

# *Evaluation of optical techniques for characterising soil organic matter quality in agricultural soils*

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1 **EVALUATION OF OPTICAL TECHNIQUES FOR CHARACTERISING SOIL**  
2 **ORGANIC MATTER QUALITY IN AGRICULTURAL SOILS**

3

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24 Soil organic matter (SOM) is one of the main global carbon pools. It is a measure of soil  
25 quality as its presence increases carbon sequestration and improves physical and  
26 chemical soil properties. The determination and characterisation of humic substances  
27 gives essential information of the maturity and stresses of soils as well as of their health.  
28 However, the determination of the exact nature and molecular structure of these  
29 substances has been proven difficult. Several complex techniques exist to characterise  
30 SOM and mineralisation and humification processes. One of the more widely accepted  
31 for its accuracy is Nuclear Magnetic Resonance (NMR) spectroscopy. Despite its  
32 efficacy, NMR needs significant economic resources, equipment, material and time.  
33 Proxy measures like the fluorescence index (FI), cold and hot-water extractable carbon  
34 (CWC and HWC) and SUVA<sub>-254</sub> have the potential to characterise SOM and, in  
35 combination, provide qualitative and quantitative data of SOM and its processes.  
36 Spanish and British agricultural cambisols were used to measure SOM quality and  
37 determine whether similarities were found between optical techniques and <sup>1</sup>H-NMR  
38 results in these two regions with contrasting climatic conditions. High correlations  
39 ( $p < 0.001$ ) were found between the specific aromatic fraction measured with <sup>1</sup>H-NMR  
40 and SUVA<sub>-254</sub> ( $R_s = 0.95$ ) and HWC ( $R_s = 0.90$ ), which could be described using a linear  
41 model. A high correlation between FI and the aromatics fraction measured with <sup>1</sup>H-

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Abbreviations: (SOM) Soil organic matter; (NMR) Nuclear magnetic resonance; (FI) Fluorescence index; (CWC) Cold-water extractable carbon; (HWC) Hot-water extractable carbon; (LOI) Loss of ignition; (DOM) Dissolved organic matter; (DOC) Dissolved organic carbon; (EEM) Excitation-emission matrix; (GS-UK) Soil with a grass cover, United Kingdom; (CC-UK) Cereal crops from United Kingdom; (CC-ES) Cereal crops from Spain; (OG-ES) Olive grove from Spain; (HIX) Humification index; (BIX) Biological/autochthonous index; (RU) Raman units; (SUVA-254) Specific absorbance at 254 nm

42 NMR ( $R_s=-0.976$ ) was also observed. In view of our results, optical measures have a  
43 potential, in combination, to predict the aromatic fraction of SOM without the need of  
44 expensive and time consuming techniques.

45 **KEYWORDS:** SOM quality; hot-water extractable carbon; cold-water extractable  
46 carbon; fluorescence index; EEM; SUVA<sub>-254</sub>; <sup>1</sup>H-NMR; aromatic fraction.

## 47 **1. Introduction:**

### 48 *1.1. The importance of organic matter*

49 Soil organic matter (SOM) is composed of organic residues that are originated from  
50 plant and animal remains and microbial products at different stages of decomposition or  
51 humification (Hur *et al.*, 2013). Additionally, it is one of the main global carbon pools,  
52 storing three times more carbon than living organisms or the atmosphere (Fischlin *et al.*,  
53 2007; Brevik, 2012). Aside from carbon sequestration, SOM is also a measure of soil  
54 quality because of the beneficial function it has on a variety of soil processes. For  
55 instance, it reduces erosion and, therefore, increases crop production by increasing the  
56 elasticity and resistance to deformation and compactability as well as porosity and water  
57 retention (Sellami *et al.*, 2008; Paradelo and Barral, 2013). Increased water retention  
58 decreases potential runoffs by improving water infiltration in to soils and provides a  
59 store of water for plant uptake, buffering against moisture and rainfall fluctuations (Lal,  
60 2004). This is of importance considering that the lack of water retention leads to a  
61 change in the hydrological patterns of agricultural areas and promotes the quantity and  
62 severity of floods and water-led erosion. Also, SOM leads to an increased vegetative  
63 cover, which ultimately reduces soil erosion (Cerdà, 1998, 2000; Novara *et al.*, 2011;  
64 Zhao *et al.*, 2013). Carbon mineralisation is crucial in SOM dynamics and along with  
65 carbon input, determines how much carbon accumulates in soil and releases nutrients  
66 that are essential for plant growth. Factors that affect mineralisation are the size of

67 labile carbon, environmental conditions and the local microbial community (Zhao *et al.*,  
68 2008; Li *et al.*, 2013). SOM and soil assemblage; SOM decomposition and transport by  
69 organisms contribute to soil stabilisation and the improvement of soil structure (Brevik  
70 *et al.*, 2015). Moreover, SOM quantity has been directly related to the preservation of  
71 soil aggregates, which in turn reduces soil erodibility (Novara *et al.*, 2011). Also, the  
72 direct processing of SOM along with its decomposition contribute to the improvement  
73 of soil chemical properties and stability (Brevik *et al.*, 2015). Therefore, optimal  
74 quantities of SOM improve structure, water retention, and nutrient holding capacity of  
75 soils, which has an effect in multiple aspects of the soil system. These are essential  
76 considering the wider context of Earth System, as SOM conservation techniques have  
77 been proven to improve the fertility of degraded soils of a wide variety of ecosystems  
78 that are the main resource of large communities of developing areas of our planet, as  
79 well as have an influence on biogeochemical cycles and climate change mitigation  
80 (Batjes, 2014; Saha *et al.*, 2014; Srinivasarao *et al.*, 2014).

### 81 *1.2. SOM carbon fractions and their importance*

82 SOM has been conceptualised as containing three pools, with different  
83 residence/turnover times (Trumbore, 2000). These pools are the active SOM (living  
84 biomass of microorganisms and partially decomposed residues; associated with 1 year  
85 turnover); the slow SOM (resistant plant material; associated to a turnover from years to  
86 centuries); and passive SOM (humic substances and inert organic matter), which has  
87 been traditionally associated with longer residence time (thousands of years) and more  
88 stability. Some authors consider that the inert organic matter should not be considered  
89 as part of the passive pool, but as a fourth pool (Trumbore, 1997; Ohno, 2002; Agren  
90 and Bosatta, 2002; Sparks, 2003; Bell and Lawrence, 2009; Dungait *et al.*, 2012).  
91 Although the traditional view has been that decomposition led to complex molecules

92 that were very stable as a result of their structure; it has recently been known that  
93 environmental conditions, organo-mineral associations and other processes influence  
94 more in SOM stability than structure, which only plays a secondary role. As a result of  
95 this new view, recent research has found that humic substances, which have always  
96 been considered high molecular mass polymers, could be simpler than originally  
97 thought (Kleber and Johnson, 2010; Schmidt *et al.*, 2011). Still, their structure is on  
98 discussion and the separation of SOM into fractions with different turnovers remains a  
99 major challenge (Kleber, 2010; Schmidt *et al.*, 2011; Schrumpf and Kaiser, 2015).  
100 Non-humic substances are composed by microbial biomass, decomposable plant  
101 material (active SOM); and resistant plant material, mainly waxes, lignified tissues and  
102 polyphenols (slow SOM) (Dungait *et al.*, 2012). Microbial biomass has been used for  
103 comparing natural and degraded ecosystems and as an early indicator of soil processes,  
104 fertility and health (García-Gil *et al.*, 2000; Brevik, 2009; Chen *et al.*, 2013).

### 105 *1.3. Current SOM quality measurements. Challenges*

106 Measures such as % Organic Matter measured by Loss of Ignition (LOI) are useful,  
107 popular and inexpensive methods to determine bulk SOM (Luke *et al.*, 2009; Salehi *et*  
108 *al.*, 2011). However, some studies have concluded that bulk SOM measurements cannot  
109 be used, on their own, as a representative indicator of carbon in soil due to their  
110 limitations (Koarashi *et al.*, 2005; Salehi *et al.*, 2011).

111 Humic substances have also been measured to determine soil quality, as their presence  
112 has been associated with a higher quality of soils as stated in section 1.2. Their study is  
113 relevant in agricultural soils, as they increase crop yield and root dry weight, although  
114 this response is not fully understood (Rose *et al.*, 2014). Therefore, the determination  
115 and characterisation of humic substances gives essential information of the maturity and  
116 stresses of soils as well as of their health.

