



Research article

Stable C and N isotope variation during anaerobic digestate composting and in the compost-amended soil-plant system

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ABSTRACT

Although the use of composts derived from anaerobic digestates as soil amendments is likely to increase in the future, there is little information concerning the fate of their C and N compounds after their incorporation into soil. This work assesses C and N concentrations and the associated changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ during the composting processes of cattle and pig slurry anaerobic digestates. In addition, the compost effect on C and N fractions and plant uptake were studied during a six-month pot experiment with rosemary plants. The results did not show $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic discrimination during composting, indicating a previous stabilization of cattle manure and pig slurry during the anaerobic digestion. This fact was also confirmed by the low C losses during the composting processes (1.2-fold and 1.05-fold for the composting piles with cattle and pig slurry anaerobic digestates, respectively). After soil addition, the composts augmented N values (from 0.41 g kg⁻¹ to around 0.56 g kg⁻¹ in low dose and 0.68 g kg⁻¹ in high dose compost amended soils) and $\delta^{15}\text{N}$ soil values (increases in the range of 50%–156%), but showed only slight differences in C and $\delta^{13}\text{C}$ values compared to unfertilised control and inorganic fertilized soils. Moreover, the rosemary leaves of the plants grown on the compost amended soils presented higher N and $\delta^{15}\text{N}$ abundance than control and inorganic fertilized plants. We conclude that $\delta^{15}\text{N}$ abundance of anaerobic digestate composts is useful to discern its N uptake and could thus be a useful tool to detect whether organic or mineral fertiliser types were used for agricultural production.

1. Introduction

Organic amendments are commonly used in organic farming systems, in which inorganic fertilizers are not allowed. They are known to have a beneficial effect on soils, as they help to maintain soil organic matter contents, to reduce the risk of runoff and erosion and to enhance the fertility and quality of soils for agricultural production (Tejada et al., 2009; Masunga et al., 2016). Moreover, their application into the soil affects the activity and proliferation of the soil microbiota (Bastida et al., 2008; Bustamante et al., 2010). In this context, anaerobic digestates originating from energy production may be used more frequently in the future after their transformation into organic amendments by composting. However, up to now their fate in agricultural systems and the contribution they make to biogeochemical cycling in the plant soil atmosphere continuum is poorly known. Our hypothesis, supported by

scientific literature, is that the influence of anaerobic digestate composts on the plant-soil system can be traced with stable nitrogen and carbon isotopes (Drollinger et al., 2019; Rijk and Ekblad, 2020; Choi et al., 2003; Flores-Delgadillo et al., 2011; Szpak et al., 2012).

Indeed, variations in $\delta^{15}\text{N}$ values of leaves result from the combination of two factors, the sources of N used by plants, and the fractionation of N isotope during transformation, assimilation, metabolism, and reallocation and loss of N from plants (Högberg, 1997; Kolb and Evans, 2002; Spangenberg et al., 2021). The $\delta^{15}\text{N}$ of soil is also subjected to changes, due to N mineralization, nitrification, denitrification, NH₃ volatilization and N leaching that discriminates against $\delta^{15}\text{N}$ and results in soil N pools with contrasting $\delta^{15}\text{N}$ signatures. Compost addition also affects soil $\delta^{15}\text{N}$. Thus, conventional and organic farming systems may have contrasting effects on their crops' signature with organic amendments being more enriched in ¹⁵N ($\delta^{15}\text{N} > +10\text{‰}$) than inorganic

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fertilizers (<+2‰). Indeed, compost amended soils tend to produce crops with a greater $\delta^{15}\text{N}$ in their tissue than those to which inorganic fertilizers have been applied (Choi et al., 2003; Bateman et al., 2007; Joergensen et al., 2019). The lower $\delta^{15}\text{N}$ signature of inorganic fertilizers depends on their origin as they are produced through the Haber-Bosch process, which uses atmospheric N_2 with $\delta^{15}\text{N}$ equal to 0‰. The greater $\delta^{15}\text{N}$ of compost N, however, is a consequence of N isotopic fractionations related to N losses during the composting process, which lead to a $\delta^{15}\text{N}$ enrichment of the remaining N (Choi et al., 2007). On the other hand, carbon isotopic fractionation occurs during soil organic matter degradation, which produces a ^{12}C enrichment of the released CO_2 and a ^{13}C enrichment in the residual soil carbon. $\delta^{13}\text{C}$ in soils also depends on plant processes, such as CO_2 stomatal uptake, carboxylation, and post photosynthetic fractionation (Farquhar et al., 1982, 1989; Badeck et al., 2005). In plants, C isotope fractionation is produced due to Rubisco discrimination against the isotope ^{13}C during the photosynthesis process (Flores et al., 2007) and may be modified by fertilization, which affects C isotope composition in the leaves as a consequence of its effects on stomatal conductance (Choi et al., 2005).

On the other hand, up to now, only few studies have investigated $\delta^{15}\text{N}$ dynamics during the composting process (Lynch et al., 2006; Kim et al., 2008; Saez et al., 2020) and little is known about $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ temporal changes from the initial composting materials to the leaves of plants grown of compost amended soils. Knowledge about these changes might be useful to assess the dynamics of anaerobic digestate compost in the plant soil system. To this end, we investigated changes of both signatures during the digestate composting process, in amended soils and plants grown on these soils during a pot experiment.

