**Response of different soil organic matter pools to biochar and organic
fertilizers**

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Highlights

- Biochar enhances C stabilization in soils treated with organic fertilizers.
- Biochar promotes sorption of C on mineral surfaces in organically-fertilized soils.
- Biochar accumulates in the free organic matter pool not protected by the soil mineral matrix
- Biochar has little impact on mineral-associated organic matter composition.

18 **Abstract**

19

20 A full understanding of the agronomic and environmental potential of biochar, and
21 especially its potential as a C sequestration strategy, requires a full comprehension of its
22 effects on native soil organic matter (SOM), as well as of its interactions with other
23 organic amendments co-applied to the soil. In a field experiment, we investigated
24 changes in quantity and quality of SOM pools characterized by different protection
25 mechanisms as affected by 20 t ha⁻¹ of biochar added alone or combined with two
26 different organic fertilizers (i.e., municipal solid waste compost and sewage sludge) 8
27 months after application. In particular, free, intra-macroaggregate, intra-microaggregate,
28 and mineral-associated SOM fractions were separated and analyzed for organic C, total
29 N, and stable isotopic ratios; further, diffuse reflectance infrared Fourier transform
30 spectroscopy was used to examine functional group composition. Soil biomass C
31 content, basal respiration, and metabolic quotient were also determined. Biochar and
32 organic fertilizer application increased significantly SOM content. Biochar accumulated
33 mainly in the free SOM pool, not protected by the soil mineral matrix. Especially
34 noteworthy was the significant interaction effect found between the biochar and organic
35 fertilizer factors on mineral-associated organic C content. This suggested a promoting
36 action of biochar on C stabilization in organically-fertilized soils through the formation
37 of organo-mineral complexes. Organically-fertilized soils had higher microbial C than
38 unfertilized soils. Basal respiration and metabolic quotient, however, were not affected
39 by any of the treatments. As a whole, our results support the potential of biochar
40 application as a strategy to sequester C in soils.

41

42 *Keywords:* Biochar; Microbial biomass C; Municipal solid waste compost; Physical
43 fractionation; Sewage sludge; Soil respiration; Stable isotopes

44 **1. Introduction**

45

46 Biochar is a C-rich product made by the pyrolysis of biomass (e.g., plant-derived
47 biomass), with the intention of climate change mitigation and soil quality enhancement
48 (e.g., Lehmann and Joseph, 2015). In particular, C in biochar is believed to form very
49 stable structures, which can be stored in soils for much longer than the biomass C from
50 which it originates, thus helping to fight global warming. Other benefits of soil
51 application of biochar have been reported to include increased water and nutrient
52 retention, increased cation-exchange capacity, correction of soil acidity, habitat for soil
53 microbes, and control of plant pathogens, among others (e.g., Lehmann, 2007; Masiello
54 et al., 2013; Lehmann and Joseph, 2015).

55 In spite of potential environmental and agricultural benefits of biochar application,
56 there are still many open questions about its potential deleterious effects (Cernansky,
57 2015). In particular, some studies have found that biochar may increase mineralization
58 rates of native soil organic matter (SOM). For example, Wardle et al. (2008) found that
59 applying biochar stimulated soil microbial activity and loss of boreal forest humus over
60 a 10-year period. Similarly, Luo et al. (2011) and Singh and Cowie (2014) reported
61 positive priming effects of biochar on native SOM in incubation experiments. On the
62 other hand, there are also studies in the literature showing no effects or even negative
63 priming effects (Liang et al., 2010; Cross and Sohi, 2011; Jones et al., 2011; Lu et al.,
64 2014; Weng et al., 2015). According to Zimmerman et al. (2011), differences in the
65 magnitude and sign of the priming effect hinges on soil and biochar type, such that
66 SOM mineralization tends to be stimulated in soils with low C content treated with
67 biochars produced at low temperatures from grasses instead of woods.