117 Traditionally, alkali and acid abstraction methods have been used, to later interpret the  
118 chemistry of the extracted functional groups (Olk and Gregorich, 2006). Afterwards,  
119 these were combined with other complex techniques that enabled scientists to obtain  
120 new information on the structure and dynamic associations of humic substances (Sutton  
121 and Sposito, 2005; Schmidt *et al.*, 2011). Despite these advances, SOM dynamics and  
122 cycling still have many questions to answer, with models differing in SOM fluxes  
123 results for the future, due to their sensitivity to SOM turnover time assumptions  
124 (Schmidt *et al.*, 2011). There are a number of powerful but complex and expensive  
125 techniques that have been used for the study of soil fluxes (Helal *et al.*, 2011). The  
126 economic resources needed, along with the time required to prepare the samples and  
127 conduct the analyses, make its use with a large number of samples difficult and delays  
128 experiments, while more work is still needed to accurately determine and define the  
129 molecular structures and linkages between the SOM components (Weishar *et al.* 2003;  
130 Helal *et al.*, 2011).

131 Nuclear Magnetic Resonance (NMR) spectroscopy is a non-destructive technique that is  
132 valuable for the characterisation of SOM and humification processes, providing  
133 information on static and dynamic properties of molecules. This is due to its high  
134 performance to assess intermolecular interactions. The relationship between SOM,  
135 contaminants and metals can also be studied with NMR (Cardoza *et al.*, 2004). Of the  
136 various variants that exist, <sup>1</sup>H-NMR spectroscopy was used in this study. This technique  
137 analyses humic and fulvic acids dissolved in neutral or alkaline solutions to characterise  
138 the components of the substance, and gives a semi-quantitative notion of aromatic,  
139 aliphatic and carboxylic groups (Hemminga and Buurman, 1997). One of the main  
140 drawbacks of this technique is the quantity of economic resources that are necessary for  
141 its regular application in research laboratories/centres. This is due to the expensive



142 deuterated solvents and NMR tubes, as well as the expensive equipment and significant  
143 sample preparation that are required (Weishar *et al.*, 2003; Cardoza *et al.*, 2004; MIT,  
144 2008). Also, the technique is time consuming not only when measuring, but when  
145 interpreting 2-D or 3-D data resulting from it (Cardoza *et al.*, 2004). Simpler methods  
146 for the characterisation of SOM are required.

147 *1.4. Proxy measures. Opportunities to improve the ability to characterise SOM*  
148 *quality*

149 Water extractable carbon is the most active component in the carbon cycle. Its quantity  
150 and biological nature is affected by the extraction temperature (Bu *et al.*, 2010). Hot-  
151 water extractable carbon (HWC) contains simple compounds such as microorganisms,  
152 soluble carbohydrates and other compounds that account for the labile fraction of SOM  
153 (Ghani *et al.*, 2003). HWC responds to land use changes in the short term and has been  
154 used to detect the effects of different land management practices and for determining  
155 the effects of soil amendments such as biochar or agricultural residues (Leifeld and  
156 Kögel-Knabner, 2005; Uchida *et al.*, 2012; Alburquerque *et al.*, 2014; Fernández-  
157 Romero *et al.*, 2014). For these reasons, it has been proven useful to obtain information  
158 about soil quality (Ghani *et al.*, 2003; Xue *et al.*, 2013).

159 Fluorescence has become popular because of its potential to characterise SOM and  
160 study humic substances, as it is non-destructive, simple, non-separative and accurate. As  
161 a result, it has been used for determining the compositional and structural properties of  
162 SOM (Chen *et al.*, 2003; Senesi and D'Orazio, 2005; Sun *et al.*, 2007; Kwiatkowska *et*  
163 *al.*, 2008; Henderson *et al.*, 2009; Hur and Kim, 2009; Tang *et al.*, 2011). The intensity  
164 and position of the peaks detected in the spectra are unique to each substance structural  
165 and functional characteristics. For instance, higher fluorescence intensities are related  
166 to a higher humification (Martins *et al.*, 2011).

167 The Fluorescence Index (FI) was developed to assess different properties of dissolved  
168 organic matter (DOM). It was defined by McKnight *et al.* (2001) as the ratio of  
169 emission intensities at 450-500 nm excited at 370 nm. The 450 nm point was chosen for  
170 specific characteristics of the experiment. Later, Cory *et al.* (2010) modified the ratio to  
171 470-520 nm to reflect corrections specific to the instruments used. This index has been  
172 correlated to the aromaticity of DOM (Korak *et al.*, 2014).

173 Fluorescence spectroscopy can be used in combination with UV-Visible spectroscopy to  
174 characterise humic substances, as absorbance measures transitions from the ground state  
175 to the excited state, as opposed to fluorescence spectroscopy (Skoog *et al.*, 2007). Its  
176 spectra are usually uniform and provide with qualitative data when a specific  
177 wavelength is selected (Hassouna, *et al.*, 2012). Also the specific absorbance at 254 nm  
178 (SUVA<sub>-254</sub>) has been recognised as a method to determine SOM aromaticity (Fuentes *et*  
179 *al.*, 2006; Chow 2006). This parameter is very useful for assessing the nature of the  
180 general composition of dissolved organic carbon (DOC), due to its high correlation with  
181 it (Weishar *et al.*, 2003).

182 Considering what has been described in sections 1.3 and 1.4 above, both the  
183 fluorescence and NMR techniques can be used in combination to determine the humic  
184 substances properties and the degree of aromaticity; while HWC could contribute  
185 further to the understanding of soil quality, given its usefulness to detect the  
186 biodegradation of soil biochemical properties (Ghani *et al.*, 2003; Saab and Martin-  
187 Neto, 2007; González-Pérez *et al.*, 2007).

188 As an illustrative and additional way to characterise and represent some of the analyses  
189 conducted and the results obtained, Excitation-Emission Matrix (EEM) spectra have  
190 been plotted. These provide information on the relative intensity of fluorescence at  
191 different excitation and emission wavelengths regions in a fast manner that is also easy

192 to interpret (Coble, 1996). Several peaks have been identified that are used to describe  
193 EEM fluorescence spectra. Peak A and C refer to humic peaks. Peak A is referred to as  
194 UVC-excited and is located at an excitation wavelength ( $\lambda_{\text{Ex}}$ ) between 240-260 nm and  
195 an emission wavelength ( $\lambda_{\text{Em}}$ ) between 400-460 nm. Peak C, also referred to as UVA-  
196 excited; is located at a  $\lambda_{\text{Ex}}$  between 320-360 nm and a  $\lambda_{\text{Em}}$  between 420-460 nm. There  
197 are also peaks that indicate biological activity material (peaks B and T, which are  
198 defined as tyrosine-like and tryptophan-like peaks respectively). B has  $\lambda_{\text{Ex}}$  of 270-280  
199 nm and  $\lambda_{\text{Em}}$  of 300-315 nm whereas T has  $\lambda_{\text{Ex}}$  between 270-280 nm and  $\lambda_{\text{Em}}$  of 345-  
200 360 nm (Birdwell and Engel, 2010).

#### 201 *1.5. Aim/objective of this study*

202 The aim of this study was to evaluate the use of fluorescence spectroscopy to measure  
203 SOM quality (specifically the grade of humification). The specific objectives were: (1)  
204 characterise water extractable SOM quality using liquid state  $^1\text{H-NMR}$ ; (2) characterise  
205 the quality of water extractable organic matter using fluorescence spectroscopy and UV-  
206 VIS; (3) compare measures of quantity and quality of water extractable organic matter  
207 with the specific organic matter fractions measured by  $^1\text{H-NMR}$  like aromaticity. If  
208 robust relationships and similarities between optical measures and  $^1\text{H-NMR}$  are found,  
209 there may be potential for fluorescence spectroscopy as a fast and more cost-effective  
210 method of organic matter characterisation.

## 211 **2. Materials and methods**

### 212 *2.1. Field sites description*

213 Cambisols were sampled in two regions with contrasting climatic conditions;  
214 Andalusia (South Spain) and Berkshire (South East England) (Table 1). Berkshire has a  
215 temperate climate, characterised generally by relatively mild winters and summers and  
216 rainfall throughout the year. The annual mean temperature ranges from 6.7 °C and

217 14.5 °C (30 years, annual mean temperature: 10.5 °C) and the average annual rainfall is  
218 635.4 mm (UK Met Office, 2014).

