



The Fate of Soil Organic Carbon from Compost: A Pot Test Study Using Labile Carbon and ^{13}C Natural Abundance

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Abstract

Recycled organic waste (OW) can be a valuable nutrient source for plant cultivation; however, knowledge is poor regarding its effect on soil carbon conservation, especially in the frame of organic-mineral fertilisation succession. In this study, four composts, green waste (GWC), anaerobically digested bio-waste (DC), sludge (SSC), and bio-waste (BWC), were compared (10 and 20 Mg volatile solids ha^{-1}) in a ryegrass pot test over two growing cycles (112 + 112 days), along with an unamended control (Ctrl) and a chemical reference (Chem), with and without mineral nitrogen (N) fertilisation. At the end of the two growth cycles, the pot soil was analysed for total- (TOC) and labile-carbon (C_L) as well as for ^{13}C isotope natural abundance ($\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ vs. Chem). At day 112, the pot test showed that Ctrl and Chem gained poor TOC (8.48 g kg^{-1}), lower than the compost at both 10 and 20 Mg volatile solids ha^{-1} (10.01 vs. 11.59 g kg^{-1}). At day 224, a deep soil TOC depletion occurred in the pot soil treated with GWC, DC and BWC at both levels (-10 and -20). However, all the compost treatments showed more depleted soil $\delta^{13}\text{C}$ vs. the references, especially Chem, thus revealing relevant compost-derived carbon conservation. Regarding the compost treatments, the carbon management index (CMI) increased over time, indicating high soil functionality, also showing a good relationship with $\delta^{13}\text{C}$, suggesting a probable increase in relative lignin which could have been linked to carbon conservation and increased functionality.

Keywords Organic waste · Recycling · Carbon storage · KMnO_4 oxidisable organic carbon · Soil functionality · $\Delta^{13}\text{C}$

1 Introduction

Soil organic carbon (SOC) is gaining increasing interest as a key factor regarding soil from multiple perspectives, gathering increasing acknowledgment for its contribution to the multifunctionality of soil management on a global scale, including environmental, social, and economic aspects (Baveye et al. 2016). Within this context, there is increasing attention regarding the preservation of and the increase in SOC, as concerns its relevance to both soil functions and climate regulation (Wiesmeier et al. 2019). Again in this context, the utilisation of recycled organic waste (OW) in agricultural soil such as animal manure, sewage sludge, anaerobic digestate and compost has substantial potential of contributing to the conservation of SOC, its storage, and

overall soil functionality (Smith et al. 1997; Ros et al. 2006). In a broader perspective, such actions help in reducing the greenhouse gas emissions caused by the alternative route of different organic wastes from less environmentally friendly disposal processes (e.g., landfilling, incineration). Moreover, these practices lead to diminished reliance on the production and application of chemical fertilisers (Amelung et al. 2020). In this setting, composting is widely acknowledged to be a reliable strategy for managing organic waste, effectively reducing its volume, degradability, and phytotoxicity, ultimately resulting in biologically stable products suitable for safe agricultural application (Grigatti et al. 2011; Onwosi et al. 2017).

Within this frame of reference, various types of organic waste, such as green waste, bio-waste, and urban and agro-industrial sludge, are composted, with an emerging trend of employing an integrated approach which combines anaerobic digestion (AD) with the composting process (Grigatti et al. 2020). The direct benefits of amending the soil with organic carbon are evident, together with the indirect enhancements in soil physical properties and increased

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nutrient availability, both of which contribute to heightened plant growth and the input of plant residues (Nayak et al. 2009; Paustian et al. 2019). It should be noted that the different origins of compost and their varying degrees of biological stability can influence their subsequent mineralisation in soil (Grigatti et al. 2014; Chen et al. 2014). Moreover, it is known that the presence of easily accessible forms of nitrogen (including mineral nitrogen fertilisers), can promote the mineralisation of soil organic carbon (Chen et al. 2014; Li et al. 2022). In this respect, numerous investigations have focused on the variations in soil organic matter resulting from single or repeated applications of compost as well as on investigations into the combined distribution of compost and mineral fertilisers (Fronning et al. 2008; Nayak et al. 2009; Maris et al. 2021). On the contrary, there is a scarcity of studies examining the fate of SOC or changes in its functionality across a sequence of compost and mineral fertiliser applications, a practice commonly adopted in agriculture (Maris et al. 2021). Addressing these topics is complicated having technical challenges associated with assessing the fate of carbon originating from recycled organic matter in soil. To tackle these challenges, the application of the stable isotope ^{13}C (or $\delta^{13}\text{C}$) tracer technique, based on natural abundance, has emerged as a valuable tool (Glaser et al. 2001; Inácio et al. 2018). However, the utility of this technique has been hindered by challenges in differentiating $\delta^{13}\text{C}$ signatures between amendments and soil, together with the substantial variability in the $\delta^{13}\text{C}$ signature of the materials added. In this regard, the microbial transformations taking place during anaerobic digestion and/or the composting of OW have the potential of mitigating the inherent variability in their ^{13}C signatures, thereby enhancing the effectiveness of this approach.