68 For given climatic and environmental conditions, the interacting mechanisms that

69 protect organic matter in the soil from mineralization conceptually include (a) selective
70 preservation and synthesis of recalcitrant structures, (b) physical disconnection from
71 decomposers, microbial enzymes, and O₂ by inclusion within macro and
72 microaggregates, and (c) inaccessibility by chemical association with mineral surfaces
73 (e.g., Piccolo, 2001; Six et al., 2002a; von Lützow et al., 2006; Schnitzer and Monreal,
74 2011, Courtier-Murias et al., 2013; Plaza et al., 2013). Despite much effort over the past
75 years, the processes and mechanisms contributing to organic matter stabilization and
76 destabilization in soils treated with biochar remain largely unknown. Particularly little
77 attention has been devoted to the interaction of biochar with other products used in
78 agriculture to ameliorate soil fertility. Biochar is a recalcitrant material potentially
79 suited to enhance C sequestration in soils, with much slower rates of decomposition
80 than other organic amendments (e.g., Bolan et al., 2012), but not a significant source of
81 plant nutrients. Thus, biochar application in combination with organic fertilizers is
82 believed to be a promising strategy to improve its benefits on soil quality and crop
83 yields (Ngo et al., 2013, 2014).

84 The objectives of this work were to (a) investigate the effects of biochar on SOM
85 pools linked to conceptual stabilization mechanisms and (b) examine whether biochar
86 interferes with the stabilization of organic C provided with two different organic
87 fertilizers (i.e., municipal solid waste compost and sewage sludge) co-applied to the
88 soil. Our hypothesis was that biochar would affect the quality and quantity of native
89 SOM and the stabilization mechanisms of organic matter in fertilized soils, mainly
90 through changes in soil microbial growth and activity.

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93 **2. Materials and methods**

94

95 *2.1. Experimental site, soil, biochar, and organic fertilizers*

96

97 The site selected for this research was located in the experimental farm “La
98 Poveda” (Spanish National Research Council, CSIC), Arganda del Rey, Madrid, Spain
99 (40°19’ N, 3°29’ W, 534 m above sea level). The site was characterized by a
100 Mediterranean climate, with an average annual rainfall of 436 mm and average annual
101 temperature of 14 °C. The soil showed a clay loam texture and was classified as a
102 Xerofluvent (Soil Survey Staff, 2014). The biochar used in this study was produced in a
103 pyrolysis plant from holm oak (*Quercus ilex* L.) chips through a slow pyrolysis process
104 at 600 °C. The municipal solid waste compost was produced using a conventional
105 windrow composting system at a waste treatment plant. The sewage sludge consists of
106 the granular end-product obtained by heat drying urban wastewater solids at about 75
107 °C at a wastewater treatment facility. The main properties of the top soil (0-15 cm),
108 biochar, and conventional amendments were reported in Fernández et al. (2014a) and
109 are summarized here in Table 1.

110

111 *2.2. Field experiment and soil sampling*

112

113 The experiment was set up as a randomized complete block design with two factors,
114 namely biochar and organic fertilizer, with four replicates. In particular, a control
115 treatment (no biochar) and a biochar application at a rate of 20 t ha⁻¹ were combined
116 with no organic fertilization, municipal solid waste compost at a rate equivalent to 75 kg
117 potentially available N (PAN) ha⁻¹, and sewage sludge at a rate equivalent to 75 kg PAN
118 ha⁻¹. All plots (5 × 3 m²) were planted with winter barley (*Hordeum Vulgare* L.) in mid-

119 November 2012. Biochar and organic fertilizers were hand broadcast in late October
120 2012 and immediately incorporated into the upper 15 cm of soil with a rotary tiller. The
121 rate of biochar used in this study can be considered medium (Jeffery et al., 2011),
122 whereas the rate of organic fertilizer was selected to meet the annual N needs of the
123 crop.

124 Barley was harvested in late June 2013. A two-way analysis of variance (ANOVA)
125 revealed a significant effect of organic fertilization on aboveground biomass ($p < 0.05$),
126 such that yields were significantly higher by 27 and 8% on the soils treated with sewage
127 sludge and municipal solid waste compost than on the unfertilized soils. There were no
128 main or interaction effects of biochar on aboveground biomass. Main and interaction
129 effects of biochar and organic fertilization on grain yield were not significant (Moreno-
130 Jiménez et al., 2016).

131 Just after barley harvest, soil samples representative of the plow layer (0-15-cm
132 depth) were collected randomly from each plot. As common in the study area, soils
133 were dry at the time of sampling. The soil samples were gently crushed and passed
134 through a 2-mm sieve for physical fractionation and microbiological analysis. An
135 aliquot of each sieved sample was ground with a ball mill for organic C and total N
136 analyses.

