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## **Identifying soil organic carbon fractions sensitive to agricultural management practices**

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25

26 **Abstract**

27 Agricultural management practices play a major role in the process of SOC  
28 sequestration. However, the large background of stable carbon (C) already present in the  
29 soil and the long period of time usually required to observe changes in soil organic  
30 carbon (SOC) stocks have increased the necessity to identify soil C fractions with a fast  
31 response to changes in agricultural management practices. Consequently, we quantified  
32 the response of total SOC, permanganate oxidizable organic carbon (POxC), particulate  
33 organic carbon (POC) and the carbon concentration of water-stable macroaggregates,  
34 microaggregates within macroaggregates and the silt-plus clay-sized fraction (M-C,  
35 mM-C, s+cM-C, respectively) to changes in management. We chose a long-term tillage  
36 and N fertilization field experiment (18 years) located in NE Spain. In the first 5 cm  
37 depth under no-tillage (NT) compared with conventional tillage (CT), the POxC fraction  
38 and total SOC increased similarly (about 59%). However, other C pools studied (i.e., M-  
39 C, M-POxC, mM-C, POC and s+cM-C) had lower increases with values ranging from  
40 17% to 31%. For the 5-20 and 20-40 cm soil depths, the POC was the most sensitive  
41 fraction to tillage with 46% and 54% decrease when NT was compared to CT,  
42 respectively. Likewise, the POC fraction presented the highest response to N  
43 fertilization in the three depths studied (i.e. 0-5, 5-20 and 20-40 cm). The mM-C and  
44 s+cM-C fractions presented the lowest sensitivity to changes in tillage and N  
45 fertilization management. Our results showed that the POC fraction had the greatest  
46 sensitivity to changes in agricultural management practices, proving its ability as an  
47 early indicator of optimized practices to sequester C in soil.

48 **Abbreviations:**

49 CT: conventional tillage; M-C: dichromate oxidizable organic carbon of the  
50 macroaggregates; mM-C: dichromate oxidizable organic carbon of the microaggregates  
51 within macroaggregates; M-POxC: permanganate oxidizable organic carbon of the  
52 macroaggregates; NT: no-tillage; POC: particulate organic carbon; POxC:  
53 permanganate oxidizable organic carbon; s+cM-C: dichromate oxidizable organic  
54 carbon of the silt-plus clay-sized soil particles of the macroaggregates.

55 **Keywords:** agricultural management-sensitive C fractions; particulate organic carbon;  
56 permanganate oxidizable carbon; soil organic carbon.

57

58 **Introduction**

59 Soils are the largest terrestrial pool of organic C with over 1550 Pg (Batjes, 1996). C  
60 sequestration in soils has been pointed out as a viable mechanism for reducing the  
61 concentration of carbon dioxide in the atmosphere (Lal, 2004). Moreover, soil organic  
62 carbon (SOC) can also improve plant productivity due to its effects on soil fertility and  
63 quality. Agricultural management practices play a major role in the process of SOC  
64 sequestration. A key issue when studying the effects of those practices on SOC levels is  
65 the period of time needed to observe changes in C stocks. This period is normally on a  
66 long-term time-scale (> 10 years). Moreover, the large background of stable organic C  
67 that is already present in the soil limits the opportunity to identify management-induced  
68 changes over short periods of time (Gregorich et al., 1994; Haynes, 2000). Both  
69 drawbacks limit our ability of testing which agronomic practices have a positive effect  
70 on SOC increase. Thus, in this context, the use of different soil C fractions with an  
71 earlier response to changes in management compared to total SOC has been pointed out  
72 as an efficient tool to identify optimized agricultural practices that increase the stock  
73 and quality of soil C. Different labile SOC pools, such as dissolved organic carbon,  
74 microbial biomass carbon and permanganate-oxidizable carbon, have recently received  
75 attention due to their sensitivity to agricultural management practices (Culman et al.,  
76 2012; Lucas and Weil, 2012). For instance, permanganate-oxidizable organic carbon  
77 has been suggested to be a more sensitive indicator than bulk SOC to tillage-induced  
78 changes (Weil et al. 2003; Melero et al. 2009). However, while fractions like microbial  
79 biomass C and N have been extensively reviewed, the significance of other fractions  
80 (e.g, permanganate-oxidizable organic carbon or particulate organic matter) is not fully  
81 well-understood (Haynes, 2005).

82 The objective of the present work was to identify which soil C fractions were most  
83 sensitive to changes in agricultural management practices. Sensitivity was implied to  
84 impart more rapid changes as early indicators of change. For this purpose we considered  
85 tillage and N fertilization as target management practices because of their impact on  
86 SOC sequestration and the abundance of literature related with these two practices  
87 (West and Post, 2002; Alvarez, 2005).