Lynch et al. (2006) have demonstrated the viability of using natural abundance ^{13}C to assess carbon storage in soil using an incubation test involving different types of compost. In addition to this methodology, researchers have often leveraged $\Delta^{13}\text{C}$ to gain deeper insights into the process of carbon mineralisation (Wang et al. 2015). Specifically, $\Delta^{13}\text{C}$ is a notation which quantifies the calculation of $\delta^{13}\text{C}$ relative to a reference, commonly a chemical reference such as in the present case. This approach factors in the fractionation processes (^{13}C vs. ^{12}C) resulting from microbial activity (Boström et al. 2007), especially in the presence of a nutrient source, mainly nitrogen, such as those introduced through chemical fertilisers (Raj et al. 2020). In addition to $\delta^{13}\text{C}$ investigations, an insightful perspective into soil functionality variations can be gleaned from the study of KMnO_4 oxidisable organic carbon, also known as Labile-C, which enables the derivation of the valuable carbon management index (CMI). As outlined by Blair et al. (1995), this index is based on the distribution of SOC within the labile and the non-labile fractions, yielding a lability index

(LI) and a comprehensive carbon pool index (CPI). These indices facilitate the assessment of the relative sustainability of different management options (e.g., cropping, fertilisation) as compared to a reference system (e.g., organic vs. chemical fertilisation). Subsequently, numerous researchers embraced the CMI as an indicator for gauging the changes in SOC quality resulting from distinct management practices (Ameer et al. 2023; Abagandura et al. 2023). Some authors have effectively combined the study of $\delta^{13}\text{C}$ and the CMI (Lefroy et al. 1993; Sandeep et al. 2016; Ghosh et al. 2019), although many of these investigations were conducted in open fields, exhibiting substantial variability and necessitating long-term assessments in order to establish robust findings. Consequently, numerous researchers encourage or are already implementing tightly controlled conditions during their research endeavours (Yılmaz and Sönmez 2017; Liao et al. 2023).

The present study, therefore, makes use of pots involving ryegrass. Ryegrass, characterised by its high nitrogen requirements and rapid growth, serves as an ideal candidate for effectively assessing the fertilisation potential of the treatments tested (Cordovil et al. 2007; Jimenez et al. 2020). The application of standardised growth conditions (soil moisture, light, temperature) ensures minimal variability in both plant and soil outcomes. This favourable combination makes it a reliable model for the investigation of both agronomic and nutritional performance as well as for changes in soil organic carbon and soil quality. To this end, the pot soil previously investigated by Grigatti et al. (2019), where four distinct composts of various origins were examined at two application rates (10 and 20 Mg VS ha^{-1}), and compared with an unamended control (Ctrl) and a chemical fertiliser (Chem). The present investigation covered two consecutive growth cycles (112 + 112 days) using ryegrass, both with and without the addition of mineral nitrogen. In this study, the fate of SOC and shifts in SOC quality within the pot soil selected were explored. To achieve this, at the end of each crop cycle (days 112 and 224), the TOC and its labile fraction were determined in the pot soil in order to measure the carbon management index. Furthermore, the study examined the pot soil $\delta^{13}\text{C}$ and $\Delta^{13}\text{C}$ in order to offer a comprehensive understanding of the fate of the organic carbon introduced into the soil by the compost.

2 Materials and Methods

2.1 Compost

The products compared in this study were derived from what was reported by Grigatti et al. (2019). In brief, a 100% green waste compost (GWC), one from anaerobically digested bio-waste + green waste (45 + 55%) (DC), one from urban and

agro-industrial sludge + green waste (50 + 50%) (SSC), and one from bio-waste + green waste (60 + 40%) (BWC) were used. The oxygen uptake rate (OUR) was determined on fresh products as described by Grigatti et al. (2007). The total organic carbon (TOC) and total nitrogen (TN) were determined with an elemental analyser; in addition, specifically for this study, the $\delta^{13}\text{C}$ (‰) of the composts compared were determined using a coupled mass spectrometer (DELTA V Advantage; Thermo Electron Germany) according to the following equation:

$$\delta\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000;$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$. The main characteristics of the composts compared are reported in Table 1.

2.2 Soil total organic carbon, $\delta^{13}\text{C}$ and KMnO_4 oxidisable carbon

A dual-stage (112 + 112) ryegrass pot test, detailed in Grigatti et al. (2019), involved the addition of four compost types (GWC, DC, SC, BWC) at two organic matter application rates: 10 and 20 Mg of volatile solids (VS) ha^{-1} , to soil taken from the upper layer of a field in the Po Valley (Bologna, Italy), the main characteristics of the soil are listed in Table 1S. In addition, an unamended control (Ctrl) and a chemical reference (Chem) were included. A randomised complete block design with four replicates was applied. After sampling at the end of the first growth period (day 112), the pot was reused in a subsequent growth season. During this second cycle, the ryegrass was fertilised to ensure a non-limiting nitrogen supply. At the conclusion of the second growth cycle (day 224), samples of pot soil were collected and utilised for the analysis described in the following section. The total organic carbon content as well as the $\delta^{13}\text{C}$ was determined on the soil samples collected at the end of the first (day 112) and second growth cycles (day 224) using an elemental analyser coupled with a mass spectrometer (DELTA V Advantage; Thermo Electron Germany). In

addition, the KMnO_4 oxidisable C (C_L) content was determined (in duplicate) on 2.5 g of pot soil according to Weil et al. (2003). The CMI was obtained according to the method of Blair et al. (1995):

$$\text{CMI}(\%) = (\text{carbon pool index}) \times (\text{lability index}) \times 100$$

where the CPI was calculated according to the following equation:

$$\text{CPI} = (C \text{ treated soil}) / (C \text{ reference soil})$$

where C treated soil is the organic carbon (g kg^{-1}) from soil treated with the organic products (GWC, DC, SSC, BWC), and C reference soil is the organic carbon (g kg^{-1}) in soil from Chem.

