#### **ORIGINAL PAPER**



# The Impact of Raw and Composted Food Waste Anaerobic Digestates on Soil Organic Carbon Management: A Pot Study

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#### Abstract

**Purpose** Ever increasing food waste production has promoted anaerobic digestion and composting for its proper management, producing a relevant amount of recycled organic waste (OW) for possible agricultural uses. However, little is known regarding soil carbon management using this type of OW.

**Methods** In this study, an anaerobic digestate from the wet digestion of food waste (WET<sub>D</sub>), and one from the dry-batch process (DRY<sub>D</sub>), along with their respective composts (WET<sub>C</sub> and DRY<sub>C</sub>), were utilized in a pot test over two growing cycles (84 + 84 days), with and without mineral nitrogen (N) fertilization, and were compared with a bio-waste compost (BW<sub>C</sub>) and a chemical reference (Chem). At the end of the two growth cycles (days 84 and 168), the ryegrass dry biomass (DW) and the N uptake were assessed.

**Results** The pot soil was analyzed for soil organic carbon (SOC) and the potassium permanganate (KMnO<sub>4</sub>) oxidizable fraction (C<sub>L</sub>) as well as  $\delta^{13}$ C and  $\Delta^{13}$ C. At day 84, the SOC (g kg<sup>-1</sup>) was the highest in DRY<sub>D</sub> and DRY<sub>C</sub> (8.53) > WET<sub>D</sub> and WET<sub>C</sub> (7.71)=BW<sub>C</sub> (7.86) > Chem (6.68), and performed similarly at day 168. At day 84, the carbon management index (CMI) was > 100% in all the organic treatments in comparison with Chem, except for WET<sub>D</sub>. At day 168, a + 30% CMI was registered in WET<sub>D</sub> and WET<sub>C</sub> > BW<sub>C</sub> > DRY<sub>D</sub> and DRY<sub>C</sub> > Chem.

**Conclusion** This pattern was related to a generally marked  $\delta^{13}$ C depletion being confirmed by  $\Delta^{13}$ C, thus indicating the conservation of the carbon form compost, this very likely being related to the preferential lignin accumulation.

Keywords Organic Waste Recycling · Ryegrass pot test · Carbon Management Index ·  $\delta^{13}$ C Natural Abundance

#### **Novelty statement**

There is increasing interest in improving soil carbon management; to this aim, the re-utilization of recycled organic waste is often claimed to be safe, successful and sustainable. However, little research has been carried out in dealing with this topic as regards food waste which is an ever-increasing source of organic matter, possibly recycled for agricultural uses. Food waste anaerobic digestates and composts can have a homogeneous <sup>13</sup>C natural abundance signature, thus allowing the study of its fate in soil. By so doing, this study represents the first attempt to use this technique in this field. Moreover, coupled with studying the carbon management index, this study represents a first insight into the context of the rational soil carbon management in a succession of organic-mineral fertilization strategies.

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# Introduction

In recent years, there has been a growing emphasis by governments in addressing greenhouse gas (GHG) emission control and soil organic carbon (SOC) conservation/storage [1-3]. Consequently, there is an increasing interest in

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strategies for managing OW which can mitigate GHG emissions and contribute to preserving or enhancing SOC stocks [4]. The anaerobic digestion of various OWs, including animal slurry, sewage sludge, and agricultural/agro-industrial residues, has emerged as a promising approach for achieving both objectives [5]. Anaerobic digestion (AD) has been recognized for its ability to reduce GHG emissions as compared to alternative processes, such as landfilling or incineration. Simultaneously, it serves as a source of renewable energy by means of biogas production, provides nutrients for crops, and offers organic carbon for soil enrichment via the use of digestates [6, 7]. In addition to the above-mentioned OWs, there has been a recent surge in the collection of food waste which, if mismanaged (e.g., incineration or landfill), poses a significant GHG impact. Hence, anaerobic digestion becomes an attractive solution for managing food waste [8, 9]. Despite its benefits, the direct application of raw anaerobic digestates to soil comes with challenges. such as ammonia (NH<sub>2</sub>) emissions and the potential release of carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), and methane  $(CH_4)$  [10–15]. To limit these issues, a common practice involves combining AD with composting to produce biologically stable products suitable for sound soil application [9]. While the nutritional aspects of anaerobic digestates and the resulting composts have been extensively studied, there is a notable research gap concerning the fate of SOC following the agricultural use of these recycled products, particularly in the case of raw and composted anaerobic digestates from food waste [16-18]. Given the crucial role of SOC in ecosystems and the growing interest in its conservation and potential increase, understanding the soil health status has become crucial [19]. Labile carbon, assessed through weak oxidation using potassium permanganate (KMnO<sub>4</sub>), is increasingly being considered an indicator for evaluating soil health and functionality, contributing to the calculation of the carbon management index (CMI) [20, 21]. The CMI aids in determining the relative sustainability of different management options as compared to a reference system (i.e. organic vs. chemical fertilization). In the literature, authors frequently use the CMI to analyze the changes in SOC quality resulting from various management practices [22, 23]; for example, Sodhi et al. [22] have utilized this approach to analyze SOC variations following long-term organic, mineral or combined fertilization or different soil management. In addition to labile carbon, the utilization of the <sup>13</sup>C natural abundance ( $\delta^{13}$ C) tracer technique can provide valuable insights into carbon mineralization processes in soils [24, 25]. More specifically the biological process due to the microbial activity occurring during the anaerobic digestion and/or the composting of OW may reduce the inherent variability of their <sup>13</sup>C signature, thus increasing the possible success of this approach [26-28]. In addition to this,

researchers have often used the  $\Delta^{13}$ C to gain deeper understanding of the carbon mineralization process in soils [29]. This approach takes into account the fractionation processes (<sup>13</sup>C vs.<sup>12</sup>C) occurring following microbial activity, especially in the presence of a nutrient source (mainly nitrogen), such as those added with chemical fertilization in soil [30, 31]. Given that anaerobic digestates and compost are used as potential fertilizer substitutes, comparing their impact on Italian ryegrass, a nitrogen-sensitive, fast-growing species, seemed to be particularly relevant in this system [32, 33].