## 89 **Materials and methods**

90 We used a long-term tillage and N fertilization experiment established in 1996 in NE  
91 Spain (Agramunt, 41° 48' N, 1° 07' E). Mean annual precipitation and ETo are 430 and  
92 855 mm, respectively. Selected soil properties at the beginning of the experiment in the  
93 0-28 cm soil layer (Ap horizon) were: pH (H<sub>2</sub>O, 1:2.5): 8.5, EC<sub>1:5</sub> (dS m<sup>-1</sup>): 0.15,  
94 CaCO<sub>3</sub> eq. (%): 40 and sand (2000-50 μm), silt (50-2 μm) and clay (<2 μm) content:  
95 465, 417 and 118 g kg<sup>-1</sup>, respectively. The soil was classified as a Typic Xerofluvent  
96 (Soil Survey Staff, 1994). Two types of tillage (NT, no-tillage, and CT, conventional  
97 intensive tillage with moldboard plowing) and two N fertilization rates (0 and 60 kg N  
98 ha<sup>-1</sup>) were compared in a randomized block design with three replications. Plot size was  
99 50 m x 6 m. The NT treatment consisted of a total herbicide application (1.5 L 36%  
100 glyphosate per hectare) for controlling weeds before sowing. The CT treatment  
101 consisted of one pass of a moldboard plow to 25 cm depth immediately followed by one  
102 or two passes with a cultivator to 15 cm depth, both in September. Nitrogen fertilizer  
103 was manually-applied and split into two applications: one-third of the dose before  
104 tillage as ammonium sulphate (21% N) and the rest of the dose at the beginning of  
105 tillering, as ammonium nitrate (33.5% N). Planting was performed in November with a  
106 disk direct drilling machine set to 2-4 cm. The cropping system consisted of a barley  
107 monocropping, as is traditional in the area. Prior to the setting up of the experiment, the  
108 historical management of the field was based on conventional intensive tillage with  
109 moldboard plowing and small grain cereals monoculture.

110 Soil sampling was performed in July 2012, right after crop harvest. For each plot, two  
111 soil pits of 0.5 m depth and 20 m apart were opened. In each pit, a composite sample  
112 was collected from three samples randomly selected. Soil samples were obtained using

113 a flat spade in three soil layers from 0 to 40 cm depth (0-5, 5-20 and 20-40 cm) and  
114 stored in crush-resistant airtight containers. Once in the laboratory, soil samples were  
115 gently sieved with an 8 mm-sieve and air-dried at room temperature. For each sample,  
116 water-stable macroaggregates ( $> 0.250$  mm) were obtained using the wet sieving  
117 method described by Elliott (1986), oven-dried at  $50$  °C during 24 h, weighed and  
118 stored. The microaggregates contained within macroaggregates were isolated by  
119 methodology described by Six et al. (2000). The microaggregates and other particles  
120 smaller than  $<0.250$  mm were washed onto a  $0.050$  mm screen by a continuous flow of  
121 water. The material on the  $0.050$  mm sieve was sieved in order to separate the stable  
122 microaggregates from the silt-plus-clay-sized material. Finally, the material on the  
123  $0.250$  mm sieve (considered particulate organic matter), the microaggregates within  
124 macroaggregates and the silt-plus-clay-sized particles ( $<0.053$  mm) were oven-dried at  
125  $50$  °C during 24 h and weighed.

126 The sand content of the macroaggregates and the microaggregates within  
127 macroaggregates was determined by dispersing a 5 g subsample of each one of these  
128 fractions in a 5% sodium hexametaphosphate. The organic C concentration of the bulk  
129 soil (SOC), the water-stable macroaggregates (M-C), the microaggregates within  
130 macroaggregates (mM-C) and the silt-plus-clay-sized fractions ( $<0.053$  mm) (s+cM-C)  
131 were determined using the dichromate wet oxidation method described by Nelson and  
132 Sommers (1996). The particulate organic C of the water-stable macroaggregates (POC)  
133 was calculated subtracting the amount of C in the microaggregates within  
134 macroaggregates (mM-C) and in the silt-plus-clay-sized fraction (s+cM-C) to the  
135 amount of C contained in the water-stable macroaggregates (M-C).