137

138 *2.3. Physical fractionation of soil organic matter*

139

140 Free, intra-macroaggregate, intra-microaggregate, and mineral-associated SOM
141 were separated using the method described by Plaza et al. (2012) with modifications
142 detailed by Plaza et al. (2013). This method is based on the densimetric procedure of
143 Golchin et al. (1994) and Sohi et al. (2001) for the fractionation of free and occluded

144 SOM and the method of Six et al. (2000, 2002b) for the breakup of macroaggregates
145 preserving microaggregates. In brief, the free organic matter in 20 g of 2-mm sieved soil
146 was separated by density flotation in a NaI solution (density, 1.85 g mL⁻¹) and
147 subsequent suction and filtration. The macroaggregates in the remaining soil were
148 broken up using the microaggregate isolator device developed by Six et al. (2000,
149 2002b) for liberating intra-macroaggregate organic matter, which was then recovered by
150 a second density separation. Finally, the intra-microaggregate organic matter was
151 isolated from the mineral-associated organic matter fraction by a third density
152 separation after ultrasonic disruption (energy input, 1500 J g⁻¹). Here NaI was used for
153 density separations instead of sodium polytungstate as reported in Plaza et al. (2012,
154 2013), because sodium polytungstate may contain some N that inevitably remains in the
155 heavy fraction after the last separation and hampers the examination of N distribution.
156 The recovered free, intra-macroaggregate, intra-microaggregate, and mineral-associated
157 organic matter fractions were oven-dried at 70 °C, weighed, and ground with a ball mill.

159 *2.4. Organic C, total N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and infrared analysis*

161 Organic C and total N contents of the whole soil samples and corresponding organic
162 matter fractions were determined by dry combustion with a Thermo Flash 2000 NC Soil
163 Analyzer. Isotopic ratios (¹³C/¹²C and ¹⁵N/¹⁴N) of biochar, municipal solid waste
164 compost, unfertilized and compost-fertilized soils with or without biochar, and
165 corresponding SOM fractions were determined in laboratory duplicates using an
166 isotopic ratio mass spectrometer (IRMS, Finnigan Delta V Advantage, Thermo Fisher
167 Scientific) coupled with an elemental analyzer (FlashEA 1112 HT, Thermo Fisher
168 Scientific). The elemental analyzer was equipped with a PorapackQS gas

169 chromatography column and connected to the IRMS through a Conflo III (Thermo
170 Fisher Scientific) interphase. The isotopic ratios were expressed in $\delta(\text{‰})$ vs. V-PDB
171 (Vienna-Pee Dee Belemnite) for C and AIR (atmospheric N₂) for N. For each run, at
172 least one in-house standard was analyzed to check the accuracy of the analysis. The
173 precision of measurements was usually $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. For
174 organic C and $\delta^{13}\text{C}$ determination, the whole soil samples and mineral-associated
175 organic matter fractions were subjected to acid fumigation (Harris et al., 2001) before
176 the analysis to remove carbonates.

177 Diffuse reflectance infrared Fourier transform (DRIFT) spectra of biochar,
178 conventional amendments, soils, and SOM fractions were recorded using a Shimadzu
179 FTIR-8400 spectrophotometer on powdered mixtures of 20 mg of sample and 200 mg
180 of KBr. The resolution was set at 4 cm⁻¹. For each determination, 128 scans were
181 recorded over the range of 4000 to 400 cm⁻¹, averaged, and corrected using KBr for the
182 background reference.

183

184 2.5. Microbiological analysis

185

186 Soil microbial biomass C was determined by the fumigation-extraction method of
187 Vance et al. (1987) after aerobic pre-incubation for 24 h at 25 °C and 40% of water
188 holding capacity. Basal respiration was determined by measuring the cumulative
189 amount of CO₂ that evolved and trapped in NaOH vials during the incubation of 50 g of
190 soil at 40% of water holding capacity in a 1-L jar for 14 days at 25 °C. Metabolic
191 quotient was calculated as the ratio of basal respiration to microbial biomass C.