To investigate the effects of soil application, wet and drybatch digestates from food waste, along with their homologous composts, were utilized as fertilizers (300 kg of available N ha<sup>-1</sup>). This study, conducted through two consecutive growth cycles (84+84 days), involved a pot test on ryegrass with and without chemical nitrogen addition (180 kg ha<sup>-1</sup>). At the conclusion of each crop cycle (days 84 and 168), cumulative plant dry biomass and N-uptake were calculated. Moreover, the pot soil was analyzed for total organic carbon and its labile fraction (KMnO<sub>4</sub> oxidizable C) to assess the carbon management index. The  $\delta^{13}$ C and the  $\Delta^{13}$ C values were analyzed to provide a comprehensive understanding of the processes occurring in the soil and the fate of the organic carbon introduced through the compost.

# **Materials and methods**

### **Organic Products**

Two anaerobic digestates and two composts were compared in this study. An anaerobic digestate was collected after two weeks of thermophilic wet digestion of (100%) slurried food waste (WET<sub>D</sub>). Its homologous compost was obtained after 10 weeks of composting of the screw-pressed WET<sub>D</sub>, following the addition of green waste (25% w: w); this was called WET<sub>C</sub>. Another anaerobic digestate was collected after four weeks of mesophilic dry-batch digestion of a mixture (90% w: w) of food- and green waste (DRY<sub>D</sub>), and its homologous compost was obtained after 8 weeks of composting of DRY<sub>D</sub> with recirculation of the solid fraction as bulking agent (50% w: w); this was called DRY<sub>C</sub>. In addition to these, a bio-waste compost without the addition of anaerobic digestates (BW<sub>C</sub>) was used as an organic reference in the pot test. The pH, electrical conductivity (EC), total solids (TS) and volatile solids (VS), in addition to NH+4-N and NO-3-N, and the oxygen uptake rate (OUR) [34], were assessed on products as they were. Total organic carbon (TOC) and total nitrogen (TN) were determined using an elemental analyzer on freeze-dried and ballmilled samples. The  $\delta^{13}$ C (‰) of the products tested were determined using a coupled mass spectrometer (DELTA

Table 1 Main characteristics of the products tested

Product	pН	TS	VS	TOC	TN	C: N	NH <sup>+</sup> <sub>4</sub> -N	NO <sup>-</sup> <sub>3</sub> -N	OUR	$\delta^{13}C$
		(%)		(%)			$(mg kg^{-1})$		$(\text{mmol O}_2 \text{ kg}^{-1} \text{ VS h}^{-1})$	(‰)
WETD	8.4	24.8	58.8	31.3	3.5	9	234	59	54	-22.23
DRYD	8.9	34.0	50.1	30.2	1.6	19	244	26	64	-23.32
WET <sub>C</sub>	7.3	63.0	39.0	24.2	2.5	10	181	207	3	-26.44
DRY <sub>C</sub>	10.0	76.0	42.5	25.5	1.8	14	199	40	10	-22.13
BW <sub>C</sub>	8.4	88.6	43.6	22.2	1.3	17	101	23	2	-26.28

TS: total solids; VS: volatile solids; TOC: total organic carbon: TN: total nitrogen; C: N: carbon to nitrogen ratio;  $NH_4^+$ -N: ammonium nitrogen;  $NO_3^-N$ : nitrate nitrogen; OUR: oxygen uptake rate;  $\delta^{13}$ C: natural <sup>13</sup>C isotopic abundance. WET<sub>D</sub>: digestate from the wet digestion of food-waste; DRY<sub>D</sub>: digestate from the dry-batch digestion of food waste; WET<sub>C</sub>: compost from WET<sub>D</sub>; DRY<sub>D</sub>: compost from DRY<sub>D</sub>; BW<sub>C</sub>: reference compost from bio-waste. VS, TOC, TN, contents are expressed on the basis of TS and are the average of two replicates (CV < 5%).  $\delta^{13}$ C values are the average of three replicates (CV < 5%).

Table 2 Nutrient content in the different organic products compared in this study

Product	Р	K	Ca	Mg	S
	$mg kg^{-1}$				
WETD	9342	4995	43,388	6937	4454
DRYD	5160	5366	72,109	5450	3549
WET <sub>C</sub>	6736	5277	40,698	11,320	3206
DRYC	7826	2946	88,260	7240	3952
BW <sub>C</sub>	3925	5057	40,547	6933	3109

 $WET_D$ : digestate from the wet digestion of food waste;  $DRY_D$ : digestate from the dry-batch digestion of food waste;  $WET_C$ : compost from  $WET_D$ ;  $DRY_D$ : compost from  $DRY_D$ ;  $BW_C$ : reference compost from bio-waste. The data are expressed on TS and are the average of two replicate (CV < 5%).