The LI was calculated according to the following equation

$$\text{LI} = (C \text{ lability treated soil}) / (C \text{ lability reference soil})$$

where the C lability treated soil is the carbon lability from soil treated with the organic products (GWC, DC, SSC, BWC), and the C lability reference soil is the carbon lability in soil from Chem. The C lability is expressed as the ratio of labile C (C_L) to non-labile C (C_{NL}). Non-labile C was calculated as the difference between the total C content and the C_L content of the soil. The data from each of the two cycles were analysed using one-way ANOVA (Statistica 7, StatSoft); the mean separation was carried out using a Tukey test ($P < 0.05$).

3 Results

3.1 Main compost characteristics

Table 1 shows the main characteristics of the compost. The data derived by Grigatti et al. (2019) demonstrated that GWC and BWC had the highest VS ($\approx 530 \text{ mg g}^{-1}$), while DC and SSC ranked below ($\approx 380 \text{ mg g}^{-1}$), being very similar to the TOC trend: GWC and BWC ($\approx 300 \text{ mg g}^{-1}$), DC

Table 1 Main characteristic of the compared composts

Compost	VS (mg g^{-1})	TOC (mg g^{-1})	N_{tot} (mg g^{-1})	C:N	OUR (mmol O_2 $\text{kg}^{-1} \text{ VS h}^{-1}$)	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	$\delta^{13}\text{C}$ (‰)
GWC	525	304	19.8	16	7	576	131	-27.91
DC	400	233	26.7	9	6	2696	2815	-27.06
SSC	375	241	27.0	9	9	6109	1678	-26.41
BWC	545	312	23.1	14	62	3106	192	-25.54

VS: volatile solids; **TOC:** total organic carbon; **N_{tot} :** total nitrogen; **C:N:** carbon to nitrogen ratio; **OUR:** oxygen uptake rate; **$\text{NH}_4^+\text{-N}$:** ammonium nitrogen; **$\text{NO}_3^-\text{-N}$:** nitrate nitrogen; **$\delta^{13}\text{C}$:** natural ${}^{13}\text{C}$ isotopic abundance. **GWC:** green waste compost; **DC:** anaerobically digested bio-waste compost; **SSC:** sludge compost; **BWC:** bio-waste compost. The data are expressed on TS, these are the average of two replicates ($\text{CV} < 5\%$); from Grigatti et al. 2019. The $\delta^{13}\text{C}$ (‰) values are the average of three replicates ($\text{CV} < 5\%$)

and SSC ($\approx 230 \text{ mg g}^{-1}$). In contrast, the total nitrogen (N_{tot}) was lowest in GWC (19.8 mg g^{-1}), while it was recorded at approximately 27.0 mg g^{-1} in DC and SSC, BWC being intermediate (23.1 mg g^{-1}), yielding a C:N ratio of approximately 15 in GWC and BWC, and approximately 9 in both DC and SSC. The stability level (OUR) showed very different outcomes, being the best in GWC, DC, and SSC ($7, 6$ and $9 \text{ mmol O}_2 \text{ kg}^{-1} \text{ VS h}^{-1}$), while it was recorded at a lower level in BWC ($62 \text{ mmol O}_2 \text{ kg}^{-1} \text{ VS h}^{-1}$). The ammonium nitrogen ($\text{NH}_4^+\text{-N}$) ranged from ≈ 600 to $\approx 6000 \text{ mg kg}^{-1}$ (GWC and SC), DC and BWC being intermediate ($\approx 3000 \text{ mg kg}^{-1}$). The nitric nitrogen ($\text{NO}_3^-\text{-N}$) was the lowest in GWC and BWC (≈ 130 and $\approx 190 \text{ mg kg}^{-1}$), the highest in DC ($\approx 2800 \text{ mg kg}^{-1}$), and intermediate in SSC ($1600 \approx \text{mg kg}^{-1}$). Moreover, the $\delta^{13}\text{C}$ (‰) values were: GWC (-27.91 ± 0.189); DC (-27.06 ± 0.004); SSC (-26.41 ± 0.364); BWC (-25.54 ± 0.181).

3.2 Total, non-labile and KMnO_4 oxidisable carbon

As reported in Table 2, at the end of the first cycle of ryegrass cultivation (day 112), the pot soil from the unamended Ctrl showed the lowest TOC (7.85 g kg^{-1}). At the same sampling time GWC_{10} and BWC_{10} had the best and the worst TOC, respectively (10.65 vs. 8.92 g kg^{-1}), Chem being

in the low range (9.11 g kg^{-1}). In this context (day 112), DC_{10} and SSC_{10} were intermediate (9.71 g kg^{-1} , on average). In contrast, no difference was detected between the treatments at high VS loading (GWC_{20} ; DC_{20} ; SSC_{20} ; BWC_{20}), averaging at 11.59 g kg^{-1} at the same sampling time (day 112). At the end of the second growth cycle (day 224), a different output was recorded since Ctrl and Chem had the worst TOC (7.30 g kg^{-1} , on average), while all the compost treatments at the low level (GWC_{10} ; DC_{10} ; SSC_{10} ; BWC_{10}), averaged a higher TOC (8.80 g kg^{-1}). At the same sampling time (day 224) SSC_{20} and GWC_{20} attained the highest TOC (11.44 and 10.02 g kg^{-1}), while DC_{20} and BWC_{20} clustered at a lower level (9.25 g kg^{-1} , on average). Table 2 also shows the non-labile carbon (C_{NL}) at day 112; this mimicked the TOC pattern. Specifically, at this sampling time, Ctrl performed the worst (7.22 g kg^{-1}), the compost treatments GWC_{10} , DC_{10} , SSC_{10} and BWC_{10} averaged 9.10 g kg^{-1} , close to Chem (8.51 g kg^{-1}), while GWC_{20} , DC_{20} , SSC_{20} and BWC_{20} performed higher C_{NL} (10.90 g kg^{-1} , on average).

