

1 **Composting modifies the patterns of incorporation of OC and N from**  
2 **plant residues into soil aggregates**

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10

11 **Abstract**

12 In order to understand if and how composting modifies the dynamics of incorporation of  
13 organic carbon and nitrogen to soil aggregates during residue decomposition, we studied  
14 samples of a soil amended with fresh or composted plant residues in the laboratory.

15 Samples from the surface horizon of a Luvisol (representative of large agricultural regions  
16 in northern Europe) were amended with fresh or composted <sup>13</sup>C-<sup>15</sup>N-labelled wheat, corn  
17 and rapeseed residues and incubated in the laboratory during three years. Aggregates of

18 samples taken at the initial time, after 45 days and after three years of incubation were  
19 fractionated by size; OC and N concentrations plus isotopic signatures of C and N were  
20 analysed in each aggregate fraction. Both fresh and composted residues addition

21 increased aggregate stability at 45 days, but the effect almost disappeared after three years.

22 The addition of the residues increased OC and N contents with respect to the control soils  
23 after three years. This additional OC and N coming from residues were initially  
24 incorporated preferentially into 0.05-0.2-mm aggregates, as indicated by isotopic

25 signature analyses, and later redistributed homogeneously among all size fractions. After

26 three years of incubation, 11% of plant residue OC remained in soil when these were  
27 added fresh, and 22% if they were previously composted, whereas similar N percentages  
28 (26-27%) remained for fresh or composted residues. Overall, a higher amount of inputs  
29 from plant residues remained in the soil in the long term when they were previously  
30 composted, with respect to non-composted residues.

31 **Keywords:** Soil organic matter;  $^{13}\text{C}$ ;  $^{15}\text{N}$ ; Isotopic fractionation; Soil aggregation;  
32 Compost.

33

### 34 **Introduction**

35 The biological interest of organic matter in ecosystems relies to a great extent on its  
36 decomposition that releases the nutrients and energy that are necessary for plants and  
37 microorganisms (Janzen, 2006), but also in its potential to offset current greenhouse gas  
38 emissions (Paustian et al., 2016) and limit climate change (Smith, 2016). Incorporation  
39 of organic carbon (OC) and nitrogen (N) in soil aggregates during plant residue  
40 decomposition is a key edaphic process with consequences for the global biogeochemical  
41 cycles of these elements. This process is responsible to a great extent for the development  
42 of soil structure, which is one of the specific features of soils, as well as for the protection  
43 and accumulation of soil organic matter (SOM). Aggregate formation and the C cycle are  
44 related through SOM protection and occlusion, as physicochemical protection offered by  
45 aggregate structures and mineral surfaces is critical for building and maintaining soil OC  
46 and N stocks (O'Brien and Jastrow, 2013).

47 The canonical theory of soil aggregation, by Tisdall and Oades (1982), states that  
48 aggregates are formed when input of plant residues to soil stimulates microbial growth  
49 and activity, which generates transient binding agents mostly involving polysaccharides  
50 in the first few weeks. In the modification of the model by Oades (1984), aggregation

51 proceeds firstly by the formation of macroaggregates ( $>250\ \mu\text{m}$ ) due to mineral  
52 associations with particulate organic matter (POM) via temporary binding agents.  
53 Afterwards, microaggregates ( $<250\ \mu\text{m}$ ) are formed once the decomposition of POM  
54 within macroaggregates by microorganisms produces exopolysaccharides and other  
55 metabolites which act as persistent binding agents. In this sense, microaggregates are  
56 increasingly considered as key constituents for soil structure, properties and functions  
57 (Six et al., 2004; Totsche et al., 2018).

58 Thus, aggregate formation and OM protection are intimately related to the dynamics  
59 of organic matter (plant residues, organic amendments) in soil. The amount of fresh  
60 organic matter and its degree of decomposition are controlling the processes involved in  
61 the formation and stabilization of aggregates (Haynes and Beare, 1996). In this sense, the  
62 nature and composition of residues entering the soil are key factors that influence these  
63 processes. The conceptual model by Monnier (1965), reviewed later by Abiven et al.  
64 (2009), explains the differences in the increase of aggregate stability as a consequence of  
65 differences in the composition of the residues. Some products, such as green manure,  
66 produce intense effects on aggregate stability within a month, while less readily  
67 decomposable materials, such as mature manure, have small initial effects but relatively  
68 large effects at the annual scale. Monnier proposed that the short-term effects of organic  
69 matter on aggregate stability are due to the turnover of microbial products and cells, while  
70 the long-term effects are due to compounds in a more advanced stage of decomposition.