Table 3 Trace element content in the different organic products compared in this study

		<u> </u>	<b>1</b>					
Product	Cd	Cr§	Hg	Ni	Cu	Zn	Pb	As <sup>§§</sup>
	$mg kg^{-1}$							
WETD	0.74	47	n.d.	25	99	211	58	1.64
DRY <sub>D</sub>	0.35	32	n.d.	13	55	122	21	2.47
WET <sub>C</sub>	0.97	102	n.d.	58	92	176	48	3.02
DRY <sub>C</sub>	0.96	91	n.d.	16	120	206	43	2.62
BW <sub>C</sub>	1.17	57	n.d.	27	126	225	72	2.34
Limits Reg. EU 2019/1009	2.00	2*	1	50	300	800	120	40*

**WET**<sub>D</sub>: digestate from the wet digestion of food waste; **DRY**<sub>D</sub>: digestate from the dry-batch digestion of food waste; **WET**<sub>C</sub>: compost from **WET**<sub>D</sub>; **DRY**<sub>D</sub>: compost from **DRY**<sub>D</sub>; **BW**<sub>C</sub>: reference compost from bio-waste. The data are expressed on TS and are the average of two replicates (CV < 5%). <sup>§</sup>Cr total; \*Cr VI. <sup>§§</sup>As total; \*As inorganic. n.d. not detectable

V Advantage; Thermo Electron Germany) and expressed according to the following equation:

$$\delta = \left[ \left( R_{sample} / R_{standard} \right) - 1 \right] \times 1000 \tag{1}$$

where  $R = {}^{13}C/{}^{12}C$ . The main characteristics of the composts compared are reported in Table 1. The nutrient content, as well as the trace element, was determined using ICP after microwave assisted acid digestion (HNO<sub>3</sub> 70% + HCl 37%) on  $\approx 250$  mg of ball-milled sample; they are reported in Tables 2 and 3.

#### Pot test

A two-stage pot experiment was conducted using soil collected from the upper layer (0-20 cm) after litter removal in a field in Bologna, Italy. The soil had the following main characteristics: pH 7.90, sand content 18.4%, clay content 39.1%, silt content 42.5%, total Kjeldahl nitrogen (TKN) 0.160%, carbon-to-nitrogen ratio (C: N) 8.3, and Olsen phosphorus (Olsen-P) 5.00 mg P kg<sup>-1</sup>. In this experiment, the four organic products (WET<sub>D</sub>, DRY<sub>D</sub>, WET<sub>C</sub>, DRY<sub>C</sub>) were applied to 1 kg of pot soil at 300 kg ha<sup>-1</sup> of available nitrogen considering a 30 cm layer (arable layer) and a bulk density of 1.3 kg dm<sup>-3</sup> (Table 1S). Furthermore, the experiment included one organic and one chemical (BW<sub>C</sub> and Chem) reference treatment at the same nitrogen rate. Two-liter pots (drilled at the bottom) pre-filled with 1 L of sand (to avoid water-logging) were filled with the amended soil and arranged in a randomized complete design with three replications.

Ryegrass was cultivated for 84 days under the following conditions: 60% water holding capacity (WHC), a 14/10hour light/dark photoperiod, and temperatures of 23°/13°C. After the initial growth period, the soil was potted for a subsequent growth season, lasting an additional 84 days. During this second cycle, the, ryegrass received nitrogen fertilization (as  $NH_4NO_3$ ) to ensure a nitrogen supply (180 kg ha<sup>-1</sup>) of available N). During the experiment, tissue samples were collected three times during both the first and the second growth cycles (every 28 days). Root samples were collected on the last day of sampling (day 84 and day 168). All the harvested tissue and root samples were dried at 70 °C in a forced-air oven until a constant weight was achieved. The dry biomasses (DWs) from the various tissue harvests were summarized. The TN content in the tissue and the root was determined using an elemental analyzer (DELTA V Advantage; Thermo Electron Germany) on ball-milled samples. The N uptake was calculated by multiplying the TN by the DW. The data were analyzed using ANOVA, and the means were separated according to the Tukey test at  $p \le 0.05$ .

# Total Organic Carbon, $\delta$ 13C and KMnO4 Oxidizable Carbon in pot soil

On soil samples collected at the end of the first (day 84) and second (day 168) growth cycles, the total organic carbon content, as well as the  $\delta^{13}$ C, were determined as previously described for the compost. In addition, the KMnO<sub>4</sub> oxidizable C (C<sub>L</sub>) content was determined on 2.5 g of pot soil according to Weil et al. [35].

The carbon management index (CMI) was obtained according to the method proposed by Blair et al. [21]:

$$CMI \ (\%) = [carbon pool index \ (CPI)] \times [lability index \ (LI)] \times 100$$
(2)

where CPI was calculated according to the following equation:

$$CPI = (C treated soil) / (C reference soil)$$
(3)

where "C treated soil" was the organic carbon (g kg<sup>-1</sup>) from the soil treated with the organic products (WET<sub>D</sub>; WET<sub>C</sub>; DRY<sub>D</sub>; DRY<sub>C</sub>; BW<sub>C</sub>), and "C reference soil" was the organic carbon (g kg<sup>-1</sup>) in the soil from Chem.