192

193 2.6. Data analysis

194

195 Data were subjected to two- and one-way ANOVA. Treatment means were
196 separated using Fisher's LSD test at the 0.05 level. R statistical software, version 3.1.1
197 (R Core Team, 2014), and the R package agricolae, version 1.2-0 (De Mendiburu,
198 2014), were used for data analysis.

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200

201 **3. Results and discussion**

202

203 *3.1. Organic C, total N, and isotopic signatures*

204

205 A summary of the two- and one-way ANOVA of the variables examined here is
206 shown in Table 2. Total soil organic C content ranges from 11.8 to 22.7 g kg⁻¹, whereas
207 total N varies between 1.10 and 1.42 g kg⁻¹. Biochar-treated soils have significantly
208 greater total organic C contents than biochar-untreated soils, while organic fertilization
209 increases not only soil organic C but also N content (Table 3). This is in agreement with
210 previous works showing that biochar and organic fertilizers, especially composted
211 materials, can increase SOM content (e.g., Senesi et al., 2007; Ngo et al., 2014;
212 Agegnehu et al., 2016). There are no significant interactions between the biochar and
213 organic fertilizer factors and no significant differences between the effects of municipal
214 solid waste compost and sewage sludge for any of these parameters (Tables 2 and 3).

215 Biochar significantly affects free organic C and N (Table 2), such that their average
216 contents are significantly higher for biochar-treated soils (Figs. 1 and 2). In a laboratory
217 incubation study, Herath et al. (2014) also recovered most biochar C in the free organic
218 matte fraction. Similarly, organic fertilization significantly affects free N contents

219 (Table 2), such that fertilized soils exhibit larger values than the unamended soils (Fig.
 220 2). In a field experiment, Fernández et al. (2014b) found most C and N supplied with
 221 organic fertilizers (municipal solid waste compost, sewage sludge compost, and
 222 thermally dried sewage sludge) in the physically unprotected free organic matter pool.
 223 They also found no organic matter accumulation in the intra-microaggregate and
 224 mineral-associated pools after three years of annual application. Here the interaction
 225 effects of biochar and organic fertilization on the soil organic C and N pools examined
 226 are not significant, except for the mineral-associated fraction (Table 2). In particular,
 227 only in the biochar-treated soils, organic fertilizers significantly increase the mineral-
 228 associated organic C pool with respect to the controls (Fig. 1). Based on incubations of
 229 biochar alone or mixed with organic fertilizers, with no soil, Ngo et al. (2013) suggested
 230 that the presence of biochar could lead to a protection of exogenous organic matter
 231 against chemical oxidation and biological degradation; in a subsequent field study, Ngo
 232 et al. (2016) showed that mixing of biochar with organic fertilizers in litterbags on the
 233 surface of a degraded Acrisol reduced C loss under tropical conditions. Our results
 234 provide evidence that biochar can promote C stabilization in organically-fertilized soils
 235 through the formation of organo-mineral complexes by intimate interaction between
 236 mineral particles and organic inputs. The lack of interaction effect between the biochar
 237 and organic fertilization factors on aboveground barley biomass suggests that, relative
 238 to organic-fertilizer-derived inputs, plant-derived organic inputs may play a secondary
 239 role in the stabilization mechanisms observed here.

240 The two-way analysis of variance shows significant main effects of biochar on the
 241 C/N ratio of whole soil and free, intra-macoraggregate, and intra-microaggregate
 242 organic matter fractions (Table 2), with greater values for biochar-treated soils (Fig. 3).
 243 Organic fertilization significantly decreases free, intra-macoraggregate, and intra-

244 microaggregate C/N ratios (Fig. 3). The interaction between the two factors is
245 significant for free and mineral-associated C/N. For the biochar-treated soils, the C/N
246 ratio of the free organic matter is lower for the organically-fertilized soils than for the
247 unfertilized soil, whereas no organic fertilization effects are observed for the soils
248 without biochar. By contrast, the mineral-associated C/N ratio of the soils without
249 biochar decreases with the application of the organic fertilizers, whereas no effects are
250 observed for the biochar-treated soils.