Therefore, the main objectives of this work were in the following: i) to assess the changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures compared to the total and water-soluble fractions of C and N throughout the composting process of anaerobic digestates from pig and cattle slurry; ii) to study the effect of the incorporation of the anaerobic digestate-derived composts on the dynamics of the C and N in the plant-soil system studied.

2. Materials and methods

2.1. Characteristics of the soil and organic amendments

The soil considered came from a semiarid abandoned agricultural area in Montelibretti (Rome, Italy, 42° 8' 7" N, 12° 44' 17" E, 232 m a.s.l.). Soil samples were manually collected using a shovel from the surface layer (0–20 cm) and were left to dry at room temperature before sieving (<2 mm) (Barra-Caracciolo et al., 2015). The soil from this site is slightly alkaline (pH = 7.6) loamy soil, with low salt concentration (0.10 dS/m) and total organic C content (0.75% C), and high content in carbonates (33.4% CaCO_3).

The composts were produced with the solid phase of the anaerobic digestates originating from cattle (CS) and pig slurry (PS) mixed with vine shoot pruning (75%:25% by dry mass), respectively. The duration of the composting processes was of 131 and 64 days for CS and PS, respectively. More details about the initial composting materials and processes is detailed elsewhere (Bustamante et al., 2012; Bustamante et al., 2013). Both composts had very adequate characteristics for being used as organic fertilizers and/or amendments: the total organic carbon to total nitrogen ratio (C/N) in both composts was below the reference value for compost maturity of 20 established for mature composts (Bustamante et al., 2012) and the N, P and K concentrations were similar to those reported in previous works of livestock derived composts (N: 2.90%, in CS, 3.03%, in PS; P: 6.95 g kg^{-1} in CS, 33.7 g kg^{-1} in PS; K: 16.4 g kg^{-1} in CS, 9.78 g kg^{-1} in PS) (Bustamante et al., 2012; Bustamante et al., 2013). Moreover, the composts verified the criteria established for mature compost, such as the germination index (GI) (>60%) and the cation exchange capacity (CEC) (>60 meq/100 g organic matter) (Bernal et al., 2009). The compost samples used corresponded to the days 0, 11, 33, 67, 95 and 131 for CS and to the days 0, 8,

16, 20, 34 and 64 for PS, which coincides with the main composting phases: initial stage, thermophilic phase (two samplings), end of the bio-oxidative phase and maturity.

2.2. Greenhouse experiment

Polyethylene pots (9 × 9 × 12 cm) were filled with 1 kg of soil mixed thoroughly with CS or PS composts at two doses (expressed on a fresh mass basis). The doses used were: a) Low dose (Low), 11.54 g compost per kg soil (equivalent dose of 30 t/ha) and b) high dose (High), 23.08 g of compost per kg soil (corresponding to a dose of 60 t/ha). The amounts of added N, P and K in every treatment are shown in Supplementary Table S1. We also established a mineral fertilisation treatment (InOrg) with incorporation of NPK in the proportion of 100:60:73 and an unfertilised control. This was obtained by adding 192 mg kg^{-1} soil of the commercial fertiliser Nitrophoska top 20 (NPK = 20:5:10), and 26 mg kg^{-1} of monopotassic phosphate (NPK = 0:52:34). The NPK ratios of amended soils were similar. The negative control treatment (Control) comprised soil without the addition of fertiliser. Six replications were established per each treatment.

Rooted cuttings of rosemary genetically identical (C3 type of photosynthesis) were planted, one per pot, corresponding to each treatment. The detailed description of the crop management during the experiment, which has a duration of six months, and of the plant and soil sample pre-analytical and analytical procedures are described elsewhere (Barra-Caracciolo et al., 2015). Soil and plant samples corresponded to the beginning (0 days) and end of the greenhouse experiment (180 days). Plant dry biomass values were also determined (Barra-Caracciolo et al., 2015) and they resulted to be 1.9-, 1.6-, 1.8-, 1.4–1.4-fold higher in InOrg, CSLow, PSLow, CSHigh and PSHigh respectively than in Control.

2.3. Analytical determinations

Organic water-soluble fraction of carbon (WSC) and nitrogen (WSN) in the compost and soil samples were extracted with deionized water (1:20, w/v) and determined using an automatic carbon analyzer (TOC Analyzer Combustion Catalytic Oxidation/NDIR Method Model (TOC-VCSN), Shimadzu Corporation, Kyoto, Japan).

Organic carbon (OC), total nitrogen (N) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) content in the samples of compost, soil and rosemary plants were determined using a CHN auto-analyser (CHN NA 1500, Carlo Erba) coupled with an isotope ratio mass spectrometer. The determined nitrogen isotope ratios are identical to those of organic nitrogen within the determination uncertainty, since inorganic nitrogen compounds constitute a very little proportion of soil total N (Sebilo et al., 2013).

Before the analyses, carbonates were removed from soil samples by adding 20 μL HCl 2N several times, until the effervescence due to acidification was no longer observable, following the standard protocol of King et al. (1998).

Carbon and nitrogen isotope ratios of the samples were expressed in parts per thousand (‰) using the delta notation (1):

$$\delta(X) = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The isotopic ratios were calculated relative to the Pee Dee Belemnite Standard (PDB) for C and relative to atmospheric N_2 for nitrogen (Sanaullah et al., 2011).

2.4. Statistical analysis

The statistical analysis of total organic C, N and stable isotopes during composting was conducted with an ANOVA analysis (significant differences at the $P < 0.05$ level). The two-way ANOVA was done

considering two variables, time and the compost mixture. Factorial analysis (FA) was also conducted to study the data set of each type of composting process, considering the total and water-soluble fractions of C and N and stable isotopes.