136 The permanganate oxidizable organic C of the bulk soil (POx C) and of the water-stable  
137 macroaggregates (M-POx C) were measured according to the method of Weil et al.  
138 (2003) and quantified by:

$$139 \text{ POx C (mg kg}^{-1}\text{soil) =}$$
$$140 = (0.02 \text{ mol L}^{-1} - (a + b \times \text{Abs})) \times (9000 \text{ mg C mol}^{-1}) (0.02 \text{ L solution} \times W^{-1})$$

141 where a is the intercept and b is the slope of the calibration obtained with the standards,  
142 Abs is the absorbance of the sample and W is the weight (kg) of the soil used.

143 The variation of each soil C fraction when using contrasting agricultural management  
144 practices was calculated. In the first case, the variation of each fraction when using NT  
145 in comparison to CT was calculated by:

$$146 \% \text{ variation}_{\text{fraction}} = ((\text{C pool})_{\text{NT}} - (\text{C pool})_{\text{CT}}) / (\text{C pool})_{\text{CT}} * 100$$

147 where (C pool)<sub>NT</sub> and (C pool)<sub>CT</sub> refer to the different C pools studied under NT and  
148 CT, respectively.

149 In the second case, the variation of each C pool when applying 60 kg mineral N ha<sup>-1</sup>  
150 compared to the control (0 kg N ha<sup>-1</sup>) was calculated by:

$$151 \% \text{ variation}_{\text{fraction}} = ((\text{C pool})_{60} - (\text{C pool})_0) / (\text{C pool})_0 * 100$$

152 where (C pool)<sub>60</sub> and (C pool)<sub>0</sub> refer to the different C pools studied under 60 and 0 kg  
153 mineral N ha<sup>-1</sup>, respectively.

154 The relationships among C fractions for a kg of whole soil were calculated by linear  
155 regression analyses with Sigmaplot 11 (Systat Software, 2008).

156

## 157 **Results and discussion**

158 For the two tillage treatments considered (i.e., NT and CT), significant linear  
159 relationships were observed between SOC and all the different soil C fractions studied  
160 (POxC, M-POxC, M-C, mM-C, POC and s+cM-C) (Fig. 1). The only exception was the  
161 POC fraction in CT. In general, according to the  $R^2$  values obtained, CT showed lower  
162 relationships than NT (Fig. 1). It has been concluded that CT results in a reduction in  
163 the proportion of labile fractions of C therefore increasing the proportion of the more  
164 recalcitrant C fractions (Zhao et al. 2012). The linear relationship between SOC and  
165 POxC under the NT management presented the highest  $R^2$  value ( $R^2$ : 0.95  $p < 0.001$ ) in  
166 agreement with previous studies (Chen et al. 2009; Culman et al. 2012). This result  
167 suggests the usefulness of the permanganate method that also eliminates the potential  
168 hazards related to the use of the dichromate (Bowman, 1998).

169 When considering the first 5 cm soil depth, NT presented the same increase in SOC and  
170 the POxC fraction (i.e., 60%, calculated as the variation in NT in relation to CT) (Table  
171 1). However, the other C pools studied (i.e., M-C, M-POxC, mM-C, POC and s+cM-C)  
172 presented lower increases, ranging from 17% to 31% (Table 1). Chen et al. (2009), in a  
173 long-term (11 years) comparison of tillage systems, reported that POxC was 2 times  
174 more sensitive to soil management than POC. Culman et al. (2012) measured different  
175 soil C fractions in 53 sites of a wide range of soil types, ecosystems and geographic  
176 areas. In 42% of those studies, they observed greater sensitivity to changes in  
177 management in the POxC fraction than in the POC, microbial biomass carbon or SOC.  
178 The last authors pointed out that POxC is closely related to smaller-sized (0.053-0.250  
179 mm) and heavier ( $>1.7 \text{ g cm}^{-3}$ ) POC fractions. Thus, we could hypothesize that, in our  
180 experiment, the increase of SOC when using NT compared to CT could be the result of  
181 an enhancement of coarse particulate material (0.250-2 mm). That fact would explain  
182 the lack of a greater sensitivity of POxC compared to SOC to detect changes in soil C  
183 when adopting NT.