71 In this sense, composting plant residues before adding them to soil may affect their  
72 dynamics of decomposition and stabilisation (Lerch et al., 2019). Composting is a process  
73 for stabilization of organic wastes, where OM is progressively processed by  
74 microorganisms and converted into “stable” or mature compost (Zucconi and de Bertoldi,  
75 1987). Obviously, the nature of the process implies a transformation of OM and the decay

76 in soil is different depending on whether residues are composted or not, and even for  
77 composted materials, depending on the degree of maturation (Bernal et al., 1998a;  
78 Francou et al., 2005). Aggregate stability in the long term will depend on the existence of  
79 permanent (non-transient) aggregation agents or binding compounds: their production is  
80 supposed to be enhanced by compost application (Whalen et al., 2003; Annabi et al.,  
81 2007) and might be different with the application of composted and non-composted plant  
82 residues (Annabi et al., 2007).

83 In view of the close linkage between aggregate structure and OM turnover, aggregate  
84 fractionation techniques are regularly used to study the carbon sequestration process  
85 (Blanco-Canqui and Lal, 2004; Six et al., 2004; O'Brien and Jastrow, 2013; Guan et al.,  
86 2015). Stable isotope analysis ( $^{13}\text{C}$  and to a lesser degree  $^{15}\text{N}$ ) is an increasingly used tool  
87 for estimating SOM dynamics (Balesdent and Mariotti, 1996; Ehleringer et al., 2000) and  
88 in association with aggregate fractionation can help determine the precise location of C  
89 and N from plant residues, their evolution and turnover rate in soils (Angers et al., 1997).

90 In order to study the processes of C and N incorporation into soil and of stable  
91 aggregate formation, as well as whether differences exist in these processes due to  
92 composting of plant residues before soil application, we have separated and characterized  
93 aggregates in samples from a three-year incubation experiment of soil with composted  
94 and non-composted isotopically-labelled plant residues (Lerch et al., 2019). We have  
95 measured aggregate stability at two moments of incubation: 45 days (short-medium term)  
96 and three years (long term). The use of isotopically labelled residues ( $^{13}\text{C}$  and  $^{15}\text{N}$ )  
97 allowed looking for differences in the incorporation of OM into aggregates. Our  
98 objectives were to better understand the effect of composting on the dynamics of C and  
99 N in soils and to investigate the potential relationships with aggregate stability.

100

101 **Materials and methods**

102 *Plant material*

103 Plant residues were a mixture of wheat straw, corn stalks and leaves, and rapeseed leaves.  
104 All these residues were enriched highly in  $^{13}\text{C}$ , some were also enriched in  $^{15}\text{N}$ . These  
105 highly enriched plants were mixed with grass clippings and wheat straw with natural  
106 isotopic contents. Boxes containing  $^{13}\text{C}$  and  $^{15}\text{N}$  enriched plant mixtures were placed in  
107 pilot composting devices where natural abundance plant residues were composted during  
108 12 weeks with forced aeration (Doublet et al., 2011). Mass loss during composting was  
109 80% dry weight, 82% for C, 73% for N.

110 The OC contents for fresh and composted residues were 39% and 35%, respectively,  
111 with C/N ratio of 11.3 and 7.7, respectively. The  $^{13}\text{C}$  signatures were 271‰ and 263‰  
112 and the  $^{15}\text{N}$  signatures were 1064‰ and 1473‰, respectively, for composted and fresh  
113 materials. The relative amount of non-cellulosic sugar measured by the acetate alditol  
114 method (Rumpel and Dignac, 2006) was 36 and 28  $\mu\text{g g}^{-1}\text{C}$  for fresh and composted plant  
115 residues, whereas the amounts of lignin were 15 and 25  $\mu\text{g g}^{-1}\text{C}$  for fresh and composted  
116 plant residues, respectively. Further details about the composition of plant residues, the  
117 composting process and analysis of the materials can be found in Lerch et al. (2019).

