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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 (H₂O, 1:2.5): 8.5, EC_{1:5} (dS m⁻¹): 0.15,
94 CaCO₃ eq. (%): 40 and sand (2000-50 μm), silt (50-2 μm) and clay (<2 μm) content:
95 465, 417 and 118 g kg⁻¹, 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⁻¹) 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 $50\text{ }^{\circ}\text{C}$ 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 $50\text{ }^{\circ}\text{C}$ 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 (POxC) and of the water-stable
137 macroaggregates (M-POxC) were measured according to the method of Weil et al.
138 (2003) and quantified by:

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

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)_{NT} and (C pool)_{CT} 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⁻¹
150 compared to the control (0 kg N ha⁻¹) 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)₆₀ and (C pool)₀ refer to the different C pools studied under 60 and 0 kg
153 mineral N ha⁻¹, 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⁻¹ 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⁻¹ 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⁻¹ 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⁻¹ as compared to the control (0 kg N ha⁻¹) (in the table shown as “Nitrogen”)
 320 at 0-5, 5-20 and 20-40 cm soil depth.

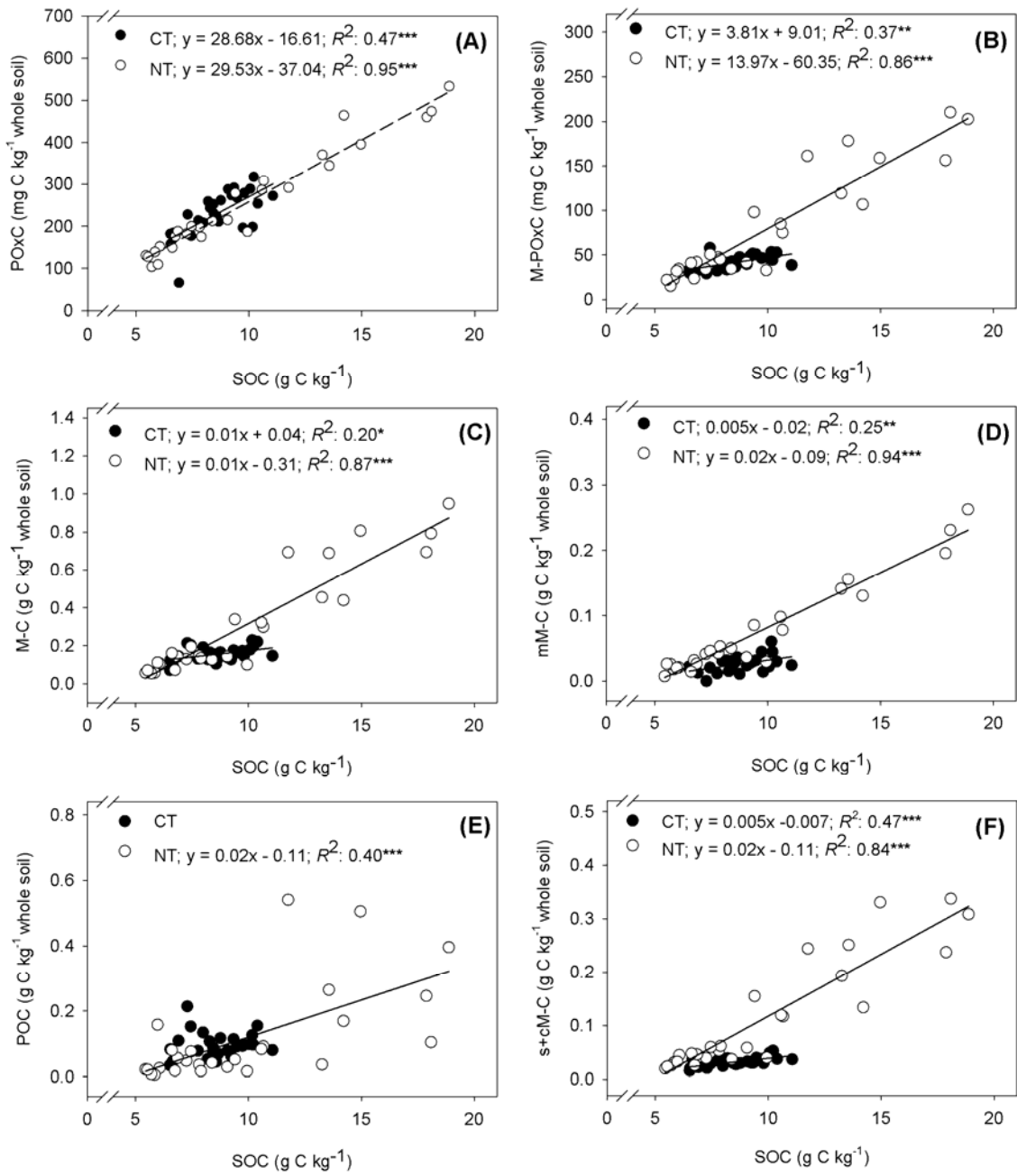
Soil depth (cm)	Management	SOC	POxC	M-C	M-POxC	mM-C	POC	s+cM-C
0-5	Tillage	60	60	28	17	31	27	29
	Nitrogen	7	3	8	37	2	21	5
5-20	Tillage	-7	-7	-15	-7	2	-46	3
	Nitrogen	11	29	19	32	0	30	6
20-40	Tillage	-16	-30	-36	-33	-23	-54	-3
	Nitrogen	20	8	17	20	3	44	-1

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.

327

328



329

330 **Fig. 1**