184 For the 5-20 and 20-40 cm depths, about 7% and 16% reduction in SOC was observed  
185 when adopting NT compared to CT, respectively (Table 1). However, when the POC  
186 fraction was considered, the decrease after the adoption of NT was 46% and 54% for the  
187 5-20 and 20-40 cm soil depths, respectively (Table 1). The POC fraction was also the



188 second most sensitive fraction when 60 kg N ha<sup>-1</sup> was applied, after the M-POxC  
189 fraction (Table 1). For the 0-5 cm soil depth, whereas total SOC increased about 7%  
190 after the application of 60 kg N ha<sup>-1</sup> compared to the control treatment, the M-POxC and  
191 POC fractions increased about 37% and 21% , respectively (Table 1). When the 20-40  
192 cm soil depth was considered, the POC was the only fraction which showed higher  
193 sensitivity compared to the total SOC (44% and 20% variation for POC and SOC,  
194 respectively when applying 60 kg N ha<sup>-1</sup> compared to the control treatment). Therefore,  
195 POC presented greater sensitivity to N fertilization management than SOC and POxC  
196 for the three soil depths studied (0-5, 5-20 and 20-40 cm), and greater sensitivity to  
197 tillage management in the 5-20 and 20-40 cm depth, where CT increased soil C  
198 compared to NT (Table 1). However, in the 0-5 cm soil depth in which NT increased  
199 soil C compared to CT, the POxC fraction was the most sensitive. Those contrasting  
200 findings could be the result of the intrinsic characteristics of the different C pools  
201 considered. Thus, whereas POC represents a partially decomposed C fraction with a  
202 short turnover time, POxC reflects a more processed fraction of soil C (Cambardella and  
203 Elliott, 1992; Haynes, 2005; Culman et al., 2012). This last aspect was demonstrated by  
204 Tirol-Padre and Ladha (2004) who observed a significant relationship between POxC  
205 and SOC but no correlation between POxC and labile C fractions such as MBC or  
206 water-soluble carbon. Similar to our findings, Awale et al. (2013) also observed a  
207 greater response to tillage management of the POC fraction when compared to the  
208 POxC fraction and Gregorich et al. (1996) observed a higher increase in POC than in  
209 bulk SOC in response to long-term fertilization of maize.

210 In our study, the mM-C and s+cM-C fractions presented low sensitivity to changes in  
211 tillage management and N fertilization (Table 1). The microaggregation that occurs  
212 within macroaggregates (mM) represents a long-term physical protection of soil C (Six  
213 et al., 2000). In turn, Chung et al. (2008) and Gulde et al. (2008) concluded that the  
214 mineral fraction of a soil can be saturated of C and, as a result, additional inputs of C  
215 due to agricultural practices will only accumulate in labile soil C pools, explaining the  
216 low response of the C contained in the microaggregates within macroaggregates (mM-  
217 C) or in the silt-plus clay-sized fraction (s+cM-C) to changes in management.

218 The results of our study showed that particulate organic carbon was the fraction with the  
219 greatest sensitivity to detect changes in SOC due to changes in agricultural management  
220 practices, whereas the permanganate oxidizable organic carbon only showed a greatest

221 sensitivity in soil surface when adopting NT instead of CT. Other fractions such as the  
222 C concentration of the microaggregates within macroaggregates or the silt-plus clay-  
223 sized soil particles showed the lowest sensitivity, although they could represent  
224 important fractions for the long-term protection of C in the soil. We conclude that  
225 particulate organic carbon presents the highest response to changes in agricultural  
226 management and can be used as an early indicator of optimized practices to sequester  
227 carbon in the soil.

228

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236

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305

306 **Figure captions**

307

308 **Fig. 1** Linear relationships between bulk soil organic carbon concentration (SOC) and  
309 (A) permanganate-oxidizable organic carbon concentration of the bulk soil (POxC) and  
310 (B) of the water-stable macroaggregates (M-POxC) and (C) dichromate oxidizable  
311 organic carbon concentration of the macroaggregates (M-C), (D) microaggregates  
312 within macroaggregates (mM-C), (E) particulate organic matter (POC) and (F) silt-plus  
313 clay-sized particles of the macroaggregates (s+cM-C) as affected by tillage (CT,  
314 conventional tillage; NT, no-tillage). \*, \*\* and \*\*\* correspond to  $P<0.05$ ,  $P<0.01$  and  
315  $P<0.001$ , respectively.

316

317 **Table 1** Percent change of different labile soil organic C pools when using (i) no-tillage  
 318 (NT) as compared to conventional tillage (CT) (in the table shown as “Tillage”) and (ii)  
 319 60 kg N ha<sup>-1</sup> as compared to the control (0 kg N ha<sup>-1</sup>) (in the table shown as “Nitrogen”)  
 320 at 0-5, 5-20 and 20-40 cm soil depth.