At the second sampling date (day 224), Ctrl and Chem also had poor C_{NL} (6.71 g kg^{-1} , on average). At this stage, the C_{NL} in GWC_{10} , DC_{10} , SSC_{10} and BWC_{10} was 8.12 g kg^{-1} (on average), while at the higher VS loading, GWC_{20} , DC_{20} , and BWC_{20} were 8.84 g kg^{-1} ; at the same time, SSC_{20} was better (10.76 g kg^{-1}). Table 2 also reports

Table 2 Total organic carbon (TOC), 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 different treatments at the different levels at the end of the first and the second growth cycle (day 112–224)

Day	Treatment	Level (Mg VS ha ⁻¹)	TOC (g kg ⁻¹)	C_{NL} (g kg ⁻¹)	C_{L} (g kg ⁻¹)	CPI	L (%)	LI	CMI (%)
112	Ctrl		7.85 c	7.22 c	0.63 b	0.86 c	8.68 a	1.23 a	106 b
	Chem		9.11 bc	8.51 bc	0.60 b		7.04 bc		
	GWC_{10}	10	10.65 ab	9.96 ab	0.70 ab	1.17 ab	7.02 bc	1.00 ac	114 ab
	DC_{10}	10	9.76 ac	9.10 ac	0.66 b	1.07 ac	7.28 ac	1.03 ac	111 ab
	SSC_{10}	10	9.66 ac	9.06 ac	0.59 b	1.06 ac	6.57 bc	0.93 bc	99 b
	BWC_{10}	10	8.92 bc	8.27 bc	0.65 b	0.98 bc	7.93 ab	1.13 ab	110 ab
	GWC_{20}	20	12.12 a	11.34 a	0.78 a	1.33 a	6.89 bc	0.98 ac	129 a
	DC_{20}	20	11.64 a	10.95 a	0.70 ab	1.28 a	6.44 bc	0.91 bc	116 ab
	SSC_{20}	20	10.83 a	10.18 ab	0.64 b	1.19 ab	6.34 bc	0.90 bc	107 ab
	BWC_{20}	20	11.78 a	11.14 a	0.64 b	1.29 a	5.79 c	0.82 c	106 b
224	Ctrl		7.21 c	6.60 d	0.61 bc	0.97 c	9.27 a	1.11 a	109 b
	Chem		7.40 c	6.83 d	0.57 c		8.32 ab		
	GWC_{10}	10	9.21 b	8.53 bc	0.68 ab	1.25 ab	8.01 ac	0.96 ab	120 ab
	DC_{10}	10	8.81 b	8.14 bc	0.67 ab	1.19 ab	8.26 ab	0.99 ab	118 ab
	SSC_{10}	10	9.14 b	8.43 bc	0.71 a	1.24 ab	8.42 ab	1.01 ab	125 a
	BWC_{10}	10	8.03 bc	7.39 cd	0.65 ac	1.09 bc	8.77 ab	1.05 a	114 ab
	GWC_{20}	20	10.02 ab	9.33 b	0.69 ab	1.35 ab	7.41 ac	0.89 ab	120 ab
	DC_{20}	20	9.05 b	8.35 bc	0.69 ab	1.22 b	8.33 ab	1.00 ab	122 ab
	SSC_{20}	20	11.44 a	10.76 a	0.70 a	1.55 a	6.36 c	0.76 c	118 ab
	BWC_{20}	20	9.50 b	8.84 b	0.67 ab	1.28 b	7.58 ac	0.91 ab	117 ab

Ctrl: unamended control soil; **Chem:** chemical reference; **GWC:** green waste compost; **DC:** anaerobically digested bio-waste compost; **SSC:** sludge compost; **BWC:** bio-waste compost. A one-way ANOVA was applied to the data from the two cycles, in each column and for each trait the different letter intervals indicate statistically different mean data according to Tukey test ($P < 0.05$)

the Labile-C (C_L) determined in the pot soil at the end of the two growth periods. At the end of the first cultivation cycle (day 112), this was in the low range in Ctrl and Chem (0.61 g kg⁻¹, on average). In comparison to these, GWC₁₀ was the best (0.70 g kg⁻¹), being higher than DC₁₀, SSC₁₀ and BWC₁₀ (0.64 g kg⁻¹, on average). At the higher loading, GWC₂₀ was the best (0.78 g kg⁻¹), slightly higher than DC₂₀ (0.70 g kg⁻¹), while SSC₂₀ and BWC₂₀ were lower (0.64 g kg⁻¹). At the end of the second growth period (day 224), Chem and Ctrl had the worst C_L (0.57 and 0.61 g kg⁻¹) values; in addition, a general C_L flattening between the different treatments at 10 and 20 Mg ha⁻¹ was also recorded: GWC (0.68 vs. 0.69); DC (0.67 vs. 0.69); SSC (0.71 vs. 0.70); BWC (0.65 vs. 0.67).