For soil and plant samples, analysis of variance (ANOVA) was performed using total C, total N, and stable isotopes as dependent variables, and fertilization as an independent factor. To study the significance of different groups of means, the Fisher post-hoc test was used, considering significant at a probability level of $P < 0.05$. For the statistical analyses, SigmaStat statistical software was used, version 4.0 (Systat Software Inc.), except FA, performed with the IBM SPSS 25 software package.

3. Results

3.1. Evolution of total, water soluble and isotopic fractions of C and N in the composting processes

Digestate of cattle slurry (CD) showed a higher carbon concentration than pig slurry digestate (PD) (Table 1). As a result, also the feedstock mixture composed of CD and vine shoot prunings (VP + CD), used for composting had a higher carbon content than the feedstock mixture composed of PD and vine shoot prunings (VP + PD). Contrarily, the percentage of nitrogen was higher in PD than in CD (Table 1). The concentrations of the water-soluble fractions of C and N were also greater in the initial mixture and final compost prepared using CD and VP (Table 1). Moreover, a positive linear correlation between $\delta^{15}\text{N}$ and the N concentration (Supplementary Fig. S1A) and a negative one between $\delta^{15}\text{N}$ and C/N were found (Supplementary Fig. S1B).

During the 131-day composting period, the carbon concentration decreased significantly ($P < 0.05$) from 40.4% to 34.6% in the mixture of VP + CD (Fig. 1 A). The decrease in carbon, however, was lower for the mixture of VP + PD. In the latter, it decreased from 33.34% to 31.82% ($P < 0.05$) during the 64-day composting period (Fig. 1A). The WSC decreased strongly during composting principally in the mixture of CS + VP, from values of 1.74% at the beginning of the process to values of 0.57% in the mature sample (Fig. 1A).

The N contents had an increasing trend, from 1.16% to 2.77% ($P < 0.001$) for CS compost (during the 131-day composting period) and only from 2.51% to 2.82% for PS compost (during the 64-day composting period) ($P < 0.001$) (Fig. 1B). On the contrary, the WSN showed a different behavior, with a clear increase in the pile with PS + VP, but with an initial decrease in the pile prepared with CS (Fig. 1B).

The $\delta^{13}\text{C}$ and the $\delta^{15}\text{N}$ did not change during compost maturation (Fig. 1C and D). In fact, $\delta^{13}\text{C}$ isotopic discrimination during composting was +0.1‰ and -0.2‰ for CS and PS respectively, whereas $\delta^{15}\text{N}$ isotopic discrimination was -0.2‰ and -0.9‰ for CS and PS.

When FA was applied to the different composting samples throughout both composting processes, the first two factors described 91.5% of the total variability (51.7% and 23.8%, respectively). A plot of the first and second factors for all the composting samples is displayed in Fig. 2. A clear separation between the type of composting mixture was observed due to the contribution of both factors. Positive contributors to

Factor 1 considered all the properties studied except TOC, while Factor 2 was principally affected by the water-soluble and isotopic fractions of C and N. Regarding the degree of stability of each composting sample, according to the composting phase, was found. This is illustrated by a higher difference in the samples from the initial composting stages (M-1 and M-2) in the composting mixture of CS + VP than in those of the compost from anaerobic pig slurry digestate (Fig. 2).

3.2. Total, water-soluble and isotopic fractions of organic carbon and total nitrogen in the amended soils

Soil organic carbon content was slightly higher (c.a. 1.05–1.1-fold) in the amended soils than in the inorganic fertilized and unamended soils ($P < 0.05$) (Table 2). Also, the nitrogen content was found to be higher in soils, which received organic amendments than in control soils and soils receiving mineral fertilizers ($P < 0.001$) (Table 2). Moreover, increasing doses of anaerobic digestate composts resulted in significant higher soil total nitrogen contents ($P < 0.05$) (N values increased around a 37% and a 66% in low dose and high dose compost-amended, respectively, in comparison with control soils). No differences in organic carbon content or total nitrogen content were found between compost CS and compost PS amended soils at any of the two different doses.

Similarly, to the results obtained for soil total organic carbon, WSC was around 1.3-, 1.8-, 1.8- and 1.9- fold higher in CS_{Low}, CS_{High}, PS_{Low} and PS_{High}, respectively with regards to control and InOrg (Fig. 3A). Moreover, the contents of WSN were c.a. 1.6-, 2- and 4.5- fold higher in CS_{High}, PS_{Low} and PS_{High} soils than in control, InOrg and CS_{Low} (Fig. 3B).

Soil $\delta^{13}\text{C}$ contents ranged between -5.74 and -6.81. In high dose amended soils, they were statistically lower (more negative) than in control and treatments with mineral fertilisers ($P < 0.05$) (Fig. 3C). On the other hand, whereas increasing doses of CS anaerobic digestate derived composts resulted in more negative soil $\delta^{13}\text{C}$ content ($P < 0.05$), no statistical difference was found in soil $\delta^{13}\text{C}$ values between low- and high-dose PS amended soils. No differences in soil $\delta^{13}\text{C}$ content were found between CS and PS amended soils at any of the two different doses.