219 Andalusia has a Mediterranean climate, which characteristics are hot and dry summers  
220 contrasted with cool and wet winters. In the province of Cordoba (Hinojosa del Duque  
221 and Pozoblanco), the annual mean temperature is 17.6 °C and the average annual  
222 rainfall is 536 mm (Aemet, 2014). As for the province of Jaen (Torredelcampo), its  
223 annual mean temperature is 16.2 °C and the average annual rainfall is 646.3 mm  
224 (REDIAM, 2007).

225 In Berkshire, samples were collected from University of Reading Farms at Sonning,  
226 Arborfield and Shinfield. The site sampled at Sonning (GS-UK-1) has been covered  
227 with grass for over 15 years. Arborfield (CC-UK-1) was permanent pasture until  
228 autumn 2012, and has been subsequently drilled with wheat or winter barley. Shinfield  
229 (CC-UK-2) has been in an arable rotation for over 20 years and sown with either winter  
230 wheat, maize or spring barley.

231 In Andalusia, soil samples were collected between the provinces of Jaen  
232 (Torredelcampo) and Cordoba (Hinojosa del Duque, Pozoblanco) that were managed  
233 with conventional tillage of cereals and olive crops. CC-ES-3 was managed with a  
234 wheat-barley-fallow cycle, whereas the other sites (CC-ES-1 and CC-ES-2) were  
235 covered by wheat crops throughout the year. OG-ES-1, OG-ES-2, and OG-ES-3 were  
236 covered by olive grove.

## 237 2.2. *Sample collection and preparation*

238 Soil samples were collected from each horizon. Total soil depths are included in Table  
239 2. Only the first horizon from each soil (specified in section 2.3) was used for the  
240 analyses. Samples were air dried and sieved with a 2 mm sieve.

241

242        2.3.        *Analytical methods*

243 Cold and hot water extractable carbon were determined following Ghani *et al.* (2003).  
244 This consists in a cold and a hot extraction of the supernatant of the samples (room  
245 temperature and 80 °C respectively). To do this, 30 ml of ultrapure water was added to  
246 3 g of soil. Then, there was a 30-minute extraction in a shaker at 20°C. After this, the  
247 sample was centrifuged during 20 minutes at 3500 rpm. Once this was done, the  
248 supernatant was extracted (cold extraction) and analysed (DOC). The resulting pellet  
249 was used for the rest of the steps. 30 ml of ultrapure water were added to the pellet,  
250 which was then shaken on a Vortex to mix the ultrapure water with the pellet. The  
251 sample was then left in a bath at 80 °C during 16 hours. The sample was centrifuged at  
252 3500 rpm during 20 minutes and was filtered with a 0.45 µm cellulose nitrate filter (hot  
253 extraction). The filtered supernatant was analysed for DOC, fluorescence, absorbance  
254 and NMR.

255 The techniques that were compared in this study were applied to the first horizon of  
256 each soil sample, as this was considered sufficient for the purposes of comparing these  
257 techniques. This horizon was at different depths depending on the soil sample (at 12 cm  
258 in GS-UK-1; 10.5 cm in CC-UK-1; 5.7 cm in CC-UK-2; 15 cm in CC-ES-1; 20 cm in  
259 CC-ES-2; 30 cm in CC-ES-3; 10 cm in OG-ES-1; 10 cm in OG-ES-2 and 18 cm in OG-  
260 ES-3).

261 Fluorescence of all the hot water extracts was measured in a Varian Eclipse  
262 Fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at an  
263 emission wavelength from 300 to 600 nm at 5-nm increments and an excitation of 240-  
264 450 nm at 5-nm increments. All samples were run in 1 cm quartz cuvettes and in  
265 triplicate.

266 FI was calculated as the ratio of intensities at 450 over 500 nm with an excitation of  
267 370 nm, as described by Cory *et al.* (2010). McKnight *et al.* (2001) introduced this  
268 index approach for the characterization of the fulvic and fraction of DOM.

269 The Humification Index (HIX) was calculated with the following formula:

$$270 \quad (\sum I_{435 \rightarrow 480}) / (\sum I_{300 \rightarrow 345}) + (\sum I_{435 \rightarrow 480})$$

271 where I is the fluorescence intensity at each wavelength (modified of Zsolnay *et al.*,  
272 1999 by Ohno, 2002).

273 The Biological/Autochthonous Index (BIX) has been calculated as the ratio of  
274 intensities at 380 nm over 430 nm with an excitation of 310 nm, as described by Huguet  
275 *et al.* (2009). This index assesses the relative contribution of autochthonous DOM in  
276 water and soil samples.

277 A subsample of the HWC extract was frozen and subsequently freeze-dried to remove  
278 all the water present. Deuterium oxide was added as a solvent to avoid disruptions in the  
279 spectrum, as other solvents have a proton signal that causes disruptions, as  
280 demonstrated by Cardoza *et al.* (2004), prior to a second freezing and a second freeze-  
281 drying. This second stage of freezing-freeze-drying was used to avoid that H<sub>2</sub>O peaks  
282 interfered in the spectra. After that, deuterium oxide (100%, density of 1.107 g/ml at  
283 25 °C) was added again as a solvent to conduct the NMR tests. NMR was measured in  
284 1.5-1.7 ml of sample with a Bruker Avance III 700 MHz NMR spectrometer (Bruker  
285 Corporation, Billerica, MA, USA). The deuterium oxide peak was used as a calibration  
286 reference and placed in 4.75 ppm.

287 Absorbance of all the hot water extracts was measured with a Varian Cary 300 UV-  
288 Visible spectrometer (Agilent Technologies, Santa Clara, CA, USA), from 200-800 nm  
289 with 1 nm intervals using a 1 cm cuvette.

290 SUVA<sub>-254</sub> has been used for this study. This is the absorbance at 254 nm divided by the  
291 DOC concentration (of the hot water extracts). Values are expressed in  $l \cdot mg^{-1} \cdot m^{-1}$ .  
292 DOC of the cold and hot water extracts was calculated with a Shimadzu TOC 5000 total  
293 organic carbon analyser (Shimadzu Corporation, Kyoto, Japan). Standards were  
294 calculated using Stock solutions of 1,000 ppm.  
295 % Organic matter measured by LOI was calculated using a modified version of Hierl *et*  
296 *al.* (2001). A soil sample of 10 g was heated at 105 °C for 24 hours. Then, the sample  
297 was weighted again ( $w_1$ ) and heated in a Muffle furnace at 550 °C for 16 hours to ignite  
298 the organic matter. The sample was weighted after this ( $w_2$ ) and the % of organic matter  
299 was obtained by weight difference between  $w_1$  and  $w_2$ .  
300 Soil pH was determined in 1:2.5 soil to water ratio. Texture was determined by laser  
301 granulometry, using a Coulter LS 230 (Beckman/Coulter Inc. Brea, CA, USA). This  
302 technique uses polarised light at three different wavelengths (450 nm, 600 nm and  
303 900 nm) to analyse the particle size distribution specifically in the 0.04  $\mu m$  to 0.4  $\mu m$   
304 range.  
305 EEM fluorescence spectra were obtained by collecting a series of emission scans of  $\lambda_{Ex}$   
306 240-450 nm at 5 nm intervals and  $\lambda_{Em}$  300-600 nm, also at 5 nm intervals. EEM spectra  
307 were plotted using RStudio v0.98.1091 (Rstudio Inc., Boston, MA, USA).  
308 Intensities are reported in Raman units (RU). Raman scattering was mitigated  
309 subtracting blanks that had been collected on ultrapure water from each spectrum.

#### 310 2.4. Statistical methods

311 Data were tested for normality to verify the model assumptions. As the data failed the  
312 normality test, non-parametric tests were used. The effect of the hot water extraction in  
313 the extractability of carbon (compared to the cold water extraction) was analysed using  
314 a Paired Sample Wilcoxon Signed Rank Test. The correlation between the different

315 analytical methods was tested using the Spearman Rank and assessing the significance  
316 of the resulted Spearman Correlation Coefficient (Minitab 16 for Windows. Minitab  
317 Inc., State College, PA, USA). Linear regressions of the Spearman Correlations were  
318 also plotted (SigmaPlot 12.0 for Windows. Systat Software Inc., San Jose, CA, USA).  
319 Differences of  $p < 0.05$  were considered statistically significant.