3.3 Carbon pool index (CPI), lability (L), lability index (LI), and carbon management index (CMI)

The CPI calculated vs. Chem is reported in Table 2. At the end of the first growth period (day 112), the CPI in pot soil was the lowest in the unamended Ctrl soil (0.86). Across the pots which were treated with compost at 10 or 20 Mg VS ha⁻¹, an overall trend was identified in which the higher dose displayed a higher CPI: GWC (1.17 vs. 1.33); DC: (1.07 vs. 1.28); SSC: (1.06 vs. 1.19); BWC (0.98 vs. 1.29). At the second sampling time (day 224), the Ctrl still had the worst CPI (0.95), while a whole clustering was still detectable among the treatments at 10 vs. 20 Mg ha⁻¹, although not always yielding statistically significant results: GWC (1.25 vs. 1.35); SSC: (1.24 vs. 1.55); BWC (1.09 vs. 1.28); only DC showed minimal variation (1.19 vs. 1.22).

Lability (L) at the end of the first growth cycle (day 112) is also reported in Table 2. At this sampling time, the Ctrl soil was the best (8.68%); among the pots treated at 10 or 20 Mg VS ha⁻¹, no significant difference was detected in either GWC (7.02 vs. 6.89%) or SSC (6.57 vs. 6.34%). Only BWC (7.93 vs. 5.79%) and DC (7.28 vs. 6.44%) showed a significant reduction, Chem being intermediate (7.04%). At the second sampling date (day 224), the unamended Ctrl soil still had the highest lability (9.27%), while no appreciable difference was recorded among the different treatments at either 10 or 20 Mg ha⁻¹ in: GWC (7.71%), DC (8.30%) and BWC (8.18%). Only SSC showed a significant reduction (down to 6.36%), while Chem was in the higher range (7.68%). Table 2 also reports the lability index (LI); at the end of the first growth period (day 112), it was the best in the Ctrl (1.23). Of the pots treated with compost at 10 and 20 Mg VS ha⁻¹, no significant difference was detected in GWC (1.00 vs. 0.98), and SSC (0.93 vs. 0.90); only DC showed some variation (1.03 vs. 0.91) together with BWC to a higher extent (1.13 vs. 0.82). At day 224, the LI of the unamended Ctrl was still the best (1.11). In the pot soil treated with compost at 10 and 20 Mg VS ha⁻¹, no significant

difference was detected in the LI from GWC (0.96 vs. 0.89) or DC (0.99 vs. 1.00); only BWC (1.05 vs. 0.91) and SSC showed some variation (1.01 vs. 0.76). Finally, Table 2 also shows the CMI (%). At the end of the first growth cycle (day 112), it was 106% in the Ctrl.

The pot soil showed some CMI variation when treated with compost at 10 or 20 Mg ha⁻¹: GWC (114 vs. 129%); SSC (99 vs. 107%); BWC (110 vs. 106%), while treatment with DC was not statistically significant (111 vs. 116%). At the end of the second growth period (day 224), the CMI was 109% in Ctrl. The CMI was very similar between the compost-treated soils at both 10 and 20 Mg VS ha⁻¹, averaging 118%, while the SSC₁₀ was 125%.

3.4 Pot soil isotopic signature $\delta^{13}C$ at the end of the two consecutive ryegrass growth cycles

As reported in Fig. 1, at the end of the first growth cycle (day 112), the isotopic signature “ $\delta^{13}C$ (‰)” of pot soil from Ctrl and Chem ranged from -25.39 to -25.55. Figure 1 also showed that, at the same sampling date (day 112), the different composts had a significantly depleted ¹³C signature (‰) in comparison to both Ctrl and Chem: GWC₁₀ (-26.58); DC₁₀ (-26.19); SSC₁₀ (-26.20); BWC₁₀ (-26.22). Always in comparison to the references (Ctrl and Chem) at the end of the first growth cycle (day 112), a more depleted ¹³C signature (‰) was recorded at increasing compost application in GWC₂₀ and DC₂₀ (-26.94 and -26.91); on the contrary, an incremental ¹³C signature (‰) was recorded in SSC₂₀ and BWC₂₀ (-25.68 and -25.91). At the end of the second growth cycle (day 224), a notable $\delta^{13}C$ (‰) increase occurred at both Ctrl and Chem reaching -24.32 and -24.32, respectively. Similarly, the pot soil treated with the different composts showed a notable $\delta^{13}C$ (‰) increase. Specifically, GWC reached very similar $\delta^{13}C$ (‰), regardless of the compost application levels of 10 and 20 Mg VS ha⁻¹ (-25.79 vs. -25.80). The other products at the two levels (10 and 20 Mg VS ha⁻¹): DC (-25.49 vs. -25.70), SSC (-25.72 vs. -25.66) and BWC (-25.25 vs. -25.10) behaved similarly. Figure 2 reports the $\Delta^{13}C$ (‰); it showed the difference between the composts compared at the two compost loadings at each time interval (day 112 and day 224). In this context, GWC₁₀ showed $\Delta^{13}C$ (‰) depletion throughout the pot test at day 112 and day 224 (-1.03 vs. 1.38), while GWC₂₀ showed similar $\Delta^{13}C$ (‰) values regardless of the sampling time (-1.39, on average). The DC behaved similarly but to a different extent (day 112 and day 224): DC₁₀ (-0.65 vs. -1.08), and DC₂₀ (-1.36 vs. -1.29). On the other hand, SSC showed additional $\Delta^{13}C$ (‰) depletion at the higher compost application level over time (day 112 and day 224): SSC₁₀ (-0.65 vs. -1.13) and SSC₂₀ (-0.35 vs. -1.15). However, BWC mimicked SSC to a lesser extent: BWC₁₀ (-0.67 vs. -0.84) and BWC₂₀ (-0.36 vs. -0.69).