Soil $\delta^{15}\text{N}$ values resulted to be c.a. a 1.5-, 1.8-, 2- and 2.5-fold higher in CS_{Low}, CS_{High}, PS_{Low} and PS_{High} soils, respectively than in the control and inorganic fertilized soils ($P < 0.001$) (Fig. 3D). PS amended soils presented statistically significant higher $\delta^{15}\text{N}$ values than CS amended soils at both, low and at high amendment doses ($P > 0.001$). Moreover, we also observed statistically higher soil $\delta^{15}\text{N}$ values in high dose amended soils with regard to the respective low dose amended soils ($P < 0.0005$ for CS and $P < 0.0001$ for PS).

3.3. Total and isotopic fractions of organic carbon and total nitrogen rosemary leaves

Leaf carbon content was a 5% higher in plants grown on PS amended soils at the low dose ($P < 0.05$) than in plants grown on control soil (Table 3), but there were no other significant differences among

Table 1

Total organic C, total N, water-soluble C (Cw), water-soluble N (Nw), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the raw materials used for composting: cattle slurry anaerobic digestate (CS), pig slurry anaerobic digestate (PS) and vine shoot pruning (VP1 for CS, VP2 for PS), in composting mixtures and in mature composts.

	Total organic C (%)	Total N (%)	C/N	Cw (%)	Nw (%)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
CD	39.77	1.42	28.01	n.d.	n.d.	-25.6	9.8
VP 1	44.71	0.69	64.80	n.d.	n.d.	-25.3	6.4
PD	30.77	2.67	11.52	n.d.	n.d.	-24.8	12.4
VP 2	43.24	0.74	58.43	n.d.	n.d.	-24.9	6.6
CD + VP1	40.40	1.16	34.83	1.74	0.43	-25.2	9.0
PD + VP2	33.34	2.51	13.28	1.18	0.37	-24.8	12.9
CS	34.60	2.77	12.49	0.57	0.23	-25.1	8.8
PS	31.82	2.82	11.28	0.81	0.61	-25.0	12.0

n.d.: not determined.

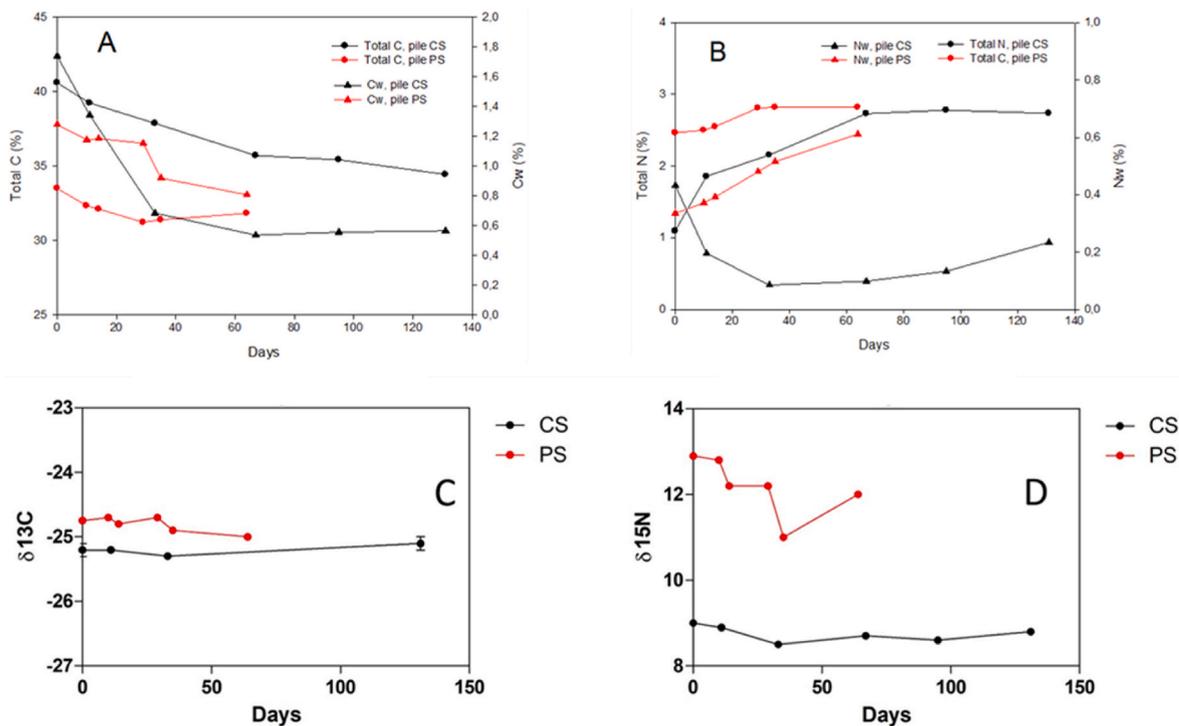


Fig. 1. Evolution of (A) total organic C and water-soluble C (Cw); (B) total and water-soluble N (Nw), (C) δ¹³C and (D) δ¹⁵N over time during compost maturation for CS and PS compost. Mean values ± standard deviation (n = 3).