118

119 *Soil incubation set up*

120 The soil is a silt loam (7% sand, 76% silt, 17% clay) Haplic Luvisol (IUSS Working  
121 Group WRB, 2014), with 8.9  $\text{g kg}^{-1}$  of total organic carbon, 1.1  $\text{g kg}^{-1}$  total N, and a pH  
122 of 6.9, obtained from the surface layer (0-28 cm) of an experimental plot located at  
123 Feucherolles (France). The bulk isotopic compositions were  $-24.8\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$  and  
124  $5.9\pm 0.2\text{‰}$  for  $\delta^{15}\text{N}$ . Soil samples (125 g dry weight equivalent) were introduced in serum  
125 bottles with Teflon® rubber stoppers crimped on with aluminium seals and amended

126 either with 360 mg of composted plant residues or 330 mg of non-composted plant  
127 residues, corresponding to a C addition of 11.5% of the soil OC. Control samples did not  
128 receive any amendment. Every sample moisture was adjusted to pF 2 (approximately 0.25  
129 g H<sub>2</sub>O g<sup>-1</sup>) and incubated at 20°C in the dark for 3 years. A whole description of the  
130 incubation set up and analyses performed during the incubation can be found in Lerch et  
131 al. (2019).

132

### 133 *Aggregate fractionation and analysis*

134 Samples from the incubation were selected for analysis at three times: initial, 45 days,  
135 three years, in order to follow the effect of the amendments in the medium and long-term.  
136 Three replicates for each incubation duration were used. The study of aggregate  
137 composition was performed following the same procedure as in Paradelo et al. (2016).  
138 Water-stable aggregates were fractionated according to sizes using the slow wetting test  
139 under controlled tension (Le Bissonnais, 1996), assumed to mime soil rehydration by  
140 gentle rain in field conditions. It is less destructive than fast wetting and enhances better  
141 discrimination between little stable and unstable soils which is the case for the agricultural  
142 silty soils developed on loess which are typical of the region (Jamagne, 2011). Briefly,  
143 five grams of calibrated air-dried aggregates (3.15–5 mm) were placed on a filter paper  
144 on a tension table and maintained at a matrix potential of -0.3 kPa for 60 min. It is  
145 important to say that the C and N composition of the calibrated aggregates is not different  
146 from that of the bulk soil (Lerch et al. 2019). Once moisture equilibrated, aggregates were  
147 transferred on a 0.05-mm sieve immersed in ethanol for the determination of aggregate  
148 size distribution. Ethanol was used for wet sieving because it reduces additional  
149 breakdown due to its low surface tension. The 0.05-mm sieve was gently moved five  
150 times with a ‘Hénin’ apparatus (Feodoroff, 1958; Hénin et al., 1958) to separate

151 aggregates <0.05 mm from those >0.05 mm. Then the >0.05-mm fraction was collected,  
152 oven-dried and gently dry-sieved by hand using a column series of six sieves: 2, 1, 0.5,  
153 0.2, 0.1 and 0.05-mm mesh. Mass percentages of aggregate-size fractions were then  
154 calculated. The mass of the <0.05-mm fraction was evaluated as the difference between  
155 the initial mass and the sum of the six other fractions. The aggregate mean weight  
156 diameter (MWD) for each sample was also calculated. Given that all samples come from  
157 the same soil, sand corrections were not performed. Three replicate fractionations were  
158 performed for each treatment.

159 The aggregate fractions were milled (<0.2 mm), and OC and N contents were  
160 measured by dry combustion in a CHN autoanalyser (Carlo Erba NA 1500). Carbon and  
161 nitrogen isotopic contents were measured by EA-IRMS (NA-1500, Carlo-Erba) and  
162 expressed with  $\delta$  notation on a per mil basis (‰), as follows:

163

$$164 \quad \delta(\text{‰}) = \frac{R_s - R_{std}}{R_{std}} \cdot 1000$$

165

166 where  $R_s$  is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  isotope ratios of the sample and  $R_{std}$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio  
167 of the VPDB standard (Coplen, 1995) or  $^{15}\text{N}/^{14}\text{N}$  ratio of atmospheric  $\text{N}_2$  (Mariotti, 1983).  
168 Precision of measurements was 0.1% for  $\delta^{13}\text{C}$  and 0.2% for  $\delta^{15}\text{N}$ .

169 In the amended soils, the contribution of labelled plant residues to aggregate OC and  
170 N was calculated as follows:

171

$$172 \quad C_R = 100 \cdot \frac{\delta^{13}C_{AS} - \delta^{13}C_{CS}}{\delta^{13}C_R - \delta^{13}C_{CS}}$$

173

$$174 \quad N_R = 100 \cdot \frac{\delta^{15}N_{AS} - \delta^{15}N_{CS}}{\delta^{15}N_R - \delta^{15}N_{CS}}$$

175

176 where  $C_R$  and  $N_R$  are the percentages of soil OC or N coming from added plant residues,  
177 AS is the amended soil and CS is the control soil.