### 320 **3. Results and discussion**

#### 321 *3.1. Soil characteristics*

322 The majority of CC-UK soils had sandy texture, with a relatively high proportion of silt  
323 (Table 2). The only exception was CC-UK-2, although the sand proportion was quite  
324 close to that of silt. This texture was similar to that of CC-ES-1 and CC-ES-2, although  
325 CC-ES-3 had a higher proportion of silt and higher proportion of clay than of sand. All  
326 the OG soils presented a texture of a majority of silt (56.7-59.5%), followed by clay. It  
327 is worth considering that silt is the most erodible fraction (Table 2).

328 pH in the UK soils was generally acid, although GS-UK-1 and CC-UK-1 had values  
329 between 3.58-5.66 and CC-UK-2 had values close to 7. ES soils had generally higher  
330 values, ranging from 5.53-6.98 (Table 2). Some authors have related pH and  
331 measurement of aromatics (Weishar *et al.*, 2003). GS-UK-1 has the lowest pH (3.58)  
332 and is the one with the highest aromatics content and highest HWC.

#### 333 *3.2. Total extractable carbon by cold and hot extractions*

334 CWC and HWC measurement results are in Table 3. HWC extracted significantly more  
335 carbon ( $P < 0.01$ ) than CWC, thus proved to be a more exhaustive extraction method.  
336 The increases in the values ranged from 161-605%. This was equivalent to higher  
337 values by a factor between 3 and 7 respectively. The most significant increase was  
338 obtained in GS-UK-1. Gregorich *et al.* (2003) obtained a similar trend in maize-cropped  
339 soils of Ottawa, Canada, and found HWC exceeding CWC by a factor of two.



340 Moreover, Landgraf *et al.* (2006) found that HWC had higher carbon concentrations  
341 than CWC by a factor that varied from 4 to 6 in surface horizons of forest soils in SE  
342 Germany.  
343 A correlation analysis was run to assess whether the C extractability differed between  
344 the different soils and locations. The correlation resulted to be high ( $R_s=0.91$ ,  $p<0.01$ ).  
345 Wang and Wang (2007) found a similar correlation ( $r=0.93$ ,  $p<0.01$ ) in forest oxisols  
346 of Southern China. Klose and Makeschin (2003) also found that HWC and CWC  
347 increased with the same proportion in forest soils of NE Germany. On the other hand,  
348 Ghani *et al.* (2003) found a positive but poor correlation.  
349 HWC and CWC data were compared with SOM (measured by LOI) to assess which of  
350 the two extraction methods would imply a higher correlation with the organic matter  
351 from the samples (Table 4). HWC implied a higher correlation with SOM measured  
352 with LOI ( $R_s= 0.70$ ,  $p<0.05$ ) than CWC ( $R_s= 0.55$ ). Despite the fact that the correlation  
353 between CWC and SOM was lower than that of HWC; other authors have found even  
354 poorer correlations. For instance, Van Migroet *et al.* (2005) found a  $r^2$  of 0.2 in forest  
355 soils in Utah, USA.

### 356 3.3. *Quality of cold-water extractable carbon using UV-Vis and Fluorescence*

357 Cold-water extractable carbon (CWC) data was compared with UV-Vis and  
358 fluorescence spectroscopy, using  $SUVA_{-254}$  and FI respectively. The Spearman Rank  
359 Correlation was calculated to evaluate the quality and reproducibility of CWC using  
360 optical techniques (Table 4).

361 There were high correlation patterns between CWC and  $SUVA_{-254}$  ( $R_s=0.82$ ,  $p<0.05$ )  
362 although not between CWC and FI ( $R_s=-0.29$ ), where the slight correlation that could  
363 be appreciated was inverse. Chow (2006) obtained a worse correlation between CWC  
364 and  $SUVA_{-254}$  ( $R^2=0.38$ ), whereas that of Van Migroet *et al.* (2005) was even lower

365 ( $R^2=0.01$ ). As Weishar *et al.* (2003) point out; some authors have found conflicting  
366 conclusions when using SUVA<sub>-254</sub> to determine the aromaticity of DOC.

### 367 3.4. *Quality of hot-water extractable carbon using UV-Vis and Fluorescence*

368 HWC data was compared with SUVA<sub>-254</sub> and FI to assess whether it had higher  
369 correlation than CWC. As it can be seen in Table 4, there is a high correlation ( $R_s=0.88$ ,  
370  $p<0.001$ ) between HWC and SUVA<sub>-254</sub> whereas that of HWC with FI is not as  
371 significant ( $R_s=-0.53$ ). In both cases, HWC was more significantly correlated to the  
372 results obtained with fluorescence and SUVA<sub>-254</sub> than CWC.

### 373 3.5. *EEM spectra general characteristics*

374 A set of reference fluorescence spectra has been represented in Figure 1. As it can be  
375 seen in Figure 1.a and 1.b, the two soils with the highest % aromatics measured with  
376 <sup>1</sup>H-NMR as well as high SUVA<sub>-254</sub> and HWC values (GS-UK-1 and CC-UK-1) showed  
377 strong peaks C (10-12 RUs), which indicate UVA-excited humic peaks as described by  
378 Coble (1996). They also showed weak peaks A.

379 On the other hand, Figure 1.c. represents the soil with the highest FI, but lowest %  
380 aromatic measured with <sup>1</sup>H-NMR as well as comparatively low values of SUVA<sub>-254</sub>  
381 (CC-ES-1). The EEM spectrum of this soil is very different from the ones represented in  
382 Figures 1.a and 1.b, with a strong peak A or UVC-excited (8 RUs), and weak peaks C  
383 and T. The presence of the latter indicates biological activity.

### 384 3.6. *Relationship between optical measures and NMR*

385 The aromaticity of isolated fulvic acid samples was calculated as the ratio of the area of  
386 aromatic hydrogen region to the total area of the <sup>1</sup>H-NMR spectrum (% aromaticity). An  
387 analogous method was used to calculate the ratio of the area of aliphatic hydrogen and  
388 carbohydrate hydrogen regions.

389 As it can be seen in Figure 2, the aromatic hydrogen region is situated in a chemical  
390 shift of 6-8ppm by frequency. On the other hand, the carbohydrate hydrogen and  
391 aliphatic hydrogen regions are situated in a chemical shift of 3-4.2 ppm and 0.5-3 ppm  
392 respectively. For Figure 2, the two soils with the highest % aromatics were chosen for  
393 representation purposes (GS-UK-1 and CC-UK-1).

394 Results of these measurements can be found in Table 3, along with the results from the  
395 optical techniques. Correlation analyses were conducted comparing the results from all  
396 of them (Table 4).

397 The largest carbon fraction of the UK soils was carbohydrates. The same trend was  
398 found in the CC-ES soils, but not in the OG-ES soils, where the largest carbon fraction  
399 was aliphatics.

400 The proportion of aromatics of the UK soils ranged from 3.91%-5.98%, whereas for the  
401 CC-ES and OG-ES soils, it ranged from 2.40-3.47% and 3.90%-4.33% respectively. As  
402 these were taken from samples in the first horizon of each soil, the low level of  
403 aromaticity can be explained by the fact that plant residues may have accumulated in the  
404 surface, while microorganisms did not have enough capacity to decompose them. As  
405 Cardoza *et al.* (2004) point out, NMR is a powerful tool for investigating humic  
406 substances interactions at the molecular level.

407 Aromatics have positive correlations for SUVA<sub>-254</sub>, CWC and HWC but are inversely  
408 correlated to FI. Correlations are high for aromatics compared with SUVA<sub>-254</sub> and  
409 HWC (Figures 3 and 4.  $R_s$  of 0.95 and 0.90 respectively;  $p < 0.001$ ), but not as good for  
410 FI (Figure 5). The correlation between aromatics and CWC, although significant, is not  
411 as strong as the one with HWC ( $R_s = 0.74$ ,  $p < 0.05$ ). Weishaar *et al.* (2003) found that  
412 SUVA<sub>-254</sub> is a good predictor of the humic fraction (higher SUVA<sub>-254</sub> indicates higher  
413 humic acid content and molecular weight in DOM solutions) and the general chemical

414 properties of HWC, although it does not predict the reactivity of HWC from different  
415 types of source materials nor information of the individual molecules of samples unless  
416 these are humic substances (Chin *et al.*, 1997; Weishar *et al.*, 2003). They obtained a  
417 good correlation between SUVA<sub>-254</sub> and aromaticity determined by <sup>13</sup>C-NMR (R=0.97),  
418 which was slightly higher than ours. Yeh *et al.* (2014) also found a high correlation  
419 between SUVA<sub>-254</sub> measurements and the aromatic fraction of samples of organic  
420 matter of river sediments of Taiwan. Jamieson *et al.* (2014) found that higher SUVA<sub>-254</sub>  
421 implied higher aromaticity in biochar from sugar maple, thus demonstrating that this  
422 effect is currently studied when characterising organic material with a high recalcitrance  
423 and that is able to retain carbon for thousands of years, due to its stability (Lehmann,  
424 2007).