The LI was calculated according to the following equation:

$$LI = (C lability treated soil) / (C lability reference soil)$$
(4)

Where the "C lability treated soil" was the carbon lability from the soil treated with the different organic products (WET<sub>D</sub>; WET<sub>D</sub>; DRY<sub>C</sub>; DRY<sub>C</sub>, and "C lability reference soil" was the carbon lability in the soil from Chem. The C lability was expressed as the ratio of labile C (C<sub>L</sub>) to non-labile C (C<sub>NL</sub>). Non-labile C was determined to be the difference between the total C content and the C<sub>L</sub> content of the soil. The data were analyzed using ANOVA, and the means were separated according to the Tukey test at  $p \le 0.05$ .

### Results

#### **Organic Products**

Table 1 reports that WET<sub>D</sub> and DRY<sub>D</sub> had similar pH values, ranging from 8.4 to 8.9, also having limited variation in TS (24.8 vs. 34.0 mg g<sup>-1</sup>), such as VS and TOC reaching approximately 55.0% and 30.0% in WET<sub>D</sub> and DRY<sub>D</sub>, respectively. Both anaerobic digestates displayed similar biological stability (OUR  $\approx$  60 mmol O<sub>2</sub> kg<sup>-1</sup> VS h<sup>-1</sup>). However, WET<sub>D</sub> and DRY<sub>D</sub> had contrasting N content (3.5 vs. 1.6%), resulting in significantly different C: N ratios (9 vs. 20). The composted products (WET<sub>C</sub> and DRY<sub>C</sub>) exhibited pH values between 7.3 and 10. They had different TS (63.0 vs. 76.0%) and VS (39.0 vs. 43.0%) but similar TOC (24.2 vs. 25.5%). Their N content was different (2.5 vs. 18%), leading to lower C: N ratios (10 vs. 14). Both composts (WET<sub>C</sub> and DRY<sub>C</sub>) demonstrated higher stability than the original digestates ( $\leq$  10 mmol O<sub>2</sub> kg<sup>-1</sup> VS h<sup>-1</sup>).

The  $\delta^{13}$ C value of the AD ranged from – 22.23 to -23.32 (WET<sub>D</sub> and DRY<sub>D</sub>). Moreover, the  $\delta^{13}$ C shift after composting showed that WET<sub>C</sub> had a strongly depleted <sup>13</sup>C signature as compared to its homologous digestate while DRY<sub>C</sub> displayed a decreasing  $\delta^{13}$ C (-26.44 and – 22.13). In this context, the reference compost (BW<sub>C</sub>) had a pH within the range of the products investigated, with higher TS and intermediate VS. As compared to the products investigated, BW<sub>C</sub> had lower TOC and N, resulting in a generally lower C: N ratio and a  $\delta^{13}$ C value of -26.28.

The nutrient content of the food waste products differed significantly, more specifically, the P and the calcium (Ca) content of WET<sub>D</sub> and DRY<sub>D</sub> varied notably, with the former ranging from  $\approx 9000$  to  $\approx 5000$  mg kg<sup>-1</sup> and the latter from  $\approx 40,000$  to  $\approx 70,000$  mg kg<sup>-1</sup> (Table 2). Other nutrients, such as potassium (K), magnesium (Mg), and sulfur (S), had narrower ranges (Table 2). In comparison to the homologous digestate, WET<sub>C</sub> showed decreasing P, Ca and S and increasing K and Mg (Table 2). While DRY<sub>C</sub> showed increasing P, Mg, and S, and decreasing K. The reference compost (BW<sub>C</sub>) was in the lower range for all the nutrient considered. According to Table 3, the trace element determined in WET<sub>D</sub> exhibited a slightly higher concentration compared to DRY<sub>D</sub>. Both composts (WET<sub>C</sub> and DRY<sub>C</sub>)

showed comparable levels of trace element, being generally more concentrated in the composts than in the homologous digestates. Furthermore,  $BW_C$  displayed a trace element content in the range of the food waste products under investigation. It should be noted that all the food waste products being compared met the requirements outlined in the EU fertilizer regulation [36].

#### Pot test

As reported in Table 4 the pot test showed that the different treatments affected the ryegrass dry biomass (P < 0.05). At the end of the first growth cycle (day 84) the DW tissue ranged from 2.76 to 4.61 g pot<sup>-1</sup> in both BW<sub>C</sub> and Chem respectively. In this context, WET<sub>D</sub> and WET<sub>C</sub> were intermediate (3.31 g pot<sup>-1</sup>, on average), while DRY<sub>D</sub> and DRY<sub>C</sub> had lower averages (2.60 g pot<sup>-1</sup>), in the same range as BW<sub>C</sub> (2.76 g pot<sup>-1</sup>). The root had a different pattern; Chem still performed the best (2.50 g  $pot^{-1}$ ), while all the other treatment separated poorly although DRY<sub>D</sub> and DRY<sub>C</sub> averaged 1.35 g pot<sup>-1</sup>, in the lower range, and WET<sub>D</sub> and WET<sub>C</sub> attained 1.54 and 1.67 g pot<sup>-1</sup>, in the same range as  $BW_C$  (1.41 g pot<sup>-1</sup>). At the end of the second growth cycle (day 168), all the other organic treatments (WET<sub>D</sub>; DRY<sub>D</sub>; WET<sub>C</sub>; DRY<sub>C</sub>) and references (BW<sub>C</sub> and Chem) averaged  $1.65 \text{ g pot}^{-1}$ . At the same sampling time (day 168), the root averaged  $1.00 \text{ g pot}^{-1}$ , regardless of the treatment. At the end of first cycle of cultivation (day 84), the N from the ryegrass tissue was the highest in Chem (193 g  $pot^{-1}$ ). The WET<sub>D</sub> and WET<sub>C</sub> ranged lower (86 g pot<sup>-1</sup>), while DRY<sub>D</sub>