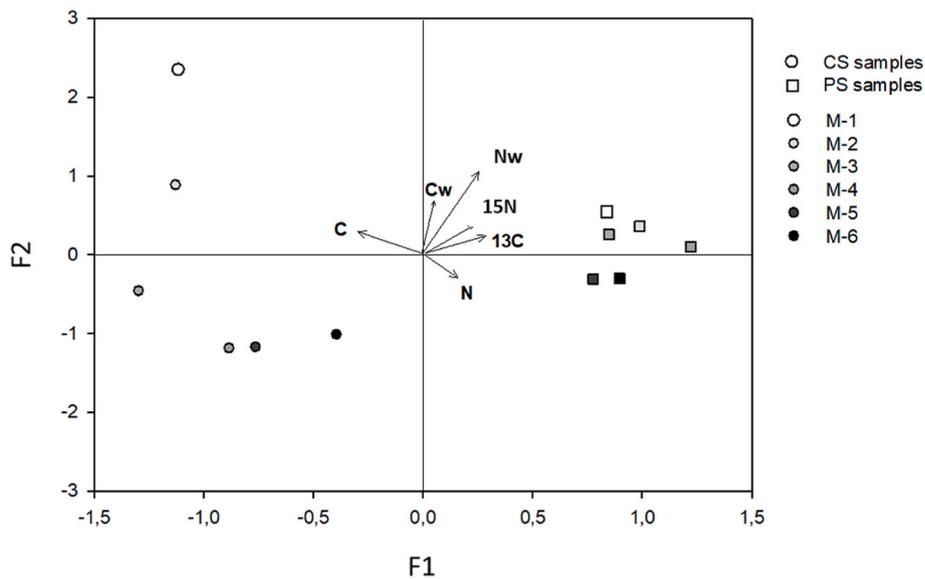


Fig. 2. Plot of the first and second factors extracted from factor analysis of total organic and water-soluble C, total and water-soluble N, δ¹³C and δ¹⁵N during both composting processes. Arrows represent the projection of variables and the different color the composting samplings.

Table 2
 Total organic C (g kg⁻¹) and total N (g kg⁻¹) of the different treated soils. Different letters indicate significant statistical differences (P < 0.05).

	Total organic C (g kg ⁻¹)	Total N (g kg ⁻¹)
Control	38.8 ^a ± 0.89	0.41 ^a ± 0.03
InOrg	38.6 ^a ± 0.43	0.41 ^a ± 0.03
CSLow	40.5 ^b ± 0.96	0.54 ^b ± 0.06
CSHigh	41.9 ^b ± 1.35	0.68 ^c ± 0.07
PSLow	40.3 ^b ± 1.12	0.59 ^b ± 0.04
PSHigh	41.0 ^b ± 1.10	0.67 ^c ± 0.06

treatments. On the other hand, leaf N concentration increased (P < 0.05) a 14%, 10%, 18% and 40% with the application of CS_{Low}, CS_{High}, PS_{Low} and PS_{High}, respectively compared to the control treatment (Table 3). N content in inorganic fertilized leaves was also lower than in leaves of organic fertilized plants (P < 0.05), with the only exception of CS amended plants at the high dose. Moreover, whereas increasing doses of compost PS resulted in increased leaf N content, no significant difference in leaf N was found between leaves of plants grown under two different doses of CS digestate compost.

No significant differences were found in leaf δ¹³C values between either different fertilization treatments or different compost doses