425 According to McKnight (2001) and other authors (Rodríguez *et al.*, 2014; Wei *et al.*,  
426 2014), there is a correlation between the FI values and those from methods that indicate  
427 the aromaticity of humic substances such as the ratio of the aromatic carbon region area  
428 in the total NMR spectra and SUVA<sub>-254</sub>. This pattern was observed in our soils, with  
429 negative correlations between FI and these 2 parameters (Rs=-0.63 and Rs=-0.52  
430 respectively), although only the trend with the aromatic carbon region area in the total  
431 NMR spectra was statistically significant (p<0.05).

432 Kim *et al.* (2006) and Rodríguez *et al.* (2014) indicated that differences in the FI higher  
433 than 0.1 would imply significant differences in the aromaticity of the samples. If this  
434 assumption is applied to our case, CC-UK-2 has differences in its aromaticity with GS-  
435 UK-1 but not with CC-UK-1. CC-ES-2 has a different aromaticity than CC-ES-1 and  
436 CC-ES-3. In the same way, OG-ES-1 has differences with OG-ES-2 and OG-ES-3.

437 When all the types are compared, there are combinations of differences in the  
438 aromaticity but a single pattern cannot be obtained.

439 Given the results above, the regressions between the aromatic carbon region measured  
440 with  $^1\text{H-NMR}$  and  $\text{SUVA}_{-254}$  and HWC are powerful. The equations we obtained can  
441 explain very significantly the relationship between these variables ( $p < 0.001$  for the  
442 results of  $^1\text{H-NMR}$  predicted with  $\text{SUVA}_{-254}$  and  $p < 0.01$  if they are predicted using  
443 HWC). These equations (Figure 3 and Figure 4) could therefore be used to predict the  
444 aromaticity of water-extractable carbon.

445 The regression of the aromaticity measured with  $^1\text{H-NMR}$  with FI, although significant  
446 ( $p < 0.05$ ), did not have the same level of confidence as the one with HWC and  $\text{SUVA}_{-254}$ .  
447 As it can be seen in Figure 5, an outlier was identified in the Regression analysis (FI  
448 value of CC-ES-3). Figure 5 showed how the regression model improved when the  
449 outlier was removed from the analysis ( $R_s = -0.98$ ). The linear regression equation that  
450 resulted after the elimination of the outlier is able to explain the  $^1\text{H-NMR}$  values using  
451 FI with a very high significance ( $p < 0.001$ ).

452 However, according to McKnight (2001), the FI would not be sufficient to estimate  
453 aromaticity and other techniques are necessary (e.g.  $\text{SUVA}_{-254}$ ), as geological processes  
454 can alter aromaticity without changing the FI.

455  $\text{SUVA}_{-254}$  did not demonstrate a particularly strong correlation with FI ( $R_s = -0.52$ , Table  
456 4). The correlation was negative (higher  $\text{SUVA}_{-254}$  led to lower FI) which coincides  
457 with authors like Kothawala *et al.* (2012) with minerals soils collected across Canada  
458 although their trend is less clear than in the study of other authors such as Williams *et*  
459 *al.* (2010), where the slope is more pronounced. The correlation of Williams *et al.*  
460 (2010) was similar to ours ( $r = -0.57$  in their study and  $R_s = -0.52$  in ours), although the  
461 statistical analysis in their case was the Pearson correlation, as their data followed a  
462 normal distribution. Hassouna *et al.* (2012) found a negative correlation as well, with  
463 slightly higher correlation in Mediterranean calcareous soils ( $r = -0.69$ ). As in the case

464 described in Figure 5, if the same outlier was removed, the correlation improved  
465 significantly ( $R_s=-0.98$ ) and the equation of the linear regression explained with a very  
466 high degree of significance ( $p<0.001$ ) the relation between SUVA<sub>-254</sub> and FI.  
467 Analogously, the same outlier could be removed in the analysis of HWC vs. FI,  
468 obtaining a significant correlation between them ( $R_s=-0.88$ ). A linear regression model  
469 explains significantly ( $p<0.01$ ) the relationship between HWC and FI and therefore  
470 similar conclusions can be stated as in the case of the correlation of SUVA<sub>-254</sub> to FI.  
471 Given the strong intrinsic relationships between the results of SUVA<sub>-254</sub>, HWC and FI;  
472 and how they are able to predict the proportion of aromaticity measured with <sup>1</sup>H-NMR,  
473 we can conclude that UV-Vis absorbance and fluorescence spectroscopy can be used to  
474 characterise the aromaticity of carbon and may be a plausible substitute for <sup>1</sup>H-NMR,  
475 given that they are more cost-effective. Other authors such as Zornoza *et al.* (2008,  
476 2015) demonstrated the effectiveness of spectroscopy (near infrared reflectance  
477 spectroscopy in their study) as an accurate, cost- and time-effective method for  
478 predicting and/or estimating soil biogeochemical properties and other soil parameters.  
479 Correlations between aliphatics and the data from the optical measures and CWC/HWC  
480 were poor (Table 4), although slight trends can be observed for SUVA<sub>-254</sub>, FI and CWC.  
481 In these cases, the correlation was positive for FI and CWC but negative for SUVA<sub>-254</sub>.  
482 Correlations were also poor between carbohydrates and the data from the optical  
483 measures and HWC/CWC, with only one trend detected. This was the inverse  
484 correlation ( $R_s=-0.35$ ) between HWC and carbohydrates.  
485 BIX and HIX did not correlate well with any of the other parameters (Table 4), with  
486 very few trends detected. Of the trends detected, HIX was positively correlated with FI  
487 ( $R_s=0.52$ ). It is worth noting that BIX was positively correlated with carbohydrates but  
488 inversely correlated to aliphatics ( $R_s=0.31$  and  $-0.31$  respectively), whereas the trend

489 was the contrary for HIX ( $R_s=-0.28$  and  $0.22$ ). A similar trend than ours was also found  
490 by Kalbitz *et al.* (2003) between HIX and carbohydrates in forest soils, arable soils and  
491 a fen area; although theirs was stronger ( $r^2=0.81$ ). Williams *et al.* (2010) studied the  
492 correlation between HIX and SUVA<sub>-254</sub> in watershed from mixed land use in Canada.  
493 They obtained a positive correlation ( $r=0.74$ ), whereas our correlation was negative. On  
494 the other hand, Yeh *et al.* (2014) did not find a clear trend between SUVA<sub>-254</sub> and HIX.  
495 The significance of our comparison between SUVA<sub>-254</sub> and HIX values is extremely  
496 low and therefore robust conclusions on this issue cannot be stated. Bu *et al.* (2010)  
497 compared HWC and HIX in various soils with different vegetation types in Wuyi  
498 Mountain (SE China), obtaining a poor negative correlation, just like ours. However,  
499 Kalbitz *et al.* (2003) found a strong correlation between the aromatic fraction of DOM  
500 and HIX ( $r^2=0.80$ ).  
501 Yeh *et al.* (2014) calculated BIX as well, obtaining that higher SUVA<sub>-254</sub> values implied  
502 lower BIX values, which coincides with our comparison in this case, although its  
503 significance is not relevant enough to establish a robust conclusion.  
504 Plotting the results from Birdwell and Engel (2010) for FI, HIX, and BIX did not led to  
505 significant correlations, which is similar to our results and, along with the authors  
506 commented above, demonstrate that a number of studies have found difficulties when  
507 correlating HIX and BIX to other parameters.

#### 508 **4. Conclusions**

509 Different techniques to measure the quantity and quality of SOM were tested in  
510 cambisols from very different climatic locations and under different cropping systems.  
511 HWC extracted a higher amount and carbon than CWC, and correlated better with the  
512 %SOM (LOI) than CWC.