251 The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of biochar, municipal solid waste compost, whole soils and the
252 corresponding SOM fractions are shown in Table 4. The $\delta^{13}\text{C}$ of biochar and compost
253 are very similar to each other (-26.65 and -25.13‰, respectively), thus resulting in no
254 clear trends in its signature either among whole soils or among SOM fractions. In
255 contrast, biochar and compost show markedly different $\delta^{15}\text{N}$ signatures (2.32 and
256 6.63‰, respectively), which are also different from that of the untreated soil (7.79‰).
257 As a consequence, the whole soil $\delta^{15}\text{N}$ signature decreases with both biochar and
258 compost application. Furthermore, only the $\delta^{15}\text{N}$ signature of free SOM is strongly
259 affected by biochar application, which indicates a preferential incorporation of biochar
260 into this fraction. This influence is less evident for the intra-macroaggregate SOM
261 fraction, whereas the intra-microaggregate and mineral-associated fractions seem to be
262 unaffected. A similar behavior is observed after the application of compost and biochar
263 with compost (Table 4).

264 Significant correlations ($R^2 > 0.82$, $p < 0.05$) are found between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
265 of all SOM fractions separated from the untreated soil and the corresponding C/N ratios.
266 In particular, free SOM shows the highest $\delta^{13}\text{C}$, the highest C/N ratio and the lowest
267 $\delta^{15}\text{N}$ (-23.34‰, 19.3 and 4.76‰ respectively); by contrast, mineral-associated SOM
268 shows the lowest $\delta^{13}\text{C}$, the lowest C/N ratio, and the highest $\delta^{15}\text{N}$ (-25.25‰, 6.1 and

269 8.34‰ respectively) (Fig. 4). Soil organic matter degradation may result in ^{13}C -
270 enrichment because bacteria preferentially metabolize ^{12}C -rich compounds (Balesdent et
271 al., 1987, 1993; Skrzypek et al., 2007). Other studies have also reported that C isotope
272 composition of plant detritus gradually changes as polysaccharides (e.g., cellulose,
273 hemicellulose) are preferentially decomposed, leaving a material that is relatively
274 enriched in lignin-derived C and depleted in ^{13}C (e.g., Benner et al., 1987; Zacccone et
275 al., 2011). Furthermore, microorganisms have been shown to preferentially use ^{14}N
276 during SOM decomposition, thus resulting in ^{15}N enrichment (Nadelhoffer and Fry,
277 1994). Consequently, our study points to the following decomposition gradient among
278 SOM fractions: mineral associated > intra-microaggregate > intra-macroaggregate >
279 free.

280

281 3.2. DRIFT

282

283 The DRIFT spectra of municipal solid waste compost and sewage sludge are similar
284 to each other and very different from that of biochar (Fig. 5). The main common
285 features of the spectra of municipal solid waste compost and sewage sludge are the
286 following: a broad band centered near 3350 cm^{-1} due to O-H stretching, two sharp bands
287 at about 2930 and 2850 cm^{-1} due to aliphatic C-H stretching, various signals between
288 1650 and 1430 cm^{-1} derived from amide, amine, and aliphatic groups, and a broad band
289 centered near 1040 cm^{-1} due to C-O stretching of polysaccharides or polysaccharide-like
290 substances. In contrast, the DRIFT spectrum of biochar is dominated by a very broad
291 band centered at about 3050 cm^{-1} due to C-H aromatic stretch and intense broad
292 absorption centered at 1460 cm^{-1} derived from aromatic C=C stretching (Baldock and
293 Smernik, 2002; Chatterjee et al., 2012).

294 The DRIFT spectra of the whole soils and the corresponding mineral-associated
295 SOM fractions, which are not affected by the biochar and organic fertilizer factors,
296 exhibit an intense peak at about 3600 cm⁻¹ and three absorption bands at about 1980,
297 1870, and 1790 cm⁻¹. These signals, which are much weaker or even absent in the
298 spectra of the free and intra-aggregate SOM fractions, may denote the presence of clay
299 minerals and are typical of soils and soil particle-size fractions (Capriel et al., 1995;
300 Spaccini et al., 2001). Unlike the whole soils and mineral-associated fractions, the
301 DRIFT spectra of the free and aggregate SOM fractions exhibit two aliphatic C-H
302 stretching bands at about 2930 cm⁻¹ and 2850 cm⁻¹, whose intensity tend to decrease
303 with biochar application. The extent of this effect is larger for the free than for the intra-
304 macroaggregate pool and is hardly noticeable for the intra-microaggregate fraction.
305 These results are in agreement with those obtained using stable isotopes, and suggest
306 that, after eight months from application, biochar is mainly located outside aggregates,
307 chemically unaltered and unprotected by the mineral matrix, and secondarily occluded
308 within macroaggregates. Our data also suggest that the composition of the mineral-
309 associated SOM fraction is not affected by any of the treatments. Paetsch et al. (2016)
310 also found a high variability in the composition of the particulate SOM isolated from
311 unamended soils and soils amended with urban waste composts and cattle manure, but
312 not in the composition of the mineral-associated fractions. In agreement with previous
313 findings (Plaza et al., 2013; Courtier-Murias et al., 2013), mineral-associated OM
314 predominantly consists of microbial biomass and microbial by-products resulting from
315 the microbial growth, transformation, and degradation processes of organic matter
316 inputs.