178

### 179 *Statistics*

180 Linear regression analysis and mixed-model ANOVA were performed in order to test the  
181 effect of aggregate size, time and treatment on aggregate composition ( $p < 0.05$ ). The  
182 homogeneity of variance was tested using the Levene test, and the normality of residuals  
183 was also checked applying the Shapiro-Wilk test to the residuals. All statistical tests were  
184 conducted using the R statistical package for MacOSX (R Core Team, 2018).

185

### 186 **Results**

187 Table 1 shows the bulk composition of the 3.15-5 mm aggregates used in the aggregate  
188 stability test. After 45 days, the OC content of the aggregates of soil amended with non-  
189 composted residues was slightly higher than that of the control soil. After 3 years,  
190 aggregates of soils amended with plant residues, composted or not were richer in OC than  
191 those of the control soil. OC concentrations decreased with time in all treatments, but  
192 decline was less marked in the amended soils: between 45 days and 3 years, 19% of initial  
193 OC was lost from the control soil, 16% from the soil amended with plant residues, and  
194 7% from the compost-amended soil. Nitrogen contents were similar in the 3.15-5 mm  
195 aggregates of all treatments at all analysed times. The C/N ratios thus decreased with time  
196 due to differential consumption of C and N by microorganisms. Isotopic  $^{13}\text{C}$  and  $^{15}\text{N}$   
197 signatures became less negative with either residue or compost inputs, and in all cases,  
198 values decreased with time. After 45 days, 4.5 and 6.7% of the aggregate bulk OC and  
199 7.7 and 8.6% of the N originated from the non-composted and composted amendment,

200 respectively. After 3 years, these proportions were 2.7 and 5.2% (OC) and 8.2 and 10%  
201 (N) for plant residue and compost addition, respectively.

202 Table 2 shows the size distribution of stable aggregates after the stability test. The  
203 trends for the control and amended soils were very similar. Large aggregate fractions (>  
204 0.5 mm) contributed more to the total aggregates at 45 days than after three years for all  
205 treatments. Soils are unstable to very unstable according to the classification of Le  
206 Bissonnais and Le Souder (1995), a typical feature of agricultural soils developed on loess  
207 at the Paris Basin. Aggregate stability, indicated by the values of MWD, decreased with  
208 the incubation time.

209 For the control soil, the contribution of 0.5-2 mm aggregates increased at the expense  
210 of <0.5 mm fractions between 0 and 45 days of incubation, but this effect was no longer  
211 observed in the long term. This short-term effect was likely due to an increase in the  
212 microbial activity as a consequence of the better cultivation conditions at the start of the  
213 laboratory incubations. After 45 days, the non-aggregated soil (<0.05 mm) decreased in  
214 all treatments, from 21% in the control soil at t=0 to 12.1 % in the control and 9.8-14.8%  
215 in the amended soils. This fraction increased again after three years. The addition of both  
216 amendments increased soil aggregate stability (MWD increasing from 0.58 in the control  
217 to 0.73-0.79 in the amended soils) after 45 days, without any significant difference  
218 between soil amended with composted and non-composted residues. However, this  
219 aggregating effect of amendments did not last, and at the end of the incubation (three  
220 years), the aggregate stability of the amended soils was not significantly different from  
221 that of the control soil. After three years, the contribution of the 0.5-1 mm size fraction  
222 of the soil amended with non-composted plant residues was higher compared to control  
223 and soil amended with compost, at the expense of 0.2-0.5 mm and 0.1-0.2 mm size  
224 fractions.