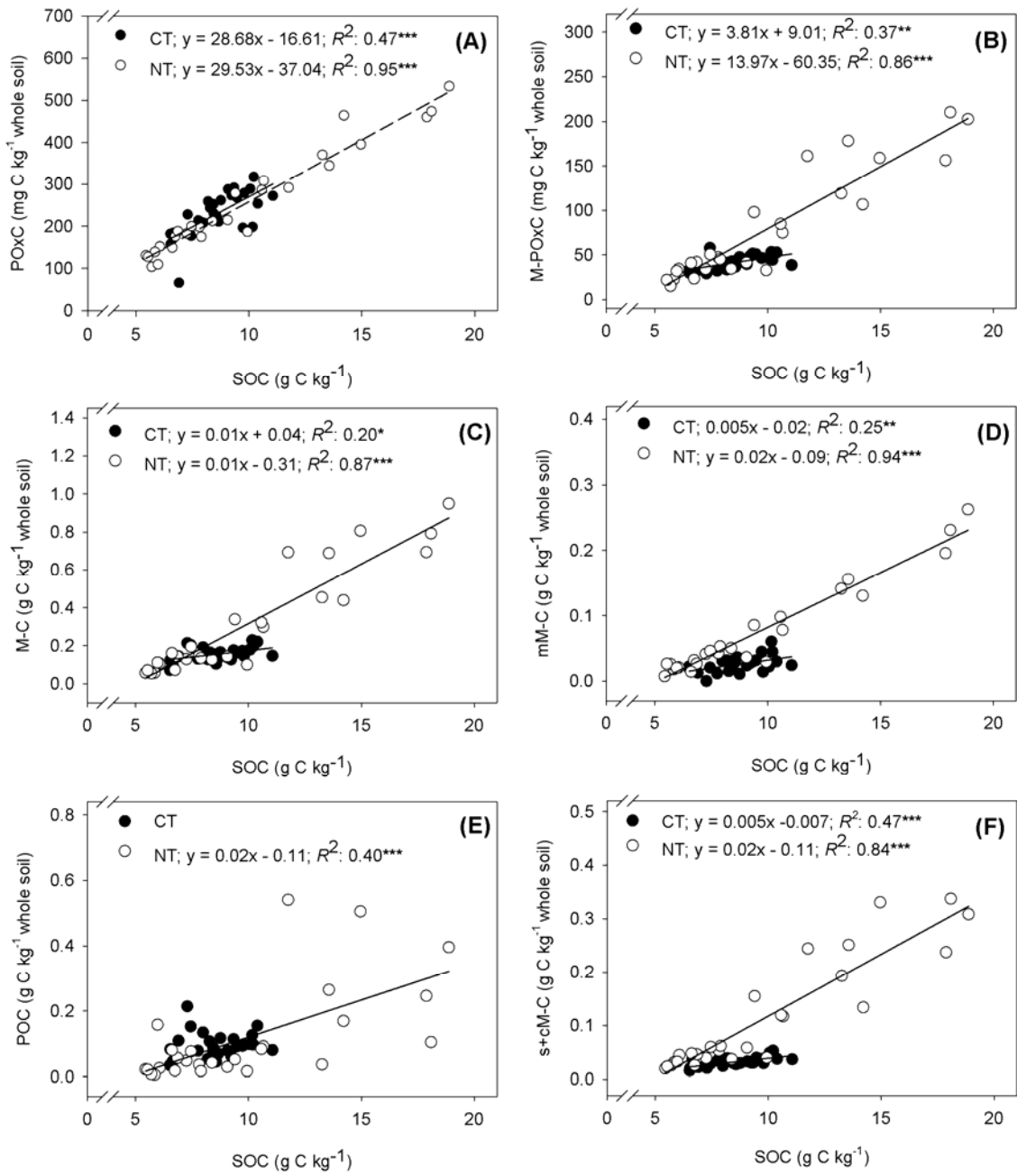
Soil depth (cm)	Management	SOC	POxC	M-C	M-POxC	mM-C	POC	s+cM-C
0-5	Tillage	60	60	28	17	31	27	29
	Nitrogen	7	3	8	37	2	21	5
5-20	Tillage	-7	-7	-15	-7	2	-46	3
	Nitrogen	11	29	19	32	0	30	6
20-40	Tillage	-16	-30	-36	-33	-23	-54	-3
	Nitrogen	20	8	17	20	3	44	-1

321

322 SOC: soil organic carbon; POxC: permanganate oxidizable organic carbon of the bulk  
 323 soil; M-C: water-stable macroaggregates-C concentration; M-POxC: macroaggregate-  
 324 permanganate oxidizable organic carbon; mM-C: microaggregates within  
 325 macroaggregates-C concentration; POC: particulate organic matter-C concentration;  
 326 s+cM-C: C concentration of the silt-plus-clay-sized fraction of the macroaggregates.

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328



329

330 **Fig. 1**