513 SUVA<sub>-254</sub> and HWC correlated significantly with the proportion of aromatics measured  
514 with <sup>1</sup>H-NMR, demonstrating their complementary nature. Linear regression models  
515 fitted to the data were able to explain the relationship between the specific fraction of  
516 aromatics measured with <sup>1</sup>H-NMR and SUVA<sub>-254</sub> and HWC, and therefore allow the  
517 aromatic fraction to be estimated at lower cost using SUVA<sub>-254</sub> and HWC.  
518 A linear regression model was also able to explain the relationship between <sup>1</sup>H-NMR  
519 measurements and FI, after an outlier was removed.  
520 BIX and HIX were not useful indexes for our purposes, as they did not correlate well to  
521 the carbon fractions measured with <sup>1</sup>H-NMR or with FI, SUVA<sub>-254</sub>, or HWC.  
522 The EEM spectra showed a difference in the peak types that appeared in the CC-UK  
523 soils and in the CC-ES soil. The fact that figures 1.a and 1.b showed a stronger peak C  
524 and figure 1.c. showed a stronger peak A indicated subtle qualitative differences in their  
525 humic fractions. This could have been caused by the different soil management (more  
526 intense agricultural activity in CC-ES-1). More work will be needed to confirm this.  
527 In view of our results, optical measures have a potential, in combination, to predict the  
528 aromatic fraction of SOM without the need of expensive and time consuming  
529 techniques like <sup>1</sup>H-NMR; which could be very useful when the equipment is not  
530 available or in instances when a high number of samples need to be analysed  
531 simultaneously.

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## 534 **6. References**

535 Aemet, 2014. Historic series of climatic data 1971-2000. Agencia Estatal de  
536 Meteorología (Spanish State Meteorological Agency). Available at:



537 <http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l>  
538 [=5402&k=and](#) [Accessed: 08<sup>th</sup> August 2014].

539 Agren, G.I., Bosatta, E., 2002. Reconciling differences in predictions of temperature  
540 response of soil organic matter. *Soil Biol. Biochem.* 34, 129–132.

541 Alburquerque, J.A., Calero, J.M., Barrón, V., Torrent, J., Campillo, M.C., Gallardo, A.,  
542 Villar, R., 2014. Effects of biochars produced from different feedstocks on soil  
543 properties and sunflower growth. *J. Plant Nutr. Soil Sci.* 177, 16–25.

544 Batjes, N. H., 2014. Projected changes in soil organic carbon stocks upon adoption of  
545 recommended soil and water conservation practices in the upper Tana River catchment,  
546 Kenya. *Land Degrad. Dev.* 25, 278–287.

547 Bell, M., Lawrence, D., 2009. Soil carbon sequestration - Myths and mysteries.  
548 Department of Primary Industries and Fisheries, Queensland, Australia. Available at:  
549 [http://www.futurebeef.com.au/wp-content/uploads/2011/09/Soil-Carbon-Sequestration-](http://www.futurebeef.com.au/wp-content/uploads/2011/09/Soil-Carbon-Sequestration-Report.pdf)  
550 [Report.pdf](#) [Accessed: 8<sup>th</sup> November 2014].

551 Birdwell, J.E., Engel, A.S., 2010. Characterization of dissolved organic matter in cave  
552 and spring waters using UV–Vis absorbance and fluorescence spectroscopy. *Org.*  
553 *Geochem.* 41, 270–280.

554 Brevik, E.C., 2009. Soil Health and Productivity, in: Verheye, W. (Ed.), *Soils, Plant*  
555 *Growth and Crop Production; Encyclopaedia of Life Support Systems (EOLSS)*,  
556 Developed under the Auspices of the UNESCO. EOLSS Publishers, Oxford, UK.  
557 Available at: <http://www.eolss.net> [Accessed 10<sup>th</sup> November 2014].

558 Brevik, E. C., 2012. Soils and climate change: Gas fluxes and soil processes. *Soil*  
559 *Horiz.* 53, 12–23.

560 Brevik, E. C., Cerdà, A., Mataix-Solera, J., Pereg, L., Quinton, J. N., Six, J., Van Oost  
561 K., 2015. The interdisciplinary nature of SOIL. *Soil* 1, 117–129.

562 Bu, X., Wang, L., Ma, W., Yu, X., McDowell, W.H., Ruan, H., 2010. Spectroscopic  
563 characterization of hot-water extractable organic matter from soils under four different  
564 vegetation types along an elevation gradient in the Wuyi Mountains. *Geoderma* 159,  
565 139–146.

566 Cardoza, L.A., Korir, A.K., Otto, W.H., Wurrey, C.J., C.K. Larive, C.K., 2004.  
567 Applications of NMR spectroscopy in environmental science. *Prog. Nucl. Mag. Res.*  
568 *Sp.* 45, 209–238.

569 Cerdà, A., 1998. The influence of aspect and vegetation on seasonal changes in erosion  
570 under rainfall simulation on a clay soil in Spain. *Can. J. Soil. Sci.* 78, 321–330.

571 Cerdà, A., 2000. Aggregate stability against water forces under different climates on  
572 agriculture land and scrubland in southern Bolivia. *Soil Till. Res.* 57, 159–166.

573 Chen, J., LeBoef, E.J., Dai, S., Gu, B., 2003. Fluorescence spectroscopic studies of  
574 natural organic matter fractions. *Chemosphere* 50, 639–647.

575 Chen, Y., Day, S.D., Wick, A.F., Strahm, B.D., Wiseman, P.E., Daniels, W.L., 2013.  
576 Changes in soil carbon pools and microbial biomass from urban land development and  
577 subsequent post-development soil rehabilitation. *Soil Biol. Biochem.* 66, 38–44.

578 Chin Y.P., Aiken, G.R., Danielsen, K.M., 1997. Binding of pyrene to aquatic and  
579 commercial humic substances: the role of molecular weight and aromaticity, *Environ.*  
580 *Sci. Technol.* 31, 1630–1635.

581 Chow, A.T., 2006. Disinfection byproduct reactivity of aquatic humic substances  
582 derived from soils. *Water Res.* 40, 1426–1430.

583 Coble, P., 1996. Characterization of marine and terrestrial DOM in seawater using  
584 excitation–emission matrix spectroscopy. *Mar. Chem.* 51, 325–346.

585 Cory, R.M., Miller, M.P., McKnight, D.M., Guerard, J.J., Miller, P.L., 2010. Effect of  
586 instrument-specific response on the analysis of fulvic acid fluorescence spectra. *Limnol.*  
587 *Oceanogr. Meth.* 8, 67–78.

588 Dungait, J., Hopkins, D., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter  
589 turnover is governed by accessibility not recalcitrance. *Glob. Chang. Biol.* 18, 1781–  
590 1796.

591 Fernández-Romero, M.L., Lozano-García, B., Parras-Alcántara, L., Collins, C.D.,  
592 Clark, J.M., 2014. Effects of land management on different forms of soil carbon in olive  
593 groves in Mediterranean areas. *Land Degrad. Dev.* (In press) DOI: 10.1002/ldr.2327.

594 Fischlin, A., Midgley, G.F., Price, J., Leemans, R., Gopal, B., Turley, C., Rounsevell,  
595 M., Dube, P. Tarazona, J., Velichko, A., 2007. Ecosystems, their properties, goods, and  
596 services, in: M. L., Canziani, O. F., Palutikof, J. P., van der Linden, P. J. & Hanson, C.  
597 E. (Eds.), *Climate Change: Impacts, Adaptation and Vulnerability. Contribution of*  
598 *Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on*  
599 *Climate Change.* Cambridge University Press, Cambridge, UK, pp. 211–272. ISBN:  
600 978 0521 88010-7.

601 Fuentes, M., González-Gaitano, G., García-Mina, J.M., 2006. The usefulness of UV–  
602 visible and fluorescence spectroscopies to study the chemical nature of humic  
603 substances from soils and composts. *Org. Geochem.* 37, 1949–1959.

604 García-Gil, J.L., Plaza, C., Soler-Rovira, P., Polo, A., 2000. Long-term effects of  
605 municipal solid waste compost application on soil enzyme activities and microbial  
606 biomass. *Soil Biol. Biochem.* 32, 1907–1913.

