. Particularly, Kim et al. [2] examined the changes in N isotope compositions during composting of livestock manure. They concluded that the ¹⁵N abundance increased during composting due to denitrification and ammonia volatilization processes, this last process being the main mechanism of N loss during composting. In addition, the chemical and/or biological N immobilization can contribute to the increase in δ^{15} N during composting [2]. Kramer et al. [31] reported a contrasted trend in δ^{15} N, observing an increase at the same time that the humification process increased and mainly attributed to microbial re-synthesis of polymerized compounds. The strong increase in δ^{15} N seems to indicate that the humification was primarily due to microbial processing rather than to preferential degradation of labile constituents.

	TN Loss (%)	δ ¹⁵ N (‰) Initial Mixture	δ^{15} N (‰) Mature Compost	Isotope Discrimination (‰)
Compost 1	46.1	5.46	7.19	1.73
Compost 2	2.51	6.66	9.51	2.85
Compost 3	13.5	6.56	11.0	4.44
Compost 4	0.96	6.96	9.75	2.79
Compost 5	54.1	7.40	10.6	3.20
Compost 6	35.9	6.65	12.3	5.65
Compost 7	0.91	9.76	13.9	4.14
Compost 8	27.1	7.16	11.0	3.84
Compost 9	24.6	7.10	8.58	1.48
Compost 10	4.76	6.15	7.39	1.24
Compost 11	13 5	10.5	10.7	0.20

Table 5. Total nitrogen losses, δ^{15} N values of the initial composting mixtures and final composts and isotope discrimination.

TN: total nitrogen. Composts 1–7, mainly constituted by sewage sludge; Composts 8–11, mainly constituted by agri-food sludge.



Figure 4. Total nitrogen concentration (%) in the initial composting mixtures and final composts.

Regarding TN concentrations (Figure 4), a trend in TN content was observed, which was consistent in all the composting piles, with an average increase in the concentration values for MS composts and AS compost (27.4 and 27.5%, respectively) (Figure 4). In the final composts, in general, MS composts showed higher TN concentrations than AS composts, probably due to the greater N contents in MS samples than in AS samples.

Regarding δ^{15} N values, this parameter also showed an increasing pattern in all the composting piles, but this increase was lower in AS composts than in MS composts (1.7 and 3.5‰, respectively). Thus, the trend of TN concentration during composting seems not to be directly related to δ^{15} N values, since the values of these parameter did not show the same trend in the final composts as TN. In this sense, different N concentrations and their δ^{15} N values in the raw materials may directly influence the δ^{15} N values of the composting mixture through δ^{15} N dilution or enrichment [19]. Kim et al. [2] observed a variable effect dilution/enrichment in the initial mixture prepared of cattle feedlot manure with rice hull and sawdust as bulking agents. These authors suggest that the relative contribution of ¹⁵N-depleted N from the bulking agent materials (isotope dilution) was greater in rice hull than in sawdust, resulting in a lower ¹⁵N of total N in rice hull compost. In our study, a different effect in the

 δ^{15} N values of the initial mixtures has been observed. In all the mixtures prepared with MS, an effect of isotope dilution was observed when the different bulking agent was added, while in all the piles prepared with AS was observed an isotope enrichment. Therefore, these results suggest that the type of sludge had a main contribution in δ^{15} N values in the initial composting mixtures.

Factorial analysis was conducted with the data of the selected parameters (EC, OM, TOC, CEC, P, δ^{15} N and TN) corresponding to the initial composting mixtures and mature composts. For this analysis, three factors (F1, F2 and F3) were used and explained 83.5% of the total explained variance, where F1 explained 41.6% of the variance, F2 explained 23.9% and F3 explained 18% of the variance. Figure 5 shows the plot of the first and second factors for all the composting mixtures, corresponding to the initial and maturity stages of composting. FA allows to observe an important differentiation among groups, depending on the degree of stabilization of the material and the type of sludge used. Additionally, the parameter δ^{15} N was positively correlated with TN and CEC, the latter being a parameter strongly associated to the humification processes during composting. This finding agrees with the previous data on the correlation between the increasing trend of δ^{15} N and the humification process [31].



Figure 5. Plot of first and second factor extracted from factor analysis of initial composting mixtures and mature composts. Arrows correspond to the projection of the variables studied. EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; CEC: cation exchange capacity; TN: total nitrogen; MS: sewage sludge; AS: agri-food sludge.

