Identifying soil organic carbon fractions sensitive to agricultural management practices

Daniel Plaza-Bonilla\textsuperscript{a*}, Jorge Álvaro-Fuentes\textsuperscript{b}, Carlos Cantero-Martínez\textsuperscript{a}

\textsuperscript{a}Departamento de Producción Vegetal y Ciencia Forestal, Unidad Asociada EEAD-CSIC, Agrotecnio Center, Universitat de Lleida, Rovira Roure 191, 25198 Lleida, Spain.

\textsuperscript{b}Departamento de Suelo y Agua. Estación Experimental de Aula Dei, Consejo Superior de Investigaciones Científicas (EEAD-CSIC), POB 13034, 50080 Zaragoza, Spain.

\textsuperscript{*}Corresponding author: D. Plaza-Bonilla.

E-mail: daniel.plaza@pvcf.udl.cat

Phone number: (+34) 973702522.

Fax number: (+34) 973238264
Abstract

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

Abbreviations:

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

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

Soils are the largest terrestrial pool of organic C with over 1550 Pg (Batjes, 1996). C sequestration in soils has been pointed out as a viable mechanism for reducing the concentration of carbon dioxide in the atmosphere (Lal, 2004). Moreover, soil organic carbon (SOC) can also improve plant productivity due to its effects on soil fertility and quality. Agricultural management practices play a major role in the process of SOC sequestration. A key issue when studying the effects of those practices on SOC levels is the period of time needed to observe changes in C stocks. This period is normally on a long-term time-scale (> 10 years). Moreover, the large background of stable organic C that is already present in the soil limits the opportunity to identify management-induced changes over short periods of time (Gregorich et al., 1994; Haynes, 2000). Both drawbacks limit our ability of testing which agronomic practices have a positive effect on SOC increase. Thus, in this context, the use of different soil C fractions with an earlier response to changes in management compared to total SOC has been pointed out as an efficient tool to identify optimized agricultural practices that increase the stock and quality of soil C. Different labile SOC pools, such as dissolved organic carbon, microbial biomass carbon and permanganate-oxidizable carbon, have recently received attention due to their sensitivity to agricultural management practices (Culman et al., 2012; Lucas and Weil, 2012). For instance, permanganate-oxidizable organic carbon has been suggested to be a more sensitive indicator than bulk SOC to tillage-induced changes (Weil et al. 2003; Melero et al. 2009). However, while fractions like microbial biomass C and N have been extensively reviewed, the significance of other fractions (e.g, permanganate-oxidizable organic carbon or particulate organic matter) is not fully well-understood (Haynes, 2005).
The objective of the present work was to identify which soil C fractions were most sensitive to changes in agricultural management practices. Sensitivity was implied to impart more rapid changes as early indicators of change. For this purpose we considered tillage and N fertilization as target management practices because of their impact on SOC sequestration and the abundance of literature related with these two practices (West and Post, 2002; Alvarez, 2005).
Materials and methods

We used a long-term tillage and N fertilization experiment established in 1996 in NE Spain (Agramunt, 41° 48’ N, 1° 07’ E). Mean annual precipitation and ETo are 430 and 855 mm, respectively. Selected soil properties at the beginning of the experiment in the 0-28 cm soil layer (Ap horizon) were: pH (H₂O, 1:2.5): 8.5, EC₁₅ (dS m⁻¹): 0.15, CaCO₃ eq. (%): 40 and sand (2000-50 μm), silt (50-2 μm) and clay (<2 μm) content: 465, 417 and 118 g kg⁻¹, respectively. The soil was classified as a Typic Xerofluvent (Soil Survey Staff, 1994). Two types of tillage (NT, no-tillage, and CT, conventional intensive tillage with moldboard plowing) and two N fertilization rates (0 and 60 kg N ha⁻¹) were compared in a randomized block design with three replications. Plot size was 50 m x 6 m. The NT treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare) for controlling weeds before sowing. The CT treatment consisted of one pass of a moldboard plow to 25 cm depth immediately followed by one or two passes with a cultivator to 15 cm depth, both in September. Nitrogen fertilizer was manually-applied and split into two applications: one-third of the dose before tillage as ammonium sulphate (21% N) and the rest of the dose at the beginning of tillering, as ammonium nitrate (33.5% N). Planting was performed in November with a disk direct drilling machine set to 2-4 cm. The cropping system consisted of a barley monocropping, as is traditional in the area. Prior to the setting up of the experiment, the historical management of the field was based on conventional intensive tillage with moldboard plowing and small grain cereals monoculture.