Fig. 1 ^{13}C isotope natural abundance [$\delta^{13}\text{C}$ (‰)] in the pot soil in the different treatments at the two application levels (10 and 20), at the end of the two growth cycles (day 112 and 224). **Ctrl**: unamended soil; **GWC**: green waste compost; **DC**: anaerobically digested bio-waste compost; **SSC**: sewage sludge compost; **BWC**: bio-waste compost; **Chem** (chemical reference). Error bars: SE n. = 4

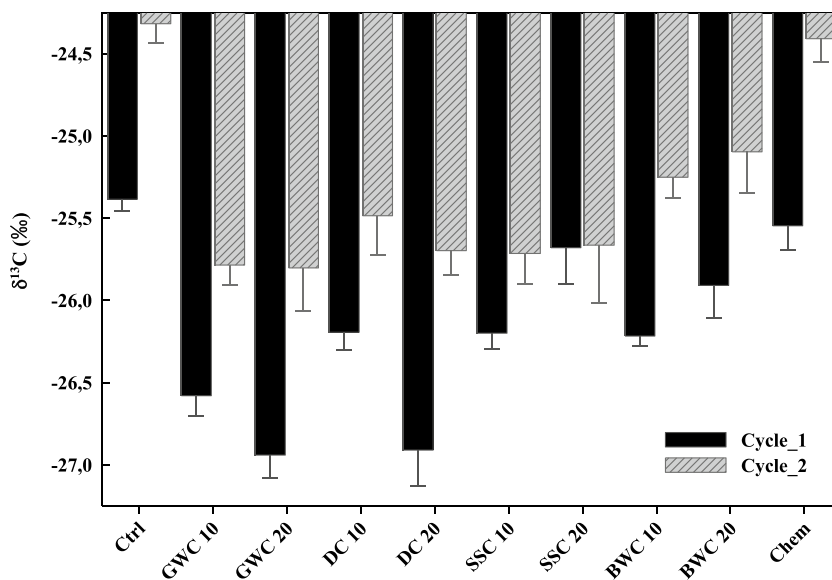
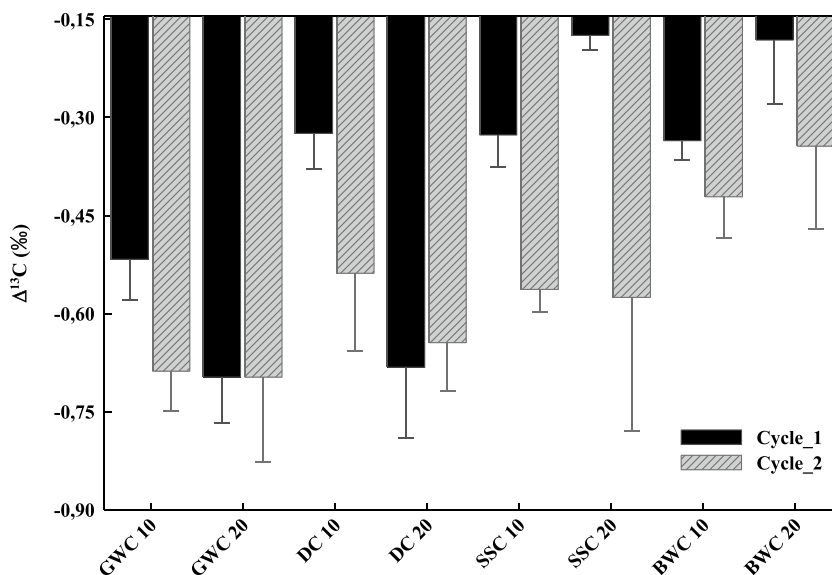


Fig. 2 ^{13}C isotope natural abundance calculated vs. **Chem** [$\Delta^{13}\text{C}$ (‰)] in pot in the pot soil in the different organic treatments at the two application levels (10 and 20), at the end of the two growth cycles (day 112 and 224). **GWC**: green waste compost; **DC**: anaerobically digested bio-waste compost; **SSC**: sewage sludge compost; **BWC**: bio-waste compost. Error bars: SE n. = 4



3.5 Relationship of the CMI to $\delta^{13}\text{C}$ (‰) and with $\Delta^{13}\text{C}$ (‰)

Figure 3a reports the relationship of the CMI with the $\delta^{13}\text{C}$ (‰) determined in the pot soil at the end of the first and the second cycles (days 112 and 224). The data showed a separate clustering of the two cycles, more notable after 112 days; the CMI ranged from 99 to 129%, and the $\delta^{13}\text{C}$ (‰) varied from -25.68 to -26.94 with a relationship described by the linear equation: $Y = -0.0381x - 22.078$ ($R^2 = 0.58$). After 224 days, a greater variation was recorded since the CMI ranged from 114 to 125%, while the $\delta^{13}\text{C}$ (‰) ranged from -25.10 to -25.80, their

relationship being described by the following equation: $Y = -0.056x - 1.889$ ($R^2 = 0.50$). Figure 3b reports the relationships between the CMI and the $\Delta^{13}\text{C}$ (‰). In this case, at the end of the first growth cycle, the CMI and the $\Delta^{13}\text{C}$ ranged from 98 to 130% and from -0.35 to -1.39, respectively, while a narrow range was recorded at the end of the second growth cycle for both the CMI (114 and 125%) and the $\Delta^{13}\text{C}$ (-0.69 and -1.39) as a result of the mineralisation of the easily degradable organic matter. The relationship between the two parameters investigated ($Y = -0.038x + 3.4158$; $R^2 = 0.64$) is very interesting, revealing the strong relationship between higher $\Delta^{13}\text{C}$ depletion and higher soil carbon functionality.