317

318 *3.3. Microbial biomass and activity*

319

320 Microbial biomass C is only affected by the organic fertilizer factor (Table 5).
321 Numerous studies available in the literature report positive effects of these kinds of
322 organic fertilizers on microbial biomass (e.g., García-Gil et al., 2000; Marando et al.,
323 2011). In a meta-analysis, Kallenbach and Grandy (2011) found that organic
324 amendments increased microbial biomass C by 36% relative to inorganic fertilizer
325 across a range of environments and agronomic practices, with the type of amendment
326 having the strongest effects. Due to the amount of C applied, biochar-treated soils show
327 much lower microbial biomass C/total organic C ratios than soils without biochar. No
328 significant main effects and interactions are found for basal respiration and metabolic
329 quotient. These results are indicative of the stability of the C added with biochar and are
330 in general agreement with the conclusions of previous studies (Kuzyakov et al., 2009,
331 2014). Biochar may add labile compounds mainly derived from incomplete biomass
332 pyrolysis that may stimulate soil microbial growth and activity, but these components
333 are rapidly mineralized and their effects on soil microorganisms are not persistent
334 (Smith et al., 2010).

335

336

337 **4. Conclusions**

338

339 Our results provide evidence that, eight months after its application, biochar
340 accumulates mainly in the free SOM fraction, and promotes C stabilization in
341 organically-fertilized soils through the formation of organo-mineral complexes by
342 intimate interaction between mineral particles and organic inputs. The chemical and
343 structural composition of the organic matter in biochar, compost, and sewage sludge

differs markedly from that of the mineral-associated SOM. However, the composition of the mineral-associated SOM fraction is not affected by any of the treatments compared to the control. This suggests a high degree of microbial transformation of the organic matter stabilized in the mineral-associated fraction of the soils treated with biochar and organic fertilizers. We hypothesize that biochar stimulates the microbial transformation of the relatively more labile organic fractions applied with organic fertilizers and the sorption of the resultant microbial by-products on soil mineral surfaces. Further research is needed to test this hypothesis, as well as the specific mechanisms involved, especially by means of long-term field experimentation. Nonetheless, the data provided in this work strongly support the potential of biochar application as a strategy to sequester C in soils and fight climate change.

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530

531 **Figure captions**

532 **Fig. 1.** Free, intra-macroaggregate, intra-microaggregate, and mineral-associated
533 organic C content of soils either unfertilized (UN), amended with municipal solid waste
534 compost (MC), or amended with sewage sludge (SS), without or with biochar (BC).
535 Error bars indicate pooled standard error. Different letters indicate statistically
536 significant differences according to Fisher's LSD test at the 0.05 level.

537 **Fig. 2.** Free, intra-macroaggregate, intra-microaggregate, and mineral-associated N
538 content of soils either unamended (UN), amended with municipal solid waste compost
539 (MC), or amended with sewage sludge (SS), without or with biochar (BC). Error bars
540 indicate pooled standard error. Different letters indicate statistically significant
541 differences according to Fisher's LSD test at the 0.05 level.

542 **Fig. 3.** Free, intra-macroaggregate, intra-microaggregate, and mineral-associated C/N
543 ratio of soils either unamended (UN), amended with municipal solid waste compost
544 (MC), or amended with sewage sludge (SS), without or with biochar (BC). Error bars
545 indicate pooled standard error. Different letters indicate statistically significant
546 differences according to Fisher's LSD test at the 0.05 level.