607 Ghani, A., Dexter, M. & Perrott, K.W., 2003. Hot-water extractable carbon in soils: a  
608 sensitive measurement for determining impacts of fertilization, grazing and cultivation.  
609 *Soil Biol. Biochem.* 35, 1231–1243.

610 González-Pérez, M., Milori, D.M.B.P., Colnago, L.A., Martin-Neto, L., Melo, W.J.,  
611 2007. A laser-induced fluorescence spectroscopic study of organic matter in a Brazilian  
612 Oxisol under different tillage systems. *Geoderma* 138, 20–24.

613 Gregorich, E.G., Beare, M.H., Stoklas, U., St-Georges, P., 2003. Biodegradability of  
614 soluble organic matter in maize-cropped soils. *Geoderma* 113, 237– 252.

615 Hassouna, M., Théraulaz, F., Massiani, C., 2012. Production and elimination of water  
616 extractable organic matter in a calcareous soil as assessed by UV/Vis absorption and  
617 fluorescence spectroscopy of its fractions isolated on XAD-8/4 resins. *Geoderma* 189–  
618 190, 404–414.

619 Helal, A.A., Murad, G.A., Helal, A.A., 2011. Characterization of different humic  
620 materials by various analytical techniques. *Arab. J. Chem.* 4, 51–54.

621 Hemminga, M.A., Buurman, P., 1997. Editorial: NMR in soil science. *Geoderma* 80,  
622 221–224.

623 Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., Khan, S.J.,  
624 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review.  
625 *Water Res.* 43, 863–881.

626 Hieri, O., Lotter, A.F., Lemke, G., 2001. Loss on Ignition as a method for estimating  
627 organic and carbonate content in sediments: Reproducibility and comparability of  
628 results. *J. Paleolimnol.* 25, 101–110.

629 Huguet, A., Vacher, L., Relexans, S., Saubusse, S., Froidefond, J.M., Parlanti, E., 2009.  
630 Properties of fluorescent dissolved organic matter in the Gironde Estuary. *Org.*  
631 *Geochem.* 40, 706–719.

632 Hur, J., Kim, G., 2009. Comparison of the heterogeneity within bulk sediment humic  
633 substances from a stream and reservoir via selected operational descriptors.  
634 *Chemosphere* 75, 483–490.

635 Hur, J., Park, S.W., Kim, M.C., Kim, H.S., 2013. Enhanced binding of hydrophobic  
636 organic contaminants by microwave-assisted humification of soil organic matter.  
637 *Chemosphere* 93, 2704–2710.

638 Jamieson, T., Sager, E., Guéguen, C., 2014. Characterization of biochar-derived  
639 dissolved organic matter using UV–visible absorption and excitation–emission  
640 fluorescence spectroscopies. *Chemosphere* 103, 197–204.

641 Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil  
642 derived dissolved organic matter as related to its properties. *Geoderma* 113, 273–291.

643 Kim, H.C., Yu, M.J., Han, I., 2006, Multi-method study of the characteristic chemical  
644 nature of aquatic humic substances isolated from the Han River, Korea. *Appl.*  
645 *Geochem.* 21, 1226–39.

646 Kleber, M., 2010. What is recalcitrant soil organic matter? *Environ. Chem.* 7, 320–332.

647 Kleber, M., Johnson, M. G., 2010. Advances in understanding the molecular structure  
648 of soil organic matter: implications for interactions in the environment. *Adv. Agron.*  
649 106, 77–142.

650 Klose, S., Makeschin, F., 2003. Effects of past fly ash deposition on the forest floor  
651 humus chemistry of pine stands in Northeastern Germany. *For. Ecol. Manage.* 183,  
652 113–126.

653 Koarashi, J., Iida, T., Asano, T., 2005. Radiocarbon and stable carbon isotope  
654 compositions of chemically fractionated soil organic matter in a temperate-zone forest.  
655 *J. Environ. Radioact.* 79, 137–56.

656 Korak, J.A., Dotson, A.D., Summers, R.S., Rosario-Ortiz, F.L., 2014. Critical analysis  
657 of commonly used fluorescence metrics to characterize dissolved organic matter. *Water*  
658 *Res.* 49, 327–338.

659 Kothawala, D.N., Roehm, C., Blodau, C., Moore, T.R., 2012. Selective adsorption of  
660 dissolved organic matter to mineral soils. *Geoderma* 189–190, 334–342.

661 Kwiatkowska, J., Provenzano, M., Senesi, N., 2008. Long term effects of a brown coal-  
662 based amendment on the properties of soil humic acids. *Geoderma* 148, 200–205.

663 Lal, R., 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123, 1–  
664 22.

665 Landgraf, D., Leinweber, P., Makeschin, F., 2006. Cold and hot water-extractable  
666 organic matter as indicators of litter decomposition in forest soils. *J. Plant Nutr. Soil*  
667 *Sci.* 169, 76–82.

668 Lehmann, J., 2007. Bio-energy in the black. *Front. Ecol. Environ.* 5, 381–387.

669 Leifeld J., Kogel-Knabner I., 2005. Soil organic matter fractions as early indicators for  
670 carbon stock changes under different land-use? *Geoderma* 124, 143–155.

671 Li, L.J., Han, X.Z., You, M.Y., Yuan, Y.R., Ding, X.L., Qiao, Y.F., 2013. Carbon and  
672 nitrogen mineralization patterns of two contrasting crop residues in a Mollisol: Effects  
673 of residue type and placement in soils. *Eur. J. Soil Biol.* 54, 1–6.

674 Luke, J. K., Brevik, E. C., Wood, G. L., 2009. Evaluation of loss on ignition method for  
675 determining organic matter of South Georgia soils. *Soil Surv. Hor.* 50, 83–85.

676 Martins, T., Saab, S.C., Milori, D.M.B.P., Brinatti, A.M., Rosa, J.A., Cassaro, F.A.M.,  
677 Pires, L.F., 2011. Soil organic matter humification under different tillage managements  
678 evaluated by Laser Induced Fluorescence (LIF) and C/N ratio. *Soil Till. Res.* 111, 231–  
679 235.

680 McKnight, D.M., Boyer, E.W., Westerhoff, P., Doran, P., Kulbe, T., Andersen, D.,  
681 2001. Spectrofluorimetric characterization of dissolved organic matter for indication of  
682 precursor organic material and aromaticity. *Limnol. Oceanogr.* 46, 38–48.

683 MIT, 2008. Things to know before you begin operating an NMR. Department of  
684 Chemistry. Instrumentation Facility. Massachusetts Institute of Technology. Available  
685 at: <http://web.mit.edu/specplab/www/PDF/DCIF-Things-2-know-NMR-m08.pdf>  
686 [Accessed 10<sup>th</sup> June 2014].

687 Novara, A., Gristina, L., Saladino, S.S., Santoro, A., Cerdà, A., 2011. Soil erosion  
688 assessment on tillage and alternative soil managements in a Sicilian vineyard. *Soil Till.*  
689 *Res.* 117, 140–147.

690 Ohno, T., 2002. Fluorescence inner-filtering correction for determining the humification  
691 index of dissolved organic matter. *Environ. Sci. Technol.* 36, 742–746.

692 Olk, D.C., Gregorich, E.G., 2006. Overview of the symposium proceedings,  
693 “Meaningful pools in determining soil carbon and nitrogen dynamics”. Soil Sci. Soc.  
694 Am. J. 70, 967–974.

695 Paradelo R., Barral, M.T., 2013. Influence of organic matter and texture on the  
696 compactability of Technosols. Catena 110, 95–99.

697 REDIAM, 2007. Historical series of meteorological data 1953-2007. Red de  
698 Información Ambiental de Andalucía (Andalusian Network of Environmental  
699 Information). Seville, Spain.

700 Rodríguez, F.J., Schlenger, P., García-Valverde, M., 2014. A comprehensive structural  
701 evaluation of humic substances using several fluorescence techniques before and after  
702 ozonation. Part I: Structural characterization of humic substances. Sci. Total  
703 Environ. 476–477, 718–730.

704 Rose, M.T., Patti, A.F., Little, K.R., Brown, A.L., Jackson, W.R., Cavagnaro, T.R.,  
705 2014. Chapter Two – A Meta-Analysis and Review of Plant-Growth Response to  
706 Humic Substances: Practical Implications for Agriculture. Adv. Agron. 124, 37–89.