225 Figure 1 shows the OC contents and  $^{13}\text{C}$  isotopic signatures of aggregate size fractions  
226 at the three sampling dates (0, 45 days, 3 years). In the control soil at  $t=0$  and after 45  
227 days, the composition of aggregates of different sizes was very homogeneous. After three  
228 years of incubation, an increase of the OC content of the larger aggregates was observed  
229 in the control soil. By contrast in the amended soils, differences in the OC contents among  
230 aggregate fractions were observed after 45 days of incubation, while they were similar  
231 after three years. In all fractions, the OC concentrations in the amended soils generally  
232 decreased with time. Regarding the isotopic signature of OC,  $\delta^{13}\text{C}$  values were higher in  
233 the amended soils with respect to the control. Similar to OC concentrations, differences  
234 between  $^{13}\text{C}$  contents of the size classes of the amended soils were larger at 45 days than  
235 after three years. The highest  $\delta^{13}\text{C}$  values were observed in small aggregates from 0.05 to  
236 0.2 mm, indicating that OC coming from the decomposition of plant residues was mostly  
237 incorporated to those fractions. In general, the incorporation of composted plant residues  
238 had a larger effect on the isotopic signature of the fractions compared to non-composted  
239 ones.

240 Figure 2 shows the N contents and  $^{15}\text{N}$  isotopic signatures of aggregate size fractions.  
241 In the control soil, although the N contents of the aggregates of different sizes were very  
242 similar, they tended to increase with aggregate size at all incubation times. In the amended  
243 soils, the trend of N content increase with aggregate size was only observed for the small  
244 aggregates ( $<0.5$  mm). A decrease of the N content of the fractions with time was  
245 observed for the amended soils. The  $^{15}\text{N}$  isotopic signature was higher in the amended  
246 soils with respect to the control in all aggregate classes, but in particular in small  
247 aggregates from 0.05 to 0.2 mm. As already noted for  $^{13}\text{C}$ , larger differences in  $\delta^{15}\text{N}$  were  
248 observed between aggregate size classes after 45 days (from 33 to 178‰), than after three

249 years (from 44 to 97%). As for OC, the incorporation of composted plant residues had a  
250 stronger effect on  $^{15}\text{N}$  isotopic signature compared to non-composted ones.

251 Figures 3 and 4 show the contribution of each aggregate fraction to total OC and N of  
252 the soil. Given that the differences in OC and N composition between aggregate classes  
253 were small (Figure 1 and 2), the contribution of each aggregate size was mostly inherited  
254 from the size distribution. Overall, most soil OC and N was present in the aggregate  
255 fractions between 0.2 and 1 mm, with differences among treatments. Initially, most OC  
256 and N was found in the 0.5-1 mm aggregates. In the non-amended control soil, the  
257 contribution of the 0.5-1 and 1-2 mm aggregates increased between 0 and 45 days of  
258 incubation, at the expenses of the fraction smaller than 0.05 mm. An opposite change is  
259 observed between 45 days and three years of incubation, even leading to a decrease of  
260 the contribution of the 0.5-2 mm aggregates with respect to their initial contribution. After  
261 three years, in the control soil, the 0.2-0.5 mm fraction represented the largest  
262 contribution to bulk soil OC and N. In the amended soils, the trends were similar to those  
263 described for the control soil, although the contribution of the 1-2 mm fraction after 45  
264 days was higher than in the control when composted residue are applied. After three years,  
265 with non-composted plant residues, the largest OC and N contributions were found in the  
266 0.5-1 mm fraction.

267 Figure 5 shows the distribution of OC and N originating from the residues in the  
268 aggregate size fractions of the amended soils after 45 days and three years of incubation.  
269 After 45 days, the distributions of C and N in size fractions were identical for composted  
270 or non-composted residues: most of the amendment C and N were recovered in the 0.5-1  
271 mm fraction in both cases. After three years, the distribution in aggregate size fractions  
272 changed and C and N from the amendments became more evenly allocated among  
273 fractions. After three years, OC and N distributions are different when the residues are

274 composted or not, maximizing in the 0.5-1 mm fraction for non-composted residues and  
275 in the 0.2-05 mm fraction for composted ones.

276 During the incubation there was an overall decrease of C and N with time, and after  
277 three years total C and N remaining from the added non-composted residues were 11.3%  
278 and 26.9%, respectively. The proportions of C and N remaining from the composted  
279 residues after three years were 26.9 and 36.3%, respectively. When we take into account  
280 the C and N loss during composting, we find however that the amounts of OC and N that  
281 remain from the initial fresh residues after composting and three year incubation in soil  
282 are 22.1% and 26.5%, respectively. Overall, N was incorporated to a higher extent than  
283 C, and organic C and N from compost were more incorporated than from fresh residues.