547 **Fig. 4.** Relationship between stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and C/N ratios of
548 untreated (control) whole soil (WS) and corresponding organic matter fractions (FR,
549 free; MA, intra-macroaggregate; MI, intra-microaggregate; and MIN, mineral-
550 associated).

551 **Fig. 5.** Diffuse reflectance infrared Fourier transform (DRIFT) spectra of biochar,
552 municipal solid waste compost, sewage sludge, and whole soils and soil organic matter
553 fractions (i.e., free, intra-macroaggregate, intra-microaggregate, and mineral-associated
554 OM), as affected by amendment with municipal solid waste compost (MC) or sewage
555 sludge (SS), with or without biochar (BC).

Table 1

Chemical properties (mean $\pm$ standard error of three laboratory replicates) of the soil, biochar, municipal solid waste compost, and sewage sludge used in this work (data from Fernández et al., 2014)

Property	Soil	Biochar	Compost	Sewage sludge
pH	8.80 $\pm$ 0.03	10.06 $\pm$ 0.01	6.79 $\pm$ 0.01	7.30 $\pm$ 0.03
EC (dS m ⁻¹)	0.11 $\pm$ 0.00	2.4 $\pm$ 0.0	10.8 $\pm$ 1.0	2.2 $\pm$ 0.2
Total organic C (g kg ⁻¹)	10.0 $\pm$ 0.1	597 $\pm$ 31	295 $\pm$ 3	277 $\pm$ 4
Inorganic C (g kg ⁻¹)	5.9 $\pm$ 0.1	14.7 $\pm$ 1.6	9.4 $\pm$ 3.8	9.3 $\pm$ 4.0
Total N (g kg ⁻¹)	1.2 $\pm$ 0.0	4.1 $\pm$ 0.6	16.0 $\pm$ 0.2	43.9 $\pm$ 0.5
C/N	8.3 $\pm$ 0.1	151 $\pm$ 18	18.45 $\pm$ 0.02	6.31 $\pm$ 0.09
P (g kg ⁻¹)	0.006 $\pm$ 0.001 ^a	2.0 $\pm$ 0.2	3.1 $\pm$ 0.2	19.2 $\pm$ 0.8
K (g kg ⁻¹)	0.21 $\pm$ 0.02 ^a	9.1 $\pm$ 0.7	6.9 $\pm$ 0.2	3.6 $\pm$ 0.1
Ca (g kg ⁻¹)	2.93 $\pm$ 0.14 ^a	52.4 $\pm$ 4.0	38.2 $\pm$ 1.6	30.3 $\pm$ 0.8
Mg (g kg ⁻¹)	0.25 $\pm$ 0.02 ^a	3.5 $\pm$ 0.3	3.5 $\pm$ 0.2	6.2 $\pm$ 0.1
Na (g kg ⁻¹)	0.069 $\pm$ 0.006 ^a	0.3 $\pm$ 0.0	4.5 $\pm$ 0.2	0.7 $\pm$ 0.0

EC, electrical conductivity.

^a Available content.

Table 2

Analysis of variance (*p*-values)

	Two-way ANOVA			One-way ANOVA
	Biochar	Organic fertilizer	Biochar × Organic fertilizer	Treatment
Total organic C	<0.001***	0.006**	0.327	<0.001***
Free organic C	<0.001***	0.374	0.274	<0.001***
Intra-macroaggregate organic C	0.465	0.035*	0.656	0.142
Intra-microaggregate organic C	0.814	0.214	0.084	0.160
Mineral-associated organic C	0.004**	<0.001***	0.010*	<0.001***
Total N	0.108	<0.001***	0.343	0.002**
Free organic N	0.002**	<0.001***	0.232	<0.001***
Intra-macroaggregate N	0.593	0.026*	0.635	0.119
Intra-microaggregate N	0.254	0.345	0.103	0.173
Mineral-associated N	0.009**	0.028*	0.035*	0.005**
Total organic C/N	<0.001***	0.140	0.100	<0.001***
Free organic C/N	<0.001***	<0.001***	0.003**	<0.001***
Intra-macroaggregate C/N	0.002**	0.007**	0.248	0.002**
Intra-microaggregate C/N	<0.001***	0.002**	0.497	<0.001***
Mineral-associated C/N	0.062	0.1658	0.002**	0.004**
Microbial biomass C	0.212	0.010*	0.078	0.015*
Microbial biomass C/total organic C	<0.001***	0.051	0.044*	<0.001***
Basal respiration	0.811	0.057	0.680	0.230
Metabolic quotient	0.336	0.863	0.105	0.313

*, **, and ***, significant at the 0.05, 0.01, and 0.001 probability level, respectively.