and DRY<sub>C</sub> attained 55 g pot<sup>-1</sup>, in the same range as BW<sub>C</sub> (60 g pot<sup>-1</sup>). At the end of the second growth cycle (day 168), the ryegrass tissue averaged 62 mg pot<sup>-1</sup> N uptake regardless of the treatment. The root N uptake at the end of the first cycle (day 84) was the best in Chem (29 mg pot<sup>-1</sup>), while all the other treatments averaged 17 mg pot<sup>-1</sup>. At the second sampling (day 168), the root N uptake was the worst in Chem (14 mg pot<sup>-1</sup>), while DRY<sub>D</sub> and DRY<sub>C</sub> attained the best (23 mg pot<sup>-1</sup>), the other treatments (WET<sub>D</sub> and WET<sub>C</sub>) being intermediate, in the same range as BW<sub>C</sub> (20 mg pot<sup>-1</sup>, on average).

#### Total, non-labile and KMnO<sub>4</sub> Oxidizable Carbon

Table 5 reports the SOC in the pot soil at the end of the first and second growth cycles (days 84 and 168). In this context at day 84, the end of the first cycle, Chem showed the poorest SOC (6.68 g kg<sup>-1</sup>). Of the food waste based treatments at the same sampling time (day 84), DRY<sub>D</sub> performed the best (8.73 g kg<sup>-1</sup>), while the other treatments (WET<sub>D</sub>; WET<sub>C</sub>;  $DRY_{C}$ ) and the organic reference (BW<sub>C</sub>) averaged at 8.00 g kg<sup>-1</sup>. At the second sampling (day 168), Chem exhibited poor SOC (6.27 g kg<sup>-1</sup>). At the same time, of the food waste based treatments, DRY<sub>D</sub> and DRY<sub>C</sub> attained the best SOC  $(7.95 \text{ g kg}^{-1}, \text{ on average})$ , in the same range of BW<sub>C</sub> (7.88 g  $kg^{-1}$ ), while WET<sub>D</sub> and WET<sub>C</sub> aligned at an intermediate value (7.22 g kg<sup>-1</sup>, on average). Table 5 also reports the  $C_{NI}$ . At day 84, this was reported to be 6.47 g kg<sup>-1</sup> in Chem. Of the food waste products compared, DRY<sub>D</sub> attained the best  $C_{NL}$  (8.46 g kg<sup>-1</sup>), while the other treatments (WET<sub>D</sub>;

 Table 4 Dry biomass (DW), nitrogen (N) content and uptake in ryegrass shoots and roots in the treatments compared at the end of the two successive cycle (days 84–168)

Treatment	Tissue		Root					
	Sampling day							
	84	168	84	168				
DW (g pot <sup>-1</sup> )								
Chem	4.61 a	1.50 ns	2.50 a	0.74 ns				
WETD	3.32 b	1.68 ns	1.54 b	0.90 ns				
WET <sub>C</sub>	3.29 b	1.76 ns	1.67 b	0.98 ns				
DRY <sub>D</sub>	2.66 c	1.60 ns	1.40 b	1.09 ns				
DRY <sub>C</sub>	2.54 c	1.75 ns	1.30 b	1.16 ns				
BW <sub>C</sub>	2.76 c	1.62 ns	1.41 b	1.24 ns				
N uptake (mg pot <sup>-1</sup> )								
Chem	193 a	59 ns	29 a	14 b				
WETD	90 b	64 ns	18 b	17 ab				
WET <sub>C</sub>	83 b	58 ns	19 b	20 ab				
DRY <sub>D</sub>	59 c	66 ns	16 b	23 a				
DRY <sub>C</sub>	52 c	65 ns	16 b	23 a				
BW <sub>C</sub>	60 c	58 ns	20 b	22 ab				

**Chem**: chemical reference (NH<sub>4</sub>NO<sub>3</sub>+KH<sub>2</sub>PO<sub>4</sub>). **WET**<sub>D</sub>: digestate from the wet digestion of food waste; **DRY**<sub>D</sub>: digestate from the dry-batch digestion of food waste; **WET**<sub>C</sub>: compost from **WET**<sub>D</sub>; **DRY**<sub>D</sub>: compost from **DRY**<sub>D</sub>; **BW**<sub>C</sub>: reference compost from bio-waste. A one-way ANOVA was applied to harvest, cumulated harvests, and root data; in each column and for each trait. Different letter intervals indicate statistically different mean data according to Tukey's test (P < 0.05)