284

## 285 **Discussion**

286 Increasing the organic matter content of agricultural soils is necessary in the current  
287 context of intensive agricultural practices that produce drastic decreases in SOM, as well  
288 as from the point of view of global cycles and the role of soil as a sink for atmospheric  
289 carbon (Dignac et al., 2017). Soil organic matter management is particularly important in  
290 the agricultural soils of the Paris sedimentary basin studied here. These intensively  
291 cultivated soils, developed on loess, are very prone to erosion and sealing due to weak  
292 soil structure, a consequence of silt-dominated textures with low clay contents, and the  
293 loss of SOM due to continuous cropping (GIS Sol, 2011; Jamagne, 2011; Darboux and  
294 Algayr, 2013). All these characteristics are common to large agricultural areas in  
295 Northern Europe and, in this context, an increase of organic matter is essential. Orienting  
296 agricultural practices towards organic matter increase necessitates to understand the fate  
297 and dynamics of the OC and N added to soils through return of plant residues to soils and  
298 through organic amendment applications.

299 In our incubation experiments, the addition of plant residues to the surface horizon of  
300 a Luvisol produced an enduring increase of soil OM contents (OC and N), as well as a  
301 transient increase of aggregate size (mainly the 1-2-mm aggregates) and stability. The  
302 stability increase, however, was only observed on the short term (45 days) and did not  
303 last for the whole duration of the incubation (three years). Since organic matter is the  
304 main aggregating agent in these low-stability soils (Puget et al., 1995, 2000; Paradelo et  
305 al., 2016), and its content progressively decreased with time due to mineralization during  
306 the incubation, this temporary effect of inputs on aggregate stability was expected. On  
307 the other hand, the degree of residue decomposition has been shown to influence its effect  
308 on aggregate stability (Annabi et al., 2007; Abiven et al., 2009, Le Guillou et al., 2011).  
309 In general, easily decomposable products have an intense and transient effect on  
310 aggregate stability, whereas more recalcitrant products have a lower- but longer-term  
311 effect (Abiven et al., 2009). Immature and mature composts have been shown to both  
312 increase aggregate stability, but through different mechanisms: increase of microbial  
313 activity for immature composts containing high contents of labile OC and diffusion of  
314 organic binding substances within the aggregates for mature composts (Annabi et al.,  
315 2007). In the Feucherolles long term observatory, C stored in the clay fractions after 15  
316 year soil amendment with green waste compost was attributed to C sequestration as  
317 microbial compounds (Paetsch et al., 2016). Since composting increases the stability of  
318 plant residues by changing its chemical composition (Zucconi and de Bertoldi, 1987), the  
319 increase in aggregate stability after 45 days was higher, as expected, for non-composted  
320 compared to composted plant residues and the reverse after three years.

321 Despite the relatively low input amounts, a significant fraction of the OC and N from  
322 the added residues still remained in the soil after three years of soil incubation: 11.3% of  
323 OC and 26.8% of N from the fresh residues, and 26.9% of OC and 36.3% of N added

324 from the compost. After three years, the OC and N remaining in soil from the compost  
325 represented 22.1% and 26.5% of the original residue OC and N, respectively. More than  
326 half of added C and N was lost in less than 45 days, but the rest was lost at a much lower  
327 pace. The rates of C and N loss were different, with a preferential loss of C, a common  
328 path given that C/N ratios in plant residues are higher than in microorganisms (Van  
329 Groeningen et al., 2017). The distribution of the remaining C and N in the aggregate size  
330 fractions after three years was very similar: this could suggest that long-term dynamics  
331 of C and N between aggregates are also similar. Composting had an effect on these  
332 dynamics, since our results suggest that compost C and N remained longer in soil and  
333 contributed more to bulk soil OC and N than C and N from non-composted residues.

334 The rates of C and N loss were different when the residues were composted or not. In  
335 the case of C, composting increased the proportion of remaining C after 45 days and three  
336 years, whereas similar proportions of the initial residue N were remaining from  
337 composted or fresh residues, indicating that changes in organic composition during  
338 composting has more influence on C dynamics than on N dynamics. Therefore, our results  
339 show that plant residue C remains longer in soil when they are composted before soil  
340 application, in agreement with other studies comparing decomposition of fresh and  
341 composted residues in similar soil types (Pascual et al., 1999; Bernal et al., 1998b).