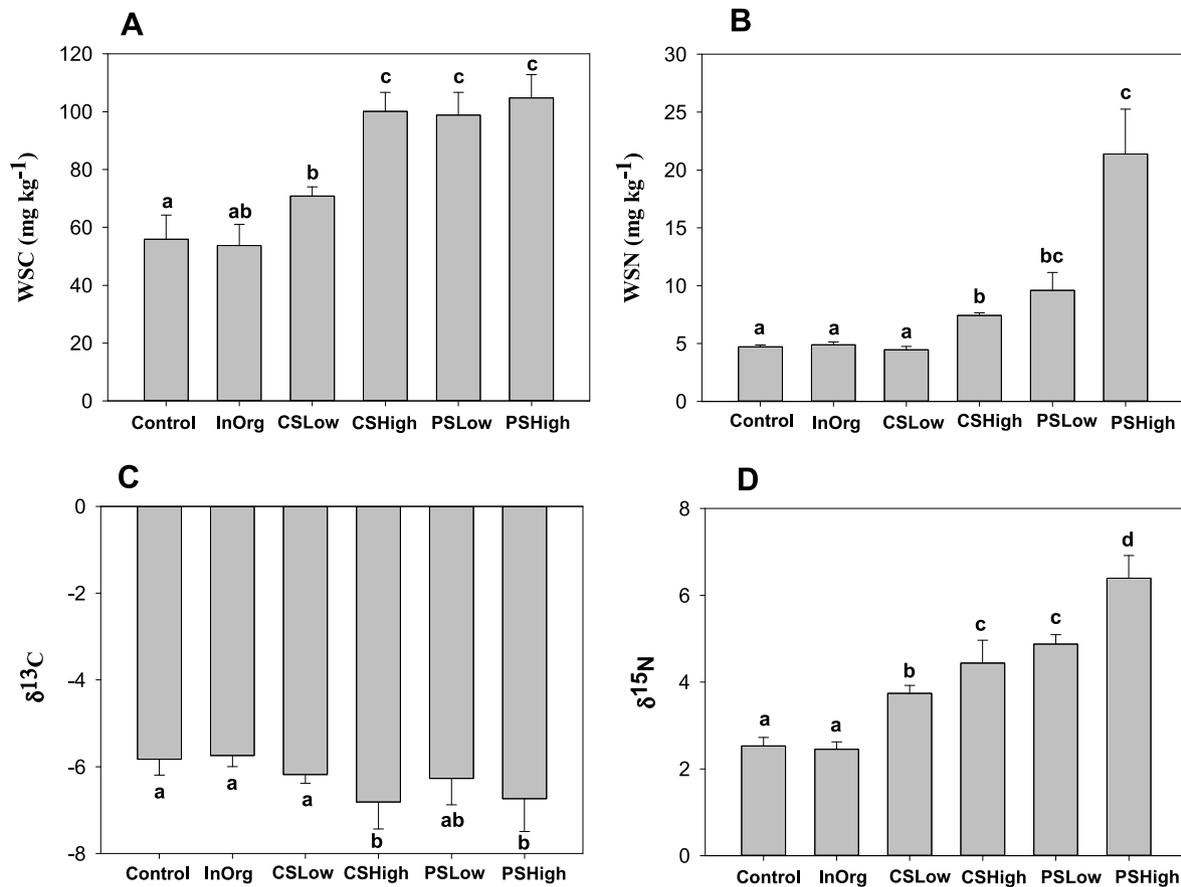


Fig. 3. Concentrations of (A) WSC, (B) WSN, (C) $\delta^{13}\text{C}$ and (D) $\delta^{15}\text{N}$ in the Control soil and in soils treated with inorganic fertilizer (InOrg), or cattle or pig anaerobic digestate derived compost at low (CSLow, PSLow) or at high (CSHigh, PSHigh) concentrations. Mean values \pm standard deviation ($n = 6$). Different letters indicate significant statistical differences ($P < 0.05$).

Table 3

Leaf total C (g kg^{-1}) and leaf total N (g kg^{-1}) of the plants grown on the different treated soils. Different letters indicate significant statistical differences ($P < 0.05$).

	Total C (g kg^{-1})	Total N (g kg^{-1})
Control	478 ^a \pm 5.58	14.68 ^a \pm 0.46
InOrg	493 ^a \pm 5.76	15.18 ^{ab} \pm 0.25
CSLow	488 ^a \pm 2.64	16.75 ^c \pm 0.35
CSHigh	485 ^a \pm 1.70	16.22 ^{bc} \pm 0.89
PSLow	503 ^b \pm 6.78	17.27 ^c \pm 2.53
PSHigh	492 ^a \pm 7.12	20.60 ^d \pm 1.2

(Fig. 4A). However, leaf $\delta^{15}\text{N}$ was different in plants grown under the various fertilization conditions. $\delta^{15}\text{N}$ values were 1.25–1.75 fold higher in leaves of plants grown soils amended with CS compost than in leaves of plants grown on control soil ($P < 0.05$) and soils, which received inorganic fertilizer ($P < 0.01$) (Fig. 4B). Similarly, leaf $\delta^{15}\text{N}$ values significantly increased 2.5–3.2-fold ($P < 0.001$) with the application of PS compared to the control treatment and inorganic treatments. Importantly, leaf $\delta^{15}\text{N}$ values of plants grown in PS amended soils were two to three-fold higher than in any other treatment.

4. Discussion

4.1. Changes in the C and N total, water-soluble and isotopic fractions during composting

The composting mixture of PS + VP presented at the initial stage of the composting process a higher carbon contents ($P < 0.05$) than the

corresponding feedstocks due to the contribution of vine pruning. Nitrogen concentrations of the initial mixtures, however, mirrored only those of initial digestates as happened with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Similar results were found by Saez et al. (2020) in a study of different types of composts derived from sewage and agri-food sludge.

During the composting process of anaerobic digestates, carbon losses were very low (1.2-fold for CS and 1.05-fold for PS). This is likely due to the C loss occurring during the anaerobic digestion process, which already led to a stabilization of organic matter. In fact, carbon losses of about (20–95%) have been reported during anaerobic digestion (Möller, 2015) due to CH_4 and CO_2 emissions, according to the type and recalcitrance of the feedstocks.

Regarding $\delta^{13}\text{C}$ values, no significant differences were observed between the beginning and the end of the maturity period. Other authors, however, have found a depletion of $\delta^{13}\text{C}$ due to the increasing relative proportion of the low degradable lignin in the composting end product (Lynch et al., 2005; Inácio et al., 2017) that shows generally more depleted $\delta^{13}\text{C}$ values (Bowling et al., 2008; Einfalt et al., 2020). Again, it is likely that, during the digestion process, there has been an increase in $\delta^{13}\text{C}$ and a stabilization of this value. Indeed, lignin is not degraded during the process of anaerobic digestion, while other compounds, such as volatile fatty acids (>90%), cellulose (>50%), hemicellulose (>80%), and raw protein are partially decomposed (Molinuevo-Salces et al., 2013; Sarker et al., 2019).

During compost maturation, the N concentration of both composts increased. The increase was higher (2.4-fold) for CS than for PS (1.12-fold), while we did not observe any change in $\delta^{15}\text{N}$. Other authors have also observed increasing N concentrations during composting, likely because of the decomposition of organic C compounds resulting in CO_2