Day	Treatment	SOC	C <sub>NL</sub>	CL	CPI	L	LI	CMI
		$(g kg^{-1})$	$(g kg^{-1})$	$(g kg^{-1})$		(%)		(%)
84	Chem	6.68 b	6.47 b	0.209 b	-	3.23 ns	-	-
	WET <sub>D</sub>	7.80 ab	7.60 ab	0.202 b	1.17 ns	2.67 ns	0.82 ns	95.9 b
	WET <sub>C</sub>	7.62 ab	7.39 ab	0.239 ab	1.14 ns	3.25 ns	1.01 ns	114.1 ab
	DRYD	8.73 a	8.46 a	0.272 a	1.31 ns	3.24 ns	1.00 ns	130.1 a
	DRY <sub>C</sub>	8.33 ab	8.07 ab	0.261 ab	1.25 ns	3.23 ns	1.00 ns	124.7 a
	BW <sub>C</sub>	7.86 ab	7.61 ab	0.247 ab	1.18 ns	3.25 ns	1.01 ns	118.3 ab
168	Chem	6.27 b	6.07 b	0.195 b	-	3.22 b	-	-
	WET <sub>D</sub>	7.03 ab	6.80 ab	0.234 ab	1.12 ab	3.45 ab	1.07 ab	120.2 b
	WET <sub>C</sub>	7.41 ab	7.11 ab	0.296 a	1.18 ab	4.17 a	1.29 a	152.7 a
	DRYD	7.83 a	7.57 a	0.261 ab	1.25 a	3.44 ab	1.07 ab	133.7 ab
	DRY <sub>C</sub>	8.07 a	7.84 a	0.222 ab	1.29 a	2.83 b	0.88 b	135.0 ab
	BWC	7.88 a	7.63 a	0.254 ab	1.26 a	3.35 b	1.04 ab	130.4 ab

**Table 5** Soil organic carbon (SOC), non-labile carbon ( $C_{NL}$ ), labile-C ( $C_L$ ), carbon pool index (CPI), lability (L), lability index (LI) and carbon management index (CMI) in the treatments compared at the end of the first and the second growth cycles (days 84 and 168)

**Chem**: chemical reference (NH<sub>4</sub>NO<sub>3</sub>+KH<sub>2</sub>PO<sub>4</sub>). **WET**<sub>D</sub>: digestate from the wet digestion of food waste; **DRY**<sub>D</sub>: digestate from the dry-batch digestion of food waste; **WET**<sub>C</sub>: compost from **WET**<sub>D</sub>; **DRY**<sub>C</sub>: compost from **DRY**<sub>D</sub>; **BW**<sub>C</sub>: reference compost from bio-waste. A one-way ANOVA was applied each trait at the two sampling dates (84 and 168). Different letter intervals indicate statistically different mean data according to the Tukey test (P < 0.05)

WET<sub>C</sub>; DRY<sub>C</sub>) averaged 7.69 g kg<sup>-1</sup>, in the same range as  $BW_C$  (7.61 g kg<sup>-1</sup>). At the second sampling time (day 168), the  $C_{NL}$  in Chem performed poorly, being 6.07 g kg<sup>-1</sup>. At the same time, of the organic treatments, DRY<sub>D</sub> and DRY<sub>C</sub> had the best  $C_{NL}$  (7.71 g kg<sup>-1</sup>, on average), in the same range as BW<sub>C</sub> (7.63 g kg<sup>-1</sup>), and higher than WET<sub>D</sub> and WET<sub>C</sub> (6.95 g kg<sup>-1</sup>, on average). Table 5 also reports  $C_L$  at day 84 which was  $0.209 \text{ g kg}^{-1}$  in Chem, in the low range. At the same sampling time (day 84),  $C_1$  varied from 0.202 to 0.272 g kg<sup>-1</sup> in WET<sub>D</sub> and DRY<sub>D</sub>, while WET<sub>C</sub>, DRY<sub>C</sub> and BW<sub>C</sub> averaged intermediate (0.249 g kg<sup>-1</sup>). At the end of the second growth cycle (day 168), Chem was 0.195 g  $kg^{-1}$  (the worst). At the same sampling time, amongst the food waste based treatments, WET<sub>C</sub> showed the best C<sub>L</sub> (0.296 g kg<sup>-1</sup>), while WET<sub>D</sub>; DRY<sub>D</sub> and DRY<sub>C</sub> averaged 0.239 g kg<sup>-1</sup>, in the same range as BW<sub>C</sub> (0.254 g kg<sup>-1</sup>).

# Carbon pool Index (CPI), Lability (L), Lability Index (LI), and Carbon Management Index (CMI)

Table 5 also reports the CPI. At the end of the first growth cycle (day 84), it was unaffected by the treatment, averaging 1.21. On the contrary, at the end of the second growth period (day 168), DRY<sub>C</sub> was the best (1.29), in the same range as BW<sub>C</sub> (1.26), followed by DRY<sub>D</sub> (1.25), WET<sub>D</sub> and WET<sub>C</sub> (1.15, on average). Lability is also shown in Table 5; at the end of the first cycle (day 84), the data showed that this parameter was unaffected by the treatments, averaging 3.15%. At the end of the second growth cycle at day 168, WET<sub>C</sub> and DRY<sub>C</sub> performed the best and the worst (4.17 and 2.83%, respectively). The other treatments (WET<sub>D</sub>; DRY<sub>D</sub>) were intermediate (3.45%, on average), in the same range as Chem (3.23%) and BW<sub>C</sub> (3.35%). The LI reported

in Table 5 showed that, at day 84, this parameter was unaffected by the treatments, averaging 0.97. At the second sampling time (day 168), WET<sub>C</sub> had the best LI (1.29), while DRY<sub>C</sub> had the worst (0.88); WET<sub>D</sub> and DRY<sub>D</sub> were intermediate (1.07, on average), in the same range as BW<sub>C</sub> (1.04). Finally, Table 5 reports the CMI. At the end of the first cycle of cultivation (day 84), this was the best in DRY<sub>D</sub> and DRY<sub>C</sub> (127.4%, on average). At the same sampling time, WET<sub>C</sub> was at a lower level (114.1%), in the same range as the organic reference BW<sub>C</sub> (118.3%), WET<sub>D</sub> was found to be the lowest (95.9%). At the second sampling time (day 168), WET<sub>C</sub> was the best (152.7%), followed by DRY<sub>D</sub> and DRY<sub>C</sub> (134.4% on average), in the same range as BW<sub>C</sub> (130.4%); WET<sub>D</sub> was found to be the lowest (be the lowest (120.2%).

