



## Comparing current chemical methods to assess biochar organic carbon in a Mediterranean agricultural soil amended with two different biochars



Irene Raya-Moreno<sup>a,\*</sup>, Rosa Cañizares<sup>a,b,1</sup>, Xavier Domene<sup>a,b</sup>, Vicenç Carabassa<sup>a</sup>, Josep M. Alcañiz<sup>a,b</sup>

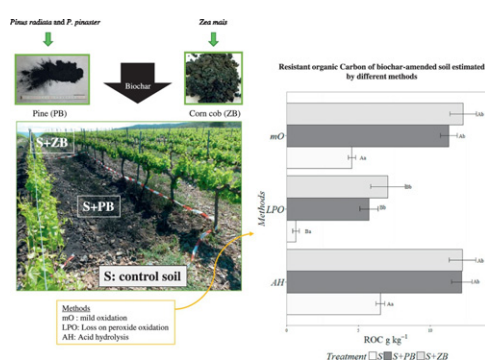
<sup>a</sup> CREAM, Cerdanyola del Vallès 08193, Spain

<sup>b</sup> Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Spain

### HIGHLIGHTS

- Resistant organic carbon (ROC) was quantified by different chemical methods in a soil amended with two biochars.
- Biochars significantly increased stable organic C content of an amended soil
- The amount and type of biochars added were stable enough not to vary along two years of field experimentation.
- Mild oxidation with potassium dichromate was a successful as indirect method to estimate recalcitrant C fraction in soil.
- Hydrogen peroxide oxidation was not a suitable method to determine biochar C in soil.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 9 November 2016

Received in revised form 17 March 2017

Accepted 18 March 2017

Available online 25 April 2017

Editor: D. Barcelo

#### Keywords:

Biochar

Soil

Resistant organic carbon

### ABSTRACT

Several methods have been proposed to quantify biochar C recalcitrance but their suitability is questionable. The aims of this work are: i) to compare the suitability of thermal or chemical oxidation and acid hydrolysis methods to quantify biochar C-pool in a biochar-amended soil,

and ii) to calculate the biochar content in the soil through a mass balance derived from the obtained data.

Two contrasted biochars from pine wood and corn cob remains were incorporated at a rate of 5 Mg C ha<sup>-1</sup> to a sandy loam vineyard soil with neutral pH and low organic carbon content, in field conditions. The analytical methods used to determine the oxidability and hydrolyzation of soil and biochar-C were: i) weight loss-on-ignition (LOI) at three temperatures (375 °C, 550 °C and 950 °C) for the assessment of organic matter, and ii) dry-combustion (TOC), strong (sO) and mild (mO) acid potassium dichromate oxidations, acid hydrolysis (AH) and peroxide oxidation (PO) for the assessment of organic C-pools. mO mainly estimated the easy oxidisable organic fraction of soil. Resistant organic carbon (ROC), estimated as non-hydrolysable organic carbon

**Abbreviations:** PB, Pine biochar; ZB, Corn cob biochar; S, Control soil; S + PB, Soil amended with pine biochar; S + ZB, Soil amended with corn cob biochar; AH, Acid hydrolysis; LOI, Loss-on-ignition; LPO, Loss-on-peroxide-hydrogen oxidation; mO, Mild potassium dichromate oxidation; PO, Peroxide-hydrogen oxidation; sO, Strong potassium dichromate oxidation; TOC, Total organic carbon (by dry combustion); BOC<sub>TOC</sub>, Biochar (of pine or corn cob) organic carbon content estimated by TOC; BOC<sub>AH</sub>, Biochar (of pine or corn cob) organic carbon content estimated by AH; BOC<sub>LPO</sub>, Biochar (of pine or corn cob) organic carbon content estimated by LPO; BOC<sub>mO</sub>, Biochar (of pine or corn cob) organic carbon content estimated by mO; CF, Correction factor for mineral losses based on LOI 550 °C; ROC<sub>AH</sub>, Resistant organic carbon values calculated from AH; ROC<sub>mO</sub>, Resistant organic carbon values calculated from mO; ROC<sub>PO</sub>, Resistant organic carbon values calculated from PO.

\* Corresponding author at: Center for Ecological Research and Forestry Applications, (CREAF), Edifici C, Campus de la UAB, 08193 Bellaterra, (Cerdanyola del Vallès), Spain.

E-mail addresses: [i.raya@creaf.uab.es](mailto:i.raya@creaf.uab.es) (I. Raya-Moreno), [r.canizares@creaf.uab.es](mailto:r.canizares@creaf.uab.es) (R. Cañizares), [x.domene@creaf.uab.es](mailto:x.domene@creaf.uab.es) (X. Domene), [v.carabassa@creaf.uab.es](mailto:v.carabassa@creaf.uab.es) (V. Carabassa), [josemaria.alcaniz@creaf.uab.es](mailto:josemaria.alcaniz@creaf.uab.es) (J.M. Alcañiz).

<sup>1</sup> The first two authors contributed equally to this work.

Soil organic matter  
Chemical oxidation  
Acid hydrolysis

by AH and as non-oxidisable by mO, led to similar values in control soil (5 g C kg<sup>-1</sup> soil), whereas different ROC values were obtained in soils amended with biochar (6–12 g C kg<sup>-1</sup> soil). The suitability of these different methods as proxies to quantify biochar C was verified through a mass balance observing differences between them. PO removes well native soil organic matter, but also attacks partially biochar's fraction, so an underestimation exists. However, mO leaves intact biochar in the amended soil. Summarising, LOI, TOC and mO were the best proxies for biochar-C quantification, especially the last one, somewhat clarifying the debate on this topic.