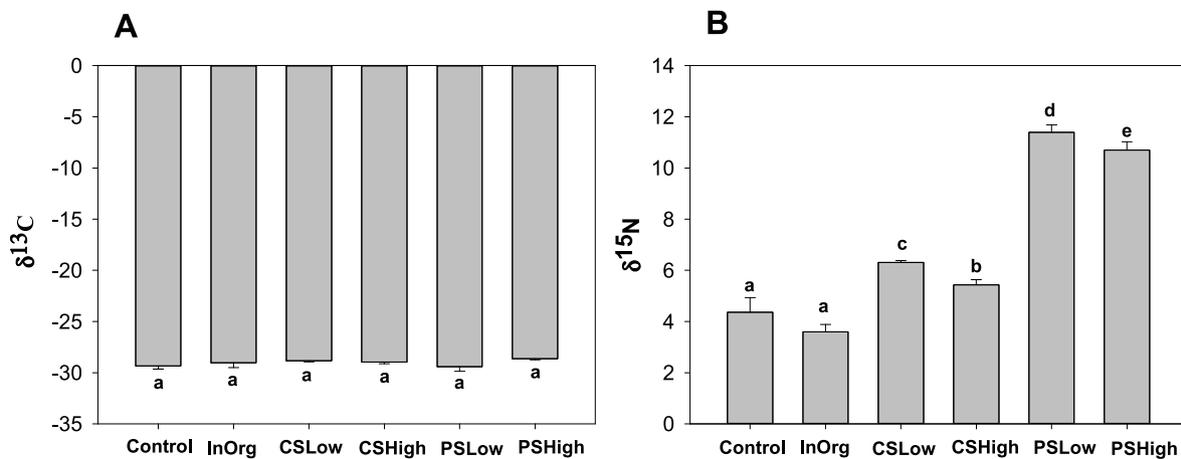


Fig. 4. Concentrations of (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$ in leaves of *Rosmarinus officinalis* plants grown on the Control soil and in soils treated with inorganic fertilizer (InOrg), or cattle or pig anaerobic digestate derived compost at low (CSLow, PSLow) or at high (CSHigh, PSHigh) concentrations. Mean values \pm standard deviation ($n = 6$). Different letters indicate significant statistical differences ($P < 0.05$).

loss and reduced compost weight (Bernal et al., 1996). An unchanged $\delta^{15}\text{N}$, however, is in contrast to the results of other authors. $\delta^{15}\text{N}$ is supposed to increase during composting due to the fractionation taking place through NH_3 volatilization (Parkinson et al., 2004) and through denitrification of NO_3^- to N_2O and N_2 (Robinson, 2001; Hao and Benke, 2008), processes that discriminate against $\delta^{15}\text{N}$ in favor of the ^{14}N isotope. The quite high initial $\delta^{15}\text{N}$ of the composting materials suggests that NH_4^+ and NO_3^- losses had already occurred prior to the composting process begun, during digestion of cattle and pig slurry. At this regard, Schievano et al. (2011) reported net N losses of 5–10% during the digestion process. It is also noticeable that digestate from pig slurry presented higher $\delta^{15}\text{N}$ values than that from cattle manure (+2.6). These differences may also be due to biological denitrification processes during the anaerobic digestion of manures.

4.2. Effect of compost incorporation on soil C and N stocks and on plant C and N

The determination of the natural abundance stable isotope constitutes a useful tool to evaluate the contribution of different sources of organic matter in soils and plants (Lynch et al., 2006; Said-Pullicino et al., 2010). In fact, isotopic composition in amended planted soils depends on various factors: 1. Soil stable $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios. 2. The isotopic composition of the organic amendments. 3. The incorporation of exogenous plant exudates through the rhizosphere, 4. The mechanisms fractioning isotopes in the carbon and nitrogen cycle.

In our experimental set-up, we found slight differences among the various soil treatments regarding soil C and WSC. Both were slightly greater in compost treated soils than in control and soils receiving mineral fertilizers ($P < 0.05$). Regarding soil $\delta^{13}\text{C}$ contents, we found statistically lower (more negative) values in high dose organic amended soils than in control and mineral fertilizer treatments. These results reflect in part the contribution of the organic amendments (richer in %C and with more negative $\delta^{13}\text{C}$ levels than soil) to the substrate (soil).

However, the slight differences in the $\delta^{13}\text{C}$ signature between the two doses of the anaerobic digestate-derived composts could be associated to lower rates of soil organic matter degradation in PSLow and CSLow with regard to the corresponding high-dose treatments. During the degradation of soil organic matter, the CO_2 released from soil respiration depletes $\delta^{13}\text{C}$ while the remaining soil organic matter becomes enriched in $\delta^{13}\text{C}$, relative to the substrate (Risk et al., 2012; Wang et al., 2015). Moreover, high dose amended soils presented a higher amount of most available C fraction (water soluble organic carbon) than PSLow and CSLow, which could explain the highest decomposition activity in these soils. Other studies have also found an increase of soil

respiration as consequence of compost application into the soil (Zhen et al., 2014).

The control soil used in this work had a N concentration of 0.41 g kg^{-1} and was influenced by the input of organic fertilizers, but not by the input of inorganic N fertilizer. Moreover, increasing doses of digestate composts resulted in increased soil nitrogen content. WSN also was higher in soils, which received a high amendment dose compared to all other treatments. Also, soil $\delta^{15}\text{N}$ values of CS and PS amended soils were higher than the value for control soils, whereas soil $\delta^{15}\text{N}$ values were similar in control and soils receiving mineral fertilizers. These results can be explained by the fact that the $\delta^{15}\text{N}$ values of inorganic fertilizers are very low (around atmospheric N, i.e., 0.3663‰) (Bateman and Kelly, 2007; Nishida and Sato, 2015), and therefore do not produce a change in the soil $\delta^{15}\text{N}$ signature. Our results coincided with the idea that both digestate composts had higher $\delta^{15}\text{N}$ ratios than mineral fertilizers and that PS digestate compost presented higher $\delta^{15}\text{N}$ values than CS digestate compost. However, other factors such as soil microbiological activity and microbial degradation of organic matter may have altered these values with regard to control soils. The major soil microbial transformations of organic matter (mineralization and ammonia volatilization) produce N volatile products (NH_3 , N_2 , and N_2O) that are depleted in $\delta^{15}\text{N}$ relative to the substrates (Högberg, 1997; Dawson et al., 2002; Makarov, 2009). Trandel et al. (2018) found that soil nitrogen isotope composition did not show that of the fertilizers used, due to the plant nitrogen uptake and microbiological activity that had occurred before the sampling. Indeed, nitrification of excess nitrogen may lead to increased soil $\delta^{15}\text{N}$ ratios.