# Pot soil $\delta^{13}C$ and $\Delta^{13}C$ at the end of the Two Ryegrass Growth Cycles

As reported in Fig. 1, at the end of the first growth cycle (day 84), the  $\delta^{13}$ C (‰) of the pot soil from Chem was – 25.40. Figure 1 also showed that, at the same sampling date, some of the treatments compared had a more depleted <sup>13</sup>C signature (‰) in comparison to Chem: WET<sub>C</sub> (-25.96); DRY<sub>D</sub> (-26.34); DRY<sub>C</sub> (-26.02), in the same range as BW<sub>C</sub> (-26.21), while WET<sub>D</sub> was in the same range as Chem at -25.51. At the second sampling on day 168, Chem was at -25.21. In comparison to these, the food waste based treatments showed more depleted  $\delta^{13}$ C (‰): WET<sub>C</sub> (-26.02); DRY<sub>D</sub> (-26.06); DRY<sub>C</sub> (-26.32), in the same range as BW<sub>C</sub> (-26.16). Also in this case, WET<sub>D</sub> was similar to Chem at -25.31. Figure 2 reports the  $\Delta^{13}$ C(‰) of the treatments compared at the two time periods (days 84 and 168). In this context, WET<sub>D</sub> had a very similar  $\Delta^{13}$ C (‰) at the two sampling



Fig. 1  $-\delta^{13}$ C isotope natural abundance [ $\delta^{13}$ C (‰)] in the pot soil in the different treatments at the end of the two growth cycles (days 84 and 168). WET<sub>D</sub>: digestate from the wet digestion of food waste; WET<sub>C</sub>: compost from WET<sub>D</sub>; DRY<sub>D</sub>: digestate from the dry-batch digestion

times (-0.107 and -0.210). Of the other food waste based products, WET<sub>C</sub> showed a slight shift to a more depleted  $\Delta^{13}$ C(‰) over time (from -0.557 to -0.817), while the  $\Delta^{13}$ C(‰) from DRY<sub>D</sub> was unaffected over time (-0.933 vs. -0.856). By contrast, DRY<sub>C</sub> had a notable  $\Delta^{13}$ C(‰) depletion (-0.611 vs. -1.111), the BW<sub>C</sub> pattern being similar, although to a lesser extent (-0.809 vs. -0.952).

## Discussion

Appropriate agricultural utilization of recycled organic matter within the context of conserving and increasing organic carbon primarily depends on the quality of the organic sources utilized. In the European Union, a crucial

of food waste; **DRY**<sub>C</sub>: compost from **DRY**<sub>D</sub>; **BW**<sub>C</sub>: reference compost from bio-waste. **Chem**: chemical reference (NH<sub>4</sub>NO<sub>3</sub>+KH<sub>2</sub>PO<sub>4</sub>). Error bars: SE n. = 3

consideration for optimal SOC management is the biological stability of recycled OW. This is widely recognized for its impact on soil organic carbon mineralization, resulting in CO<sub>2</sub> losses, and potential emissions of other GHGs, such as N<sub>2</sub>O and CH<sub>4</sub> [37]. According to the EU fertilizer regulation, the digestates and composts compared in this study demonstrated either unstable (WET<sub>D</sub> and DRY<sub>D</sub>) or stable (WET<sub>C</sub> and DRY<sub>C</sub>) characteristics based on the OUR threshold set at 25 mmol O<sub>2</sub> kg<sup>-1</sup> VS h<sup>-1</sup> for sound soil utilization [36]. The varying biological stability observed aligned with the different biological processes utilized. Anaerobic digestion, especially when integrated with composting, is known to efficiently reduce the easily degradable organic matter [37–39]. In addition, the wet and dry processes, due to their distinct organic carbon loading capacities, require different



Fig. 2 –  $\delta^{13}$ C isotope natural abundance calculated vs.Chem [ $\Delta^{13}$ C(‰)]in the pot soil in the different organic treatments at the end of the two growth cycles (days 84 and 168). WET<sub>D</sub>: digestate from the wet digestion of food waste; WET<sub>C</sub>: compost from WET<sub>D</sub>; DRY<sub>D</sub>:

amounts of green waste, influencing the nitrogen content and C: N ratio of digestates and composts [40-42]. This difference in the C: N ratio not only affected plant nutrition as proven by the pot test, but also influenced the total organic carbon applied to the soil. Plans for fertilization typically align with crop nitrogen requirements regardless of the organic matter content of the products; in this study WET<sub>D</sub> and WET<sub>C</sub> provided approximately 7 Mg ha<sup>-1</sup> organic carbon, while the dry-batch food waste products (DRY<sub>D</sub> and DRY<sub>C</sub>), with their higher C: N ratio, supplied higher organic carbon ( $\approx 10-15$  Mg ha<sup>-1</sup>). Consequently, pot soils treated with  $DRY_D$  and  $DRY_C$  exhibited the highest SOC at the end of both cultivation cycles. For a more in-depth insight, it therefore appeared that, in the digestate and the compost treatments following the external mineral fertilizer application, the SOC decreased by 10 and 3%, respectively. This occurred regardless of their origin, in agreement with the different stabilization level previously mentioned. However, in the pots treated with the anaerobic digestates, and even more in those treated with compost, the native soil carbon

digestate from the dry-batch digestion of food waste; **DRY**<sub>C</sub>: compost from **DRY**<sub>D</sub>; **BW**<sub>C</sub>: reference compost from bio-waste. **Chem**: chemical reference (NH<sub>4</sub>NO<sub>3</sub> + KH<sub>2</sub>PO<sub>4</sub>). Error bars: SE n. = 3

appeared to be protected from mineralization, as suggested by the  $\delta^{13}$ C which will be additionally discussed below.