342 Regarding the distribution of the exogenous organic matter into soil aggregates, OC  
343 and N were incorporated in all size fractions of water-stable aggregates, regardless of  
344 being composted or not. In the short term, it was incorporated unevenly in all aggregate  
345 fractions, with most soil OC and N found within the 0.5-1 mm fraction, whereas in the  
346 long term the 0.2-0.5 mm fraction became the main contributor, although the distribution  
347 showed less variations among sizes (Figures 1-2). Therefore, OC and N from plant  
348 residues incorporated to the soil are first creating larger stable aggregates and are later

349 broken down, redistributing OC and N among aggregate size classes. This redistribution  
350 leads to an overall transfer of OM from larger to smaller aggregates (Figures 3-4), and to  
351 an increase of the 0.2-0.5 and <0.05 mm fractions after three years for both C and N. This  
352 is in agreement with other studies that also observed that organic matter is initially  
353 incorporated into coarse stable aggregates, and progressively redistributed among all  
354 aggregate classes with time (Angers et al., 1997; Puget et al., 2000; Guan et al., 2015).  
355 Therefore, our results indicate that C occlusion in microaggregates only happens after  
356 occlusion at the macroaggregate scale, in agreement with the aggregation model by Oades  
357 (1984).

358 In terms of C balance, if we take into account losses during composting, 22% of C  
359 initial residue C remains in soil when composted and 11% when fresh residues are directly  
360 applied to soil. Thus, the loss of OC from plant residues considering both composting and  
361 mineralization in soil was 78% of the initial OC, whereas loss of OC from non-composted  
362 residues was 89%. Therefore, composting reduces the loss of C. Furthermore, our  
363 findings that OC and N from composted residues are preferentially stored in  
364 microaggregates on the long term might further reduce organic matter losses by protecting  
365 C and N from decomposition by improving soil water repellency (Goebel et al., 2011),  
366 reducing soil erosion and limiting priming effect (Lerch et al., 2019). Storage of N in  
367 microaggregates might also limit N losses by leaching and favour nutrient uptake by crops  
368 from different size aggregates.

369

## 370 **Conclusions**

371 A study of aggregate stability and C and N incorporation to aggregates after amendment  
372 of a silty-loam Luvisol with composted and non-composted plant residues was performed  
373 using an isotopic tracer technique ( $^{13}\text{C}$  and  $^{15}\text{N}$ -labeled wheat and maize residues). The

374 addition of plant residues produced an enduring increase of SOM content (OC and N), as  
375 well as a transient increase of aggregate stability. Exogenous OM was incorporated to  
376 soil in a non-homogeneous manner that is related to the three-dimensional structure of  
377 soil: this is, during amendment decomposition, C and N incorporate differently to  
378 different size aggregates. In the short term, they were mainly stored in aggregates larger  
379 than 0.5 mm (macroaggregates) but after three years of incubation a shift happened and  
380 C and N were transferred to smaller aggregates. Therefore, on the long term, OM was  
381 transferred from larger aggregates to smaller aggregates. Microaggregates were more  
382 enriched with amendment C and N with the composted residues compared to the non-  
383 composted plant residues. This might improve soil functioning by limiting losses of C  
384 and nutrients through leaching or erosion. Overall, the fact that more C and N from initial  
385 plant residues are transferred to the soil when they are composted than when they were  
386 not and that these C and N were preferentially transferred in the smaller fractions of the  
387 soil, suggests that composting is a better option than using fresh materials from the point  
388 of view of C sequestration and nutrient preservation in soils.

389

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393

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507

508 **FIGURE CAPTIONS**

509

510

511 Figure 1. Organic C concentrations and isotopic signature in aggregate size classes. Bars  
512 show confidence intervals at  $P < 0.05$ , and different letters denote significant differences  
513 between treatments at  $P < 0.05$  (when letters are not shown it means that no significant  
514 differences were found). Black letters are for C contents and white letters for isotopic  
515 signatures.

516

517 Figure 2. Nitrogen concentrations and isotopic signature in aggregate size classes. Bars  
518 show confidence intervals at  $P < 0.05$ , and different letters denote significant differences  
519 between treatments at  $P < 0.05$  (when letters are not shown it means that no significant  
520 differences were found). Black letters are for C contents and white letters for isotopic  
521 signatures.

522

523 Figure 3. Contribution of each aggregate size fraction to soil OC stocks. The striped  
524 section of the bars indicates the amount of soil OC that comes from the plant residues  
525 added (please note that these values have been multiplied by a factor of ten, for clarity).  
526 Bars show confidence intervals in the t-test at  $P < 0.05$ .