On the other hand, carbon assimilation by plants takes place through photosynthesis, however, carbon partitioning between ^{12}C and ^{13}C may occur through different processes such as, CO_2 diffusion through stomata, carboxylation by RuBisCo, respiration and photorespiration.

It is also known that there is a strong link between plant $\delta^{13}\text{C}$ values and soil water content (Klaus et al., 2016), and between the N cycle and the plant-soil C dynamics (Ostle et al., 2009). At this regard Yan et al., 2020 found a correlation between foliar $\delta^{13}\text{C}$ values and foliar $\delta^{15}\text{N}$ values and N concentrations. Nonetheless, plant C is very stable, and they observed just little changes. In our study no difference in leaf C content and/or $\delta^{13}\text{C}$ was found among treatments, indicating that soil fertilization did not alter plant physiological processes. Nogués et al. (2015) already showed that organic amendments did not alter CO_2 assimilation through photosynthesis in *Rosmarinus officinalis* plants.

Many factors influence plant N isotopic compositions. Plant $\delta^{15}\text{N}$ integrates, (1) fractionation processes occurring in the soil and (2) isotopic discrimination during the different physiological mechanisms occurring in the entire plant system that include uptake, assimilation,

allocation and loss of N from plants (Coomstock, 2001; Fogel et al., 2008; Fuertes-Mendizábal et al., 2018). Thus, the application of different N sources to the soil to maintain plant growth could be expected to influence plant $\delta^{15}\text{N}$ signatures, showing the N sources used by the crop.

In our case, $\delta^{15}\text{N}$ resulted to be higher in plants grown on CS compost amended soils than in any other case, though leaves of plant grown in PS compost amended soils also presented a higher $\delta^{15}\text{N}$ content than leaves of plants grown on control and inorganic fertilized soils. These results are in agreement with Choi et al. (2003) and Bateman et al. (2007) who showed that application of organic amendments produced crops with a higher $\delta^{15}\text{N}$ in their tissue than those applied with inorganic fertilizers.

Interestingly, plant $\delta^{15}\text{N}$ signature was higher than those of soil in every treatment. This difference can be considered as a measure of isotopic enrichment during plant growth. Plant-soil $\delta^{15}\text{N}$ difference was 1.83‰ for control, 1.14‰ for inorganic fertilizer, 2.56‰ for CSLow, 1‰ for CSHigh, 6.5‰ for PSLow, and 4.3‰ for PSHigh. At this regard, Trandel et al. (2019) reported that nitrogen fractionation did not occur when conventional inorganic fertilizers were used due to the fact that nitrogen was easily available from the soil source in the form of ammonia and nitrate and consequently the influx of nitrogen into the plant was high. On the contrary to inorganic fertilizers, composts usually had low concentrations of WSN, and the organic nitrogen contained in them undergoes mineralization prior to be available for the crop, making it more susceptible to fractionation (Evans et al., 2001; Bateman et al., 2007). Therefore, we consider that nitrogen contained in the pig slurry digestate-derived compost has undergone more fractionations processes leading to $\delta^{15}\text{N}$ enrichment from the soil to plant leaves than the nitrogen enclosed in control soil, inorganic fertilizer soil and cattle digestate compost. In this context, Bustamante et al. (2019) also reported that the pig slurry digestate derived compost was the treatment that produced a different behavior compared to the rest of treatments in another important characteristic of the rosemary plant leaves, such as the metabolite composition.

5. Conclusions

We investigated whether stable isotope ratios can be used to assess the dynamics of digestate composts in the plant soil system. Our results showed that isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of composting mixtures mirrored mainly that of the corresponding digestates without any contribution from the bulking material (vine pruning), indicating that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values had already stabilized through organic matter decomposition and denitrification processes during the anaerobic digestion of cattle manure and pig slurry, respectively. After soil addition, soils receiving digestate-derived composts presented higher $\delta^{15}\text{N}$ values than control and soils receiving mineral fertilization. In addition, leaves of rosemary plants grown on compost amended soils had higher $\delta^{15}\text{N}$ abundance than those of control and mineral fertilized plants. Thus, we can conclude that stable N ratios may be an adequate tool to trace plant uptake of N derived from anaerobic digestate-derived composts.

Credit author statement

I. Nogués: (Conceptualization) (Investigation) (Data curation) (Validation) (Methodology) (Writing - original draft) (Writing -review & editing) (Funding acquisition); C. Rumpel: (Supervision) (Project administration) (Resources) (Methodology) (Investigation) (Validation) (Writing -review & editing); M. Sebilo: (Supervision) (Methodology) (Investigation) (Validation) (Project administration) (Writing -review & editing) (Resources); V. Vauy: (Investigation) (Methodology) (Validation); R. Moral: (Investigation) (Methodology) (Validation) (Resources); M.A. Bustamante (Conceptualization) (Investigation) (Methodology) (Validation) (Data curation) (Writing - original draft) (Writing -review & editing) (Funding acquisition).

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.117063>.

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