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## 1. Introduction

Biochar has received much attention in recent years due to its properties as a potential soil amendment (Lehmann and Joseph, 2015). Biochar is a solid carbonaceous material obtained from the thermochemical conversion of biomass in an oxygen-limited environment and intended for use as soil amendment (International Biochar Initiative, 2012). Furthermore, the application of biochar to soil provides additional environmental benefits, such as long-term C sequestration in soil (Shackley et al., 2013), compensating CO<sub>2</sub> emissions (Fang et al., 2014) and playing a key role in a global C-negative strategy (Cheng et al., 2006; Qin et al., 2016).

Biochar stability could vary depending on biomass feedstock and pyrolysis procedure, which influences aromatic C condensation (Lehmann et al., 2006; Wang et al., 2015). Most biochar C is very stable due to its chemical structure, which is rich in aromatic C structures (Baldock and Smernik, 2002; Calvelo Pereira et al., 2011; Singh et al., 2012), highly resistant to microbial decomposition (Shindo, 1991; Cheng et al., 2008). However, biochar contains a minor labile fraction that consist mostly of carbohydrates, proteins and fatty acids that have not been completely charred (Lehmann and Joseph, 2009). This minor organic fraction alone might not contribute to efficient C sequestration in soil due to its fast turnover rates, although it may play a key role in soil structure owing to physical protection processes that might increase its residence time in soil (Plaza et al., 2016).

While there can be no doubt that the application of biochar to agricultural soils increases the recalcitrant fraction of soil organic carbon (SOC), and hence C sequestration in soil, the most suitable methods for its assessment are still under debate. Various attempts have been made to correlate analytical determinations -chemical, biological and physical methods- with SOC pools (Strosser, 2010; von Lützow et al., 2007) but, in the specific case of chemical methods, their validity has been questioned (Naisse et al., 2013). A variety of current thermal and chemical soil analysis methods have been used for this purpose as dry combustion, loss-on-ignition (LOI), and loss-on-hydrogen peroxide oxidation (LPO) being the most widespread methods to determine soil organic matter (Gustafsson et al., 1997; Mikutta et al., 2005). On the other hand, dichromate oxidation has been widely used to assess soil organic carbon (e.g. Walkley and Black, 1934) and acid hydrolysis (AH) to discriminate the recalcitrant C fraction (Rovira and Vallejo, 2007). However, the accuracy of these methods for the quantification of biochars is unclear due to the diverse composition of carbon pools and their vulnerability to chemical attack. As an example of this, acid dichromate oxidation has been suggested by several authors as an alternative to test biochar C reactivity in soil (Calvelo Pereira et al., 2011; Knicker et al., 2007). This procedure is a modification of the broadly used classic methods to estimate organic carbon (e.g. Walkley and Black, 1934; Mebius, 1960). The K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>/H<sub>2</sub>SO<sub>4</sub> concentration, temperature and time of digestion are the key parameters which determine the oxidation degree because not all biochar C is always oxidised by dichromate (Rumpel et al., 2006). Calvelo Pereira et al. (2011) proposed acid dichromate oxidation as a method to evaluate the most reactive fraction of biochar, suggesting that resistant C to that oxidation could reflect the most stable fraction and the degree of biochar aromatisation.

The main aims of this field study, carried out in a soil amended with two different biochars, were: i) to evaluate which of the widely used chemical methods for the assessment of soil organic matter (SOM) or SOC are the most suitable for discriminating resistant soil and biochar organic carbon pools, and ii) to calculate the biochar content in soils through a mass balance derived from the available analytical data and confirm if these methods are good proxies to quantify biochar.

## 2. Materials and methods

Table 1 summarise and define the acronyms used in this paper.

### 2.1. Biochar characterisation

Two biochars were tested in this study, one obtained as a residue of the gasification of mixed pine wood splinters (*Pinus radiata* and *P. pinaster*) at 600–900 °C (PB), and the other by slow pyrolysis of corn cobs (*Zea mays*) at 450–500 °C (ZB). Both biochars were grounded and sieved to 2 mm prior to analysis and field application.

Biochar C and H content were determined using a Flash 2000 C.E. Elemental Analyzer (Thermo Fisher Scientific) at 1020 °C, N content by a Flash EA 1112 Elemental Analyzer (Thermo Fisher Scientific) at

**Table 1**

List of acronyms used in this paper by alphabetical order.

General acronyms	Definition
<b>Biochars</b>	
PB	Pine biochar
ZB	Corn cob biochar
<b>Treatment</b>	
S	Control soil
S + PB	Soil amended with pine biochar
S + ZB	Soil amended with corn cob biochar
<b>Methods</b>	
AH	Acid hydrolysis (HCl, 100 °C, 17 h)
LOI	Loss-on-ignition (375 °C, 550 °C, 950 °C)
LPO	Loss-on-peroxide-hydrogen oxidation
mO	Mild potassium dichromate oxidation (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> , 60 °C, 8 h)
PO	Peroxide-hydrogen oxidation
sO	Strong potassium dichromate oxidation (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> , 150 °C, 10 min)
TOC	Total organic carbon (by dry combustion)
Specific acronyms	Definition
BOC <sub>TOC</sub>	Biochar (of pine or corn cob) organic carbon content estimated by TOC
BOC <sub>AH</sub>	Biochar (of pine or corn cob) organic carbon content estimated by AH
BOC <sub>LPO</sub>	Biochar (of pine or corn cob) organic carbon content estimated by LPO
BOC <sub>mO</sub>	Biochar (of pine or corn cob) organic carbon content estimated by mO
CF	Correction factor for mineral losses based on LOI 550 °C
ROC <sub>AH</sub>	Resistant organic carbon values calculated using AH
ROC <sub>mO</sub>	Resistant organic carbon values calculated using mO
ROC <sub>PO</sub>	Resistant organic carbon values calculated using PO

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