527

528 Figure 4. Contribution of each aggregate size fraction to soil N stocks. The striped  
529 section of the bars indicates the amount of soil N that comes from the plant residues  
530 added (please note that these values have been multiplied by a factor of ten, for clarity).  
531 Bars show confidence intervals in the t-test at  $P < 0.05$ .

532

533

534 Figure 5. Fractions of OC and N remaining in the soil from plant residues (left, non-

535 composted plant residues; right, composted plant residues) as a function of aggregate size.

536

537 Table 1. Composition of the soil used for the study. PR: plant residue, CPR: composted  
 538 plant residue. TOC: total organic carbon.

Time		TOC (mg g <sup>-1</sup> )	N (mg g <sup>-1</sup> )	C/N	$\delta^{13}\text{C}_{\text{VPDB}}$ (‰)	$\delta^{15}\text{N}$ (‰)	Fraction of soil OC coming from plant residues (%)	Fraction of soil N coming from plant residues (%)
0	Control	10.2	1.0	10.1	-25.0	4.8	-	-
45 d	Control	9.8	1.0	10.0	-25.5	6.3	-	-
	PR	10.2	1.0	9.9	-12.5	119	4.5	7.7
	CPR	9.6	1.1	9.0	-5.7	97	6.7	8.6
3 y	Control	7.9	0.9	8.5	-25.0	5.1	-	-
	PR	8.6	1.0	8.3	-17.2	125	2.7	8.2
	CPR	8.9	1.1	8.2	-9.5	111	5.2	10.0

539

540

541 Table 2. Aggregate size distribution (% weight) and mean weight diameters (MWD).  
 542 Mean  $\pm$  standard deviation (n=3). PR: plant residue; CPR: composted plant residue.  
 543 Different letters denote significant differences between treatments at  $p < 0.05$  (when letters  
 544 are not shown it means that no significant differences were found).

Time	Treatment	Aggregate size (mm)							MWD (mm)
		2-3.15	1-2	0.5-1	0.2-0.5	0.1-0.2	0.05-0.1	< 0.05	
0	Control	0.9 $\pm$ 0.4	9.5 $\pm$ 1.9ac	38.1 $\pm$ 3.4b	23.1 $\pm$ 3.0ab	4.2 $\pm$ 0.4a	3.2 $\pm$ 0.2ab	21.0 $\pm$ 7.5	0.55 $\pm$ 0.06ac
45 d	Control	0.6 $\pm$ 1.0	16.3 $\pm$ 4.2ac	42.7 $\pm$ 5.2b	20.2 $\pm$ 3.2ab	3.9 $\pm$ 0.2a	3.1 $\pm$ 0.3ab	12.1 $\pm$ 8.0	0.58 $\pm$ 0.12ac
	PR	1.2 $\pm$ 0.4	21.1 $\pm$ 2.6bc	41.2 $\pm$ 2.4b	15.2 $\pm$ 2.6a	3.3 $\pm$ 0.4a	3.1 $\pm$ 0.4ab	14.8 $\pm$ 2.7	0.73 $\pm$ 0.17bc
	CPR	1.8 $\pm$ 0.9	22.6 $\pm$ 6.7c	43.1 $\pm$ 2.8b	16.6 $\pm$ 3.9a	3.6 $\pm$ 1.7a	2.6 $\pm$ 0.3a	9.8 $\pm$ 4.8	0.79 $\pm$ 0.10c
3 y	Control	0.4 $\pm$ 0.2	3.8 $\pm$ 2.4a	22.7 $\pm$ 5.0a	32.5 $\pm$ 2.1c	7.7 $\pm$ 0.6bc	5.1 $\pm$ 0.3bc	27.8 $\pm$ 8.2	0.38 $\pm$ 0.07a
	PR	0.2 $\pm$ 0.2	8.8 $\pm$ 0.4ab	32.9 $\pm$ 3.2ab	26.6 $\pm$ 3.8bc	5.7 $\pm$ 1.1ab	5.0 $\pm$ 1.0bc	20.9 $\pm$ 2.2	0.49 $\pm$ 0.02ab
	CPR	0.2 $\pm$ 0.2	4.1 $\pm$ 3.6a	25.7 $\pm$ 4.7a	33.9 $\pm$ 3.0c	8.9 $\pm$ 1.3c	6.8 $\pm$ 1.7c	20.4 $\pm$ 3.2	0.40 $\pm$ 0.08a

545