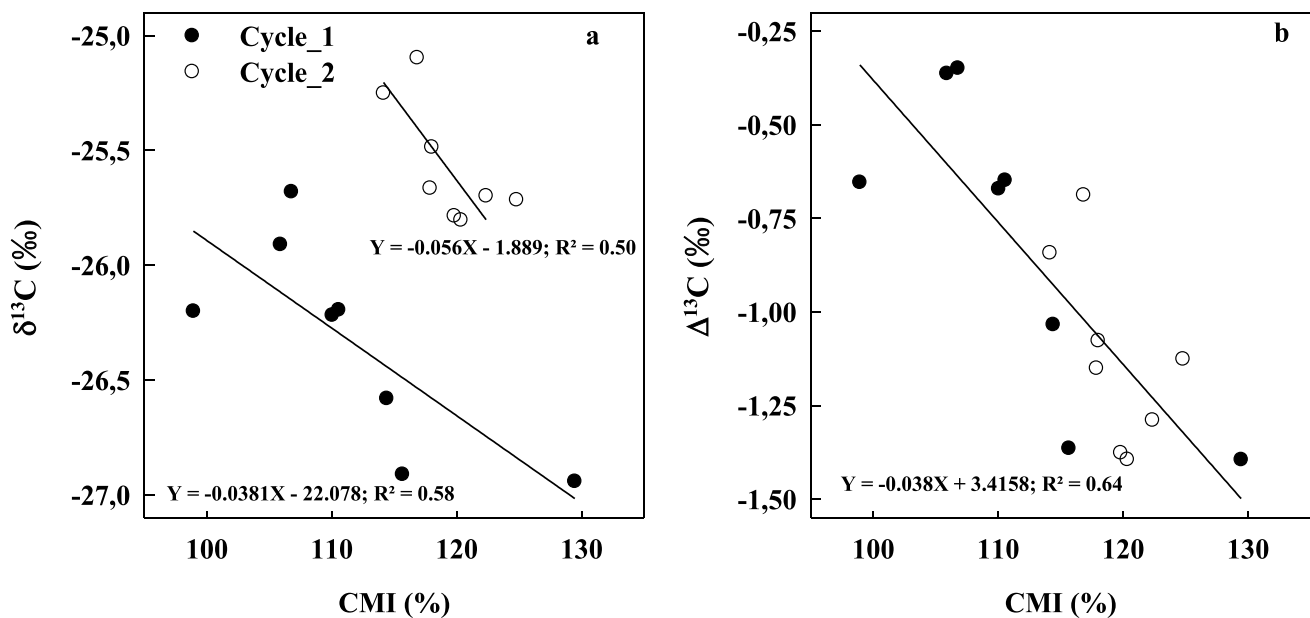


Fig. 3 Relationship between the carbon management index (CMI) and the ^{13}C isotope natural abundance [$\delta^{13}\text{C}$ (‰)] (a) and the ^{13}C isotope natural abundance calculated vs. **Chem** [$\Delta^{13}\text{C}$ (‰)] (b) in the

pot soil in the different treatments at the end of the two growth cycles (day 112 and 224)

4 Discussion

The data on the composts compared in this work have shown that the different raw materials and processes adopted played a key role in determining the main physicochemical properties of the tested products. Specifically, the green waste and bio-waste composts (GWC and BWC), formed from pure lignocellulosic residues or a mixture of lignocellulosic and food waste, showed a higher amount of organic matter than digestate and sludge composts. This aligns with the different composting processes used: single composting for GWC and BWC versus coupled anaerobic digestion/composting for DC and SSC, as previously reported by Grigatti et al. (2019). In addition most of the compared samples achieved high biological stability (OUR), meeting agricultural usability standards under the European Fertilizer Regulation (EU Reg. 1009/2019). This not only avoids undesirable CO_2 emissions from the soil but also promotes greater SOC conservation, as supported by previous works (Grigatti et al. 2007; 2020). In this scenario, the natural abundance of ^{13}C may be helpful in studying the fate of the organic carbon added with compost to the soil (Lynch et al. 2006), especially with the low $\delta^{13}\text{C}$ inherent variability of the selected products. In this regard, the $\delta^{13}\text{C}$ (‰) levels of GWC and BWC showed the widest interval with values in agreement with different raw materials and processes. The literature reports some information regarding the $\delta^{13}\text{C}$ (‰) of compost derived from sewage sludge; Lynch et al. (2006), reported a $\delta^{13}\text{C}$ (‰) signature very close to the sewage sludge based compost from this