3.4. Composting Process: Characteristics of the Composts

Table 6 summarises the selected chemical properties of the final composts. In general, AS- and MS-derived composts presented pH values close to neutrality. However, AS-derived composts had higher electrical conductivity (EC) values than MS-derived composts, despite of the higher EC values observed in MS samples. This fact could be due to the characteristics of the bulking agent used in AS pile (palm trunk), which showed the highest EC values (Table 3). Probably due to these higher contents in soluble salts, which may negatively affect the germination and the early stage of plant growth [35], these composts reached lower values of the germination index (GI) than MS compost at the end of the process (Table 6). However, the GI values reached in all the composts of our study indicate absence of phytotoxicity [21]. All the composts had OM concentrations higher than 35%, AS composts showing in general the highest OM contents, with similar values to those obtained

by Vico et al. [9] and Morales et al. [11] during the co-composting of agri-food sludge. CEC and CEC/TOC ratio are parameters usually considered to evaluate compost maturity [36,37]. The values of the CEC and CEC/TOC ratio differed greatly among the obtained composts (Table 6). In general, MS composts showed higher mean values of these parameters than AS composts, although in all cases both parameters showed values higher than those established as reference values in the literature [38]. In the MS mixtures, the humification processes were probably more intense, which would lead to the formation of functional groups responsible for the cation exchange capacity [11]. Concerning the P and K concentrations, the values observed were similar to those found in other studies of composting using sewage sludge [39,40] and/or agri-food sludge [11] and higher than those reported by Awasthi et al. [41] in compost of sewage sludge with biochar. The AS composts presented the highest K content according to the results previously found in other studies of composting using agri-food organic materials [9,11].

	pН	EC (ds/m)	OM (%)	P (%)	K (%)	CEC (meq/100 g OM)	CEC (meq/g TOC)	GI (%)
Compost 1	6.1	7.8	61.5	1.6	1.4	135	2.7	68
Compost 2	6.7	3.4	44.3	1.8	1.1	99	2.0	131
Compost 3	7.1	8.1	47.8	3.3	1.8	135	2.5	106
Compost 4	7.2	8.2	40.7	2.7	1.6	166	2.7	81
Compost 5	5.9	4.9	56.8	3.1	1.1	194	3.8	77
Compost 6	5.7	5.5	59.6	3.3	0.9	159	3.1	77
Compost 7	6.0	7.2	45.9	2.1	1.3	152	3.1	69
Compost 8	6.9	6.7	42.7	1.6	1.6	82	1.5	72
Compost 9	7.0	12.7	76.0	2.4	2.6	117	2.2	94
Compost 10	6.7	13.1	74.9	2.5	2.4	91	1.9	71
Compost 11	7.2	12.3	71.2	2.7	2.4	120	2.4	80
MS-compost ¹	6.1	6.5	50.9	2.6	1.3	148	2.8	87
AS-compost ²	6.7	11.2	66.2	2.3	2.3	102	2.0	79

Table 6. Chemical characteristics and maturity properties of the final composts.

¹ Mean value for all the sewage sludge-derived composts. ² Mean value for all the agri-food sludge derived-composts. EC: electrical conductivity; OM: organic matter; CEC: cation exchange capacity; GI: germination index. Composts 1–7, mainly constituted by sewage sludge; Composts 8–11, mainly constituted by agri-food sludge.

4. Conclusions

The results in this study evidence that the use of ¹⁵N abundance in composting studies may be a suitable parameter to monitor the N losses throughout the composting process. Moreover, the results obtained show higher values of δ^{15} N in the sewage sludge samples compared to agri-food sludge samples, while the bulking agents showed a wide variation in the δ^{15} N values, even between wastes within the same plant species. The incorporation of the bulking agents to the composting mixtures produced an isotope dilution in the mixtures with sewage sludge, but an isotope enrichment in the agri-food derived mixtures, which indicates that the type of sludge had a main contribution in δ^{15} N during composting in relation to total nitrogen contents seems to indicate that there is isotopic discrimination and confirms that exist other processes associated to this parameter, such as the humification processes during composting. Therefore, further studies concerning the changes of δ^{15} N in the different stages of the composting should be carried out to increase the knowledge concerning the nitrogen dynamics during the composting process.

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