As mentioned in the Introduction, in the light of improving soil health, studying labile-C and the CMI, can contribute to sustainable land management practices more than the SOC measurement [43–45]. Regarding the above, some information about the utilization of raw and composted sewage sludge which improves the CMI in sandy soil has been reported by Kalisz et al. [46], and some information concerning the positive role of biochar on CMI by Yang et al. [47], while van Midden et al. [48] reported the positive, and rapid, effect of the liquid fraction of a "general" anaerobic digestate on labile-C on the microbial community, thus showing the digestate solid fraction had higher long-term effect on fungi activity. The wet anaerobic digestate (WET<sub>D</sub>) tested in this study showed the lowest CMI, thus indicating a poor effect on carbon management in the context of one simulated growing season, this also being detectable in the context of two seasons. On the contrary, the composted product (WET<sub>C</sub>) had a higher CMI, also increasing over time,

thus proving the efficiency of composting on this parameter. The other food waste products from dry-batch digestion (DRY<sub>D</sub> and DRY<sub>C</sub>) showed a steadier CMI, thus suggesting the possible role of a higher green waste addition. The green waste addition could have played a key role regarding this parameter since the possible effect of lignin on the KMnO<sub>4</sub> oxidizable C and on the derived CMI has been recognized in the literature [49]. Christy et al. [50] reported the effect of a phenolic compound (such as lignin) in the assessment of KMnO<sub>4</sub> oxidizable C, also suggesting a general increase in this fraction over time, due to its resistance to degradation and its consequent preferential accumulation. This issue has been widely debated; however, KMnO4 oxidizable C has often been recognized as reflecting practices which promote organic matter accumulation or stabilization, and can therefore be a useful indicator of long-term soil C sequestration [51]. In this context, the analysis of  $\delta^{13}$ C, and more importantly of  $\Delta^{13}$ C, can be useful for a complete understanding. It is widely acknowledged that, during the soil organic matter mineralization process, the microbial fractionation of <sup>13</sup>C vs.<sup>12</sup>C occurs, generally leading to an enrichment of <sup>13</sup>C and consequently less <sup>13</sup>C depleted soil [52, 53]. Simultaneously, lignin, which is highly resistant to degradation, tends to build up preferentially over cellulose and hemicellulose, both of which are abundant in composts [28]; therefore, investigating  $\delta^{13}$ C could provide valuable insights into the differences between treatments and sampling times, including the effects of chemical nitrogen fertilization. The data presented in the present study revealed that, after both the first and the second growth cycle, the compost-treated pots were those which especially exhibited a greater depletion of  $\delta^{13}$ C as compared to the standard chemical fertilization treatment. At the same time, the dry-batch derived digestate also showed a similar trend. This was in agreement with the probable preferential build-up of lignin as a driving force for the  $\delta^{13}$ C pattern observed in the above-mentioned treated soil after the first cultivation cycle. This finding could be additionally supported by the different initial  $\delta^{13}C$ values of the food waste products tested. In fact, WET<sub>D</sub> and DRY<sub>C</sub> had very similar ranges (-22.23 and -22.13), but resulted in significantly different pot soil  $\delta^{13}$ C values after 168 days (-25.30 and -26.32), this also being proven by the very different  $\Delta^{13}$ C values (-0.21 and -1.11). These outputs were consistent with the findings of Christy et al. [50]; in addition, they were also consistent with the general increase in the CMI over time. More specifically, Christy et al. [50] pointed out that labile C assessment via  $KMnO_4$ oxidation can be affected by the presence of many different organic molecules from the entire spectrum of organic matter; however, they are particularly abundant in polyphenolic compounds, such as lignin and the tannins [54].

# Conclusion

The characteristics of the anaerobic digestates from food waste varied significantly depending on the processes applied. However, the raw digestates exhibited poor stabilization. The composting effectively enhanced the biological stability of the raw digestates, having minimal impact on their fertilizing capacity but promoting greater soil organic carbon conservation. In addition, not only was the total soil organic carbon affected, its quality was also affected. When compared to chemical fertilization, all the organic treatments (except for  $WET_D$ ) had a higher carbon management index. This index also increased over time, following chemical fertilization. This trend corresponded to a substantial depletion of the soil  $\delta^{13}$ C, thus indicating the conservation of the organic carbon applied with the food waste products. This trend was ascribed to the preferential accumulation of lignin, thus suggesting that waste management processes incorporating higher quantities of green waste could significantly contribute to increasing soil organic carbon and its quality within a succession of organic-chemical fertilization practices.

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**Data Availability** Enquiries regarding data availability should be directed to the authors.

#### Declarations

**Competing Interest** The authors have no relevant financial or non-financial interests to disclose.

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