Soil sampling was performed in July 2012, right after crop harvest. For each plot, two soil pits of 0.5 m depth and 20 m apart were opened. In each pit, a composite sample was collected from three samples randomly selected. Soil samples were obtained using
a flat spade in three soil layers from 0 to 40 cm depth (0-5, 5-20 and 20-40 cm) and
stored in crush-resistant airtight containers. Once in the laboratory, soil samples were
gently sieved with an 8 mm-sieve and air-dried at room temperature. For each sample,
water-stable macroaggregates (> 0.250 mm) were obtained using the wet sieving
method described by Elliott (1986), oven-dried at 50 °C during 24 h, weighed and
stored. The microaggregates contained within macroaggregates were isolated by
methodology described by Six et al. (2000). The microaggregates and other particles
smaller than <0.250 mm were washed onto a 0.050 mm screen by a continuous flow of
water. The material on the 0.050 mm sieve was sieved in order to separate the stable
microaggregates from the silt-plus-clay-sized material. Finally, the material on the
0.250 mm sieve (considered particulate organic matter), the microaggregates within
macroaggregates and the silt-plus-clay-sized particles (<0.053 mm) were oven-dried at
50 °C during 24 h and weighed.
The sand content of the macroaggregates and the microaggregates within
macroaggregates was determined by dispersing a 5 g subsample of each one of these
fractions in a 5% sodium hexametaphosphate. The organic C concentration of the bulk
soil (SOC), the water-stable macroaggregates (M-C), the microaggregates within
macroaggregates (mM-C) and the silt-plus-clay-sized fractions (<0.053 mm) (s+cM-C)
were determined using the dichromate wet oxidation method described by Nelson and
Sommers (1996). The particulate organic C of the water-stable macroaggregates (POC)
was calculated subtracting the amount of C in the microaggregates within
macroaggregates (mM-C) and in the silt-plus-clay-sized fraction (s+cM-C) to the
amount of C contained in the water-stable macroaggregates (M-C).
The permanganate oxidizable organic C of the bulk soil (POxC) and of the water-stable macroaggregates (M-POxC) were measured according to the method of Weil et al. (2003) and quantified by:

\[
POxC \text{ (mg kg}^{-1}\text{soil}) = \frac{(0.02 \text{ mol L}^{-1}-(a+b \times \text{Abs})) \times (9000 \text{ mg C mol}^{-1})(0.02 \text{ L solution x W}^{-1})}{\text{Abs is the absorbance of the sample and W is the weight (kg) of the soil used.}}
\]

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

\[
\% \text{ variation fraction} = \frac{((C \text{ pool})_{NT} - (C \text{ pool})_{CT})}{(C \text{ pool})_{CT}}\times 100
\]

where (C pool)_{NT} and (C pool)_{CT} refer to the different C pools studied under NT and CT, respectively.

In the second case, the variation of each C pool when applying 60 kg mineral N ha\(^{-1}\) compared to the control (0 kg N ha\(^{-1}\)) was calculated by:

\[
\% \text{ variation fraction} = \frac{((C \text{ pool})_{60} - (C \text{ pool})_0)}{(C \text{ pool})_0}\times 100
\]

where (C pool)_{60} and (C pool)_0 refer to the different C pools studied under 60 and 0 kg mineral N ha\(^{-1}\), respectively.