707 Saab, S.C., Martin-Neto, L., 2007. Condensed aromatic rings and E<sub>4</sub>/E<sub>6</sub> ratio: humic  
708 acids in gleysoils studied by NMR cp/mas <sup>13</sup>C, and dipolar dephasing. Quim. Nova 30,  
709 260–263.

710 Saha, D., Kukal, S.S., Bawa, S.S., 2014. Soil organic carbon stock and fractions in  
711 relation to land use and soil depth in the degraded Shiwaliks Hills of lower Himalayas.  
712 Land Degrad. Dev. 25, 407–416.

713 Salehi, M.H., Beni, O.H., Harchegani, H.G., Borujeni, I.E., 2011. Refining Soil Organic  
714 Matter Determination by Loss-on-Ignition. Pedosphere 21, 473–482.



715 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A.,  
716 Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse,  
717 D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an  
718 ecosystem property. *Nature* 478, 49–56.

719 Schrumpf, M., Kaiser, K., 2015. Large differences in estimates of soil organic carbon  
720 turnover in density fractions by using single and repeated radiocarbon inventories.  
721 *Geoderma* 239–240, 168–178.

722 Sellami, F., Hachicha, S., Chtourou, M., Medhioub, K., Ammar, E., 2008. Maturity  
723 assessment of composted olive mill wastes using UV spectra and humification  
724 parameters. *Bioresource Technol.* 99, 6900–6907.

725 Senesi, N., D’Orazio, V., 2005. Fluorescence spectroscopy, in: Hillel, D. (Ed.),  
726 *Encyclopedia of Soils in the Environment*. Academic Press, Elsevier Science, pp. 35–  
727 52. ISBN: 978-0-12-348530-4.

728 Skoog, D.A., Holler, F.J., Crouch, S.R., 2007. *Principles of Instrumental Analysis*.  
729 Brooks/Cole Publishing. Belmont, CA, USA. ISBN: 978-0-495-01201-6.

730 Sparks, D.L., 2003. *Environmental Soil Chemistry*, second ed. Academic Press,  
731 Elsevier Science, San Diego, USA. ISBN 0-12-656446-9.

732 Srinivasarao, CH., Venkateswarlu, B., Lal, R., Singh, A. K., Kundu, S., Vittal, K. P.  
733 R., Patel, J. J., Patel, M. M., 2014. Long-term manuring and fertilizer effects on  
734 depletion of soil organic carbon stocks under pearl millet- cluster bean-castor rotation in  
735 Western India. *Land Degrad. Dev.* 25, 173–183.

736 Sun, W.L., Ni, J.R., Xu, N., Sun, L.Y., 2007. Fluorescence of sediment humic substance  
737 and its effect on the sorption of selected endocrine disruptors. *Chemosphere* 66, 700–  
738 707.

739 Sutton, R., Sposito, G., 2005. Molecular Structure in Soil Humic Substances: The New  
740 View. *Environ. Sci. Technol.* 39, 9009–9015.

741 Tang, Z., Yu, G., Liu, D., Xu, D., Shen, Q., 2011. Different analysis techniques for  
742 fluorescence excitation–emission matrix spectroscopy to assess compost maturity.  
743 *Chemosphere* 82, 1202–1208.

744 Trumbore, S., 1997. Potential responses of soil organic carbon to global  
745 environmental change. *P. Natl. Acad. Sci. USA* 94, 8284-8291.

746 Trumbore, S., 2000. Age of soil organic matter and soil respiration: Radiocarbon  
747 constraints on belowground C dynamics. *Ecol. Appl.* 10, 399–411.

748 Uchida, Y., Nishimurab, S., Akiyama, H., 2012. The relationship of water-soluble  
749 carbon and hot-water-soluble carbon with soil respiration in agricultural fields. *Agr.*  
750 *Ecosyst. Environ.* 156, 116–122.

751 UK Met Office, 2014. Reading 1981–2010 climatic averages. Available at:  
752 <http://www.metoffice.gov.uk/public/weather/climate/gcpc9wzww> [Accessed 8<sup>th</sup>  
753 November 2014].

754 Van Miegroet, H., Boettinger, J.L., Baker, M.A., Nielsen, J., Evans, D., Stum, A., 2005.  
755 Soil carbon distribution and quality in a montane rangeland-forest mosaic in northern  
756 Utah. *Forest Ecol. Manag.* 220, 284–299.

757 Wang, Q.K., Wang S.L., 2007. Soil organic matter under different forest types in  
758 Southern China. *Geoderma* 142, 349–356.

759 Wei, Z., Zhao, X., Zhu, C., Xi, B., Zhao, Y., Yu, X., 2014. Assessment of humification  
760 degree of dissolved organic matter from different composts using fluorescence  
761 spectroscopy technology. *Chemosphere* 95, 261–267.

762 Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K.,  
763 2003. Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical  
764 Composition and Reactivity of Dissolved Organic Carbon. *Environ. Sci. Technol.* 37,  
765 4702–4708.

766 Williams, C.J., Yamashita, Y., Wilson, H.F., Jaffé, R., Xenopoulos, M.A., 2010.  
767 Unravelling the role of land use and microbial activity in shaping dissolved organic  
768 matter characteristics in stream ecosystems. *Limnol. Oceanogr.* 55, 1159–1171.

769 Xue, S., Li, P., Liu, G., Li, Z., Zhang, C., 2013. Changes in Soil Hot-Water Extractable  
770 C, N and P Fractions During Vegetative Restoration in Zhifanggou Watershed on the  
771 Loess Plateau. *J. Integr. Agr.* 12, 2250–2259.

772 Yeh, Y.L., Yeh, K.J., Hsu, L.F., Yu, W.C, Lee, M.H., Chen, T.C., 2014. Use of  
773 fluorescence quenching method to measure sorption constants of phenolic  
774 xenoestrogens onto humic fractions from sediment. *J. Hazard. Mater.* 277, 27–33.

775 Zhao, G., Mu, X., Wen, Z., Wang, F., Gao, P., 2013. Soil erosion, conservation, and  
776 eco-environment changes in the Loess Plateau of China. *Land Degrad. Dev.* 24, 499–  
777 510.

778 Zhao, M., Zhou, J., Kalbitz, K., 2008. Carbon mineralization and properties of water-  
779 extractable organic carbon in soils of the south Loess Plateau in China. *Eur. J. Soil*  
780 *Biol.* 44, 158–165.

781 Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V., Mataix-  
782 Beneyto, J., 2008. Near infrared spectroscopy for determination of various physical,  
783 chemical and biochemical properties in Mediterranean soils. *Soil Biol. Biochem.* 40,  
784 1923–1930.

785 Zornoza, R., Acosta, J. A., Bastida, F., Domínguez, S. G., Toledo, D. M., Faz A., 2015.  
786 Identification of sensitive indicators to assess the interrelationship between soil quality,  
787 management practices and human health. *Soil* 1, 173–185.

788 Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., Saccomandi, F., 1999.  
789 Differentiating with fluorescence spectroscopy the sources of dissolved organic matter  
790 in soils subjected to drying. *Chemosphere* 38, 45–50.

791 **Figure captions**

792 **Figure 1.** EEM plots from: a) GS-UK-1; b) CC-UK-1; c) CC-ES-1

793 **Figure 2.** Liquid state  $^1\text{H-NMR}$  spectra from: a) GS-UK-1; b) CC-UK-1

794 **Figure 3.** Correlation between % Aromatics and  $\text{SUVA}_{-254}$ . A linear regression model  
795 was calculated to explain the relationship between the variables. The equation was  $y$  [%  
796 Aromatics] =  $0.250 + 0.950 \times [\text{SUVA}_{-254}]$ ,  $p < 0.001$

797 **Figure 4.** Correlation between % Aromatics and HWC. A linear regression model was  
798 calculated to explain the relationship between the variables. The equation was  $y$  [%  
799 Aromatics] =  $0.500 + 0.900 \times [\text{HWC}]$ , ( $p < 0.01$ )

800 **Figure 5.** Correlation between % Aromatics and FI. A linear regression model was  
801 calculated to explain the relationship between the variables. The equation was  $y$  [%  
802 Aromatics] =  $8.17 - 0.633 \times$  [FI], ( $p < 0.05$ ), represented by the solid line. The removal  
803 of an outlier resulted in the equation:  $y$  [% Aromatics] =  $8.89 - 0.976 \times$  [FI], which  
804 explained the relationship with a higher significance ( $p < 0.001$ ). The second equation is  
805 represented by the dashed line