study. Dai et al. (2009) also reported a similar range for composted biosolids, while there is less information regarding the compost based on bio-waste anaerobic digestates. Some information regarding the $\delta^{13}\text{C}$ (‰) of cattle, and pig slurry digestates is reported by Nogués et al. (2023) who also described the $\delta^{13}\text{C}$ value of composted products, this characteristic being notably affected by the addition of bulking material, rich of lignin. It is widely recognized in the literature that lignin has a depleted $\delta^{13}\text{C}$ signature compared to whole plant material (Lynch et al. 2006). Furthermore, lignin is generally very resistant to biological degradation, being more preserved than other plant tissue components such as cellulose and hemicellulose, both following mineralization processes in soil and also during composting (Lynch et al. 2006), and better discussed below. While there have been many studies examining the effects of compost application or combined applications of organic and mineral fertilisers on the fate of soil organic carbon (Li et al. 2022), there is less research which has considered these aspects in relation to simulated field cultivation which alternates organic and chemical fertilisation management (Tang et al. 2018). In this field some studies have demonstrated that alternating organic-chemical fertilisation can maximise plant nutrient utilisation efficiency (mainly N and P), thus preventing overfertilisation (Grigatti et al. 2019), and at the same time, increasing soil carbon functionality (Grigatti 2023). In the present study results proved the positive effect of compost utilisation on the soil organic carbon storage capacity, especially in comparison with common chemical

fertilisation. These outcomes are in agreement with the literature following the application of various types of compost to the soil (Cooper and DeMarco 2023; Badewa et al. 2023), however as mentioned earlier, exploring SOC functionality can provide additional insights into the topic. In order to gain a deeper understanding, the Authors carried out an investigation on the oxidisable carbon (C_L) using $KMnO_4$. This fraction is known to be highly sensitive to variations in soil management (Blair et al. 1995), and can also be utilised for calculating the CMI. However, after the first cycle, a minimal variation in C_L was observed across the different treatments, similarly to what reported by Grigatti et al. (2023) in a pot study on composted anaerobic digestates derived from sewage sludge and bio-waste. Following the nitrogen fertilisation, the C_L values for the various organic treatments converged to similar levels, indicating the mineralisation of this active portion (Albuquerque et al. 2009). However, only modest variations in the CMI, with a slight increase observed passing from the first to the second cycle; these values consistently remained greater than 100%. This proved the high soil organic carbon functionality of the compost-treated soil. At the same time the present study revealed after the first growth cycle there was an overall greater depletion of $\delta^{13}C$ in the compost-treated pots in comparison to both the references (Ctrl and Chem). This behaviour was very likely related to the ^{13}C vs. ^{12}C fractionation process generally occurring during the soil organic matter mineralisation (Lynch et al. 2006; Atere et al. 2020). At the same time, it has generally been recognised that lignin, highly resistant to degradation, may present a preferential build-up over cellulose and hemicellulose in which composts are rich (Lynch et al. 2006). This is the most likely reason for the more depleted $\delta^{13}C$ which was determined in the major part of the compost-treated soil after the first cycle of cultivation. The different composts provided nutrients ensuring plant growth, and also feeding the soil microbial community since these microorganisms are responsible for ^{13}C vs. ^{12}C fractionation; in this light the study of $\Delta^{13}C$ can represent a more informative insight when carried out under optimal conditions. The data showed that the soil treated with GWC had highly depleted $\Delta^{13}C$ (which was only poorly affected by the application level over time), in accord with the higher lignin content of this product. The other compost treatments having greater depletion at the end of the second cycle, suggested intense mineralization of the other fractions (cellulose, hemicellulose), and consequent lignin conservation, especially at this stage. The study of the relationship between the CMI and $\Delta^{13}C$ appeared to be very helpful in giving the whole picture of soil organic carbon functionality and its conservation. In fact, the $\Delta^{13}C$ and the CMI had a wide range at the end of the first growth cycle, while having a narrow range at the end of the second growth cycle as a result of the mineralisation of the easily degradable organic

matter. Very interesting the linear relationship between the two parameters investigated ($Y = -0.038x + 3.4158$; $R^2 = 0.64$), revealing the strong relationship between higher $\Delta^{13}C$ depletion and higher soil carbon functionality.

5 Conclusions

Different raw materials and processes yield composts with different characteristics, which can play a role in carbon storage and its functionality once distributed to agricultural soils. This study proved that compost-treated soils exhibited higher organic carbon levels in comparison to the chemically fertilized soil after a simulated growing season with ryegrass. Being this effect consistent across the different application levels (10 and 20 Mg of volatile solids ha^{-1}). Notably, the observed outcomes persisted even after the mineral nitrogen application in a sequence of organic-chemical fertilization during a second simulated growth season. These outputs were confirmed by the remarkable depletion of the natural ^{13}C isotopic abundance ($\delta^{13}C$) observed in the compost-treated soils, thus indicating compost-derived carbon conservation, especially in the lignin-rich products. Furthermore, the soil organic carbon functionality determined via the study of $KMnO_4$ -oxidisable carbon (labile carbon) was enhanced in compost-treated soils, as evidenced by the carbon management index (CMI), emphasizing the advantages of using compost. The study, which focused on the natural abundance of $\delta^{13}C$ and labile carbon, suggested a potential correlation between the functional aspects of soil organic carbon and the resilient component of compost, lignin. These findings can contribute significantly to the broader understanding of sustainable agricultural practices and effective soil organic carbon management strategies through compost utilization. Lignin, emerging as a key factor in soil organic matter potential storage and soil functionality deserves deeper exploration in future studies about agricultural use of compost.

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Declarations

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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