The relationships among C fractions for a kg of whole soil were calculated by linear regression analyses with SigmaPlot 11 (Systat Software, 2008).
Results and discussion

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

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

For the 5-20 and 20-40 cm depths, about 7% and 16% reduction in SOC was observed when adopting NT compared to CT, respectively (Table 1). However, when the POC fraction was considered, the decrease after the adoption of NT was 46% and 54% for the 5-20 and 20-40 cm soil depths, respectively (Table 1). The POC fraction was also the
second most sensitive fraction when 60 kg N ha\(^{-1}\) was applied, after the M-POxC fraction (Table 1). For the 0-5 cm soil depth, whereas total SOC increased about 7% after the application of 60 kg N ha\(^{-1}\) compared to the control treatment, the M-POxC and POC fractions increased about 37% and 21%, respectively (Table 1). When the 20-40 cm soil depth was considered, the POC was the only fraction which showed higher sensitivity compared to the total SOC (44% and 20% variation for POC and SOC, respectively when applying 60 kg N ha\(^{-1}\) compared to the control treatment). Therefore, POC presented greater sensitivity to N fertilization management than SOC and POxC for the three soil depths studied (0-5, 5-20 and 20-40 cm), and greater sensitivity to tillage management in the 5-20 and 20-40 cm depth, where CT increased soil C compared to NT (Table 1). However, in the 0-5 cm soil depth in which NT increased soil C compared to CT, the POxC fraction was the most sensitive. Those contrasting findings could be the result of the intrinsic characteristics of the different C pools considered. Thus, whereas POC represents a partially decomposed C fraction with a short turnover time, POxC reflects a more processed fraction of soil C (Cambardella and Elliott, 1992; Haynes, 2005; Culman et al., 2012). This last aspect was demonstrated by Tirol-Padre and Ladha (2004) who observed a significant relationship between POxC and SOC but no correlation between POxC and labile C fractions such as MBC or water-soluble carbon. Similar to our findings, Awale et al. (2013) also observed a greater response to tillage management of the POC fraction when compared to the POxC fraction and Gregorich et al. (1996) observed a higher increase in POC than in bulk SOC in response to long-term fertilization of maize.

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

The results of our study showed that particulate organic carbon was the fraction with the greatest sensitivity to detect changes in SOC due to changes in agricultural management practices, whereas the permanganate oxidizable organic carbon only showed a greatest
sensitivity in soil surface when adopting NT instead of CT. Other fractions such as the C concentration of the microaggregates within macroaggregates or the silt-plus clay-sized soil particles showed the lowest sensitivity, although they could represent important fractions for the long-term protection of C in the soil. We conclude that particulate organic carbon presents the highest response to changes in agricultural management and can be used as an early indicator of optimized practices to sequester carbon in the soil.

Acknowledgments

José Luis Arrúe, Javier Bareche, Carlos Cortés, Silvia Martí, João D. Nerone, Jonatan Ovejero and Miquèl Segalàs are gratefully acknowledged. We also want to thank Belén Perpiñà for her guidance during the spectrophotometry analysis. This research was supported by the Comisión Interministerial de Ciencia y Tecnología of Spain (AGL 2004-07763-C02-02 and AGL2010-22050-C03-01/02). D. Plaza-Bonilla was awarded a FPU fellowship by the Spanish Ministry of Education.
References

Alvarez, R. A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use and Management 21, 38-52


Figure captions

**Fig. 1** Linear relationships between bulk soil organic carbon concentration (SOC) and (A) permanganate-oxidizable organic carbon concentration of the bulk soil (POxC) and (B) of the water-stable macroaggregates (M-POxC) and (C) dichromate oxidizable organic carbon concentration of the macroaggregates (M-C), (D) microaggregates within macroaggregates (mM-C), (E) particulate organic matter (POC) and (F) silt-plus clay-sized particles of the macroaggregates (s+cM-C) as affected by tillage (CT, conventional tillage; NT, no-tillage). *, ** and *** correspond to $P<0.05$, $P<0.01$ and $P<0.001$, respectively.
Table 1 Percent change of different labile soil organic C pools when using (i) no-tillage (NT) as compared to conventional tillage (CT) (in the table shown as “Tillage”) and (ii) 60 kg N ha\(^{-1}\) as compared to the control (0 kg N ha\(^{-1}\)) (in the table shown as “Nitrogen”) at 0-5, 5-20 and 20-40 cm soil depth.

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

SOC: soil organic carbon; POx C: permanganate oxidizable organic carbon of the bulk soil; M-C: water-stable macroaggregates-C concentration; M-POx C: macroaggregate-permanganate oxidizable organic carbon; mM-C: microaggregates within macroaggregates-C concentration; POC: particulate organic matter-C concentration; s+cM-C: C concentration of the silt-plus-clay-sized fraction of the macroaggregates.
Fig. 1