Table 3

Total organic C content, total N, and C/N ratio (mean $\pm$ pooled standard error) of soils either unamended (UN), or amended with municipal solid waste compost (MC) or sewage sludge (SS), without or with biochar (BC)

	Total organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N
UN	11.8 $\pm$ 0.7 d	1.10 $\pm$ 0.05 c	10.7 $\pm$ 0.6 c
MC	14.2 $\pm$ 0.7 c	1.31 $\pm$ 0.05 ab	10.8 $\pm$ 0.6 c
SS	12.7 $\pm$ 0.7 cd	1.19 $\pm$ 0.05 bc	10.6 $\pm$ 0.6 c
BC+UN	19.8 $\pm$ 0.7 b	1.08 $\pm$ 0.05 c	18.6 $\pm$ 0.6 a
BC+MC	22.6 $\pm$ 0.7 a	1.42 $\pm$ 0.05 a	15.9 $\pm$ 0.6 b
BC+SS	22.7 $\pm$ 0.7 a	1.33 $\pm$ 0.05 ab	17.2 $\pm$ 0.6 ab

Different letters indicate statistically significant differences according to Fisher's LSD test at the 0.05 level.

Table 4

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean $\pm$ standard deviation) of biochar, municipal solid waste compost, and whole soils and soil organic matter fractions (i.e., free, intra-macroaggregate, intra-microaggregate, and mineral-associated), as affected by amendment with municipal solid waste compost (MC), with or without biochar (BC).

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Biochar	-26.65 ± 0.06	2.32 ± 0.27
Municipal solid waste compost	-25.13 ± 0.07	6.63 ± 0.12
Whole soils		
UN	-24.44 ± 0.14	7.79 ± 0.15
MC	-20.51 ± 0.23	6.03 ± 0.17
BC+UN	-23.08 ± 0.04	5.54 ± 0.25
BC+MC	-22.61 ± 0.02	5.83 ± 0.47
Free organic matter		
UN	-23.34 ± 0.02	4.76 ± 0.19
MC	-24.24 ± 0.03	5.25 ± 0.18
BC+UN	-25.95 ± 0.13	3.42 ± 0.13
BC+MC	-25.80 ± 0.10	3.91 ± 0.24
Intra-macroaggregate organic matter		
UN	-24.28 ± 0.01	5.03 ± 0.16
MC	-24.69 ± 0.04	4.62 ± 0.03
BC+UN	-24.98 ± 0.04	4.60 ± 0.24
BC+MC	-25.01 ± 0.07	4.62 ± 0.09
Intra-microaggregate organic matter		
UN	-24.61 ± 0.03	5.42 ± 0.12
MC	-24.84 ± 0.03	5.37 ± 0.14
BC+UN	-24.93 ± 0.02	5.40 ± 0.09
BC+MC	-24.98 ± 0.06	5.24 ± 0.24
Mineral-associated organic matter		
UN	-25.25 ± 0.13	8.34 ± 0.12
MC	-25.10 ± 0.07	8.36 ± 0.11
BC+UN	-25.20 ± 0.03	8.39 ± 0.00
BC+MC	-25.28 ± 0.16	8.04 ± 0.22

Table 5

Microbial biomass C content, microbial biomass C/total organic C ratio, basal respiration, and metabolic quotient (mean $\pm$ pooled standard error) of soils either unamended (UN), or amended with municipal solid waste compost (MC) or with sewage sludge (SS), without or with biochar (BC)

	Microbial biomass C (mg kg ⁻¹)	Microbial biomass C/Total organic C (%)	Basal respiration (mg CO ₂ -C kg ⁻¹ day ⁻¹)	Metabolic quotient (mg CO ₂ -C g ⁻¹ microbial biomass C day ⁻¹)
UN	315 $\pm$ 40 c	2.67 $\pm$ 0.23 b	30.6 $\pm$ 5.0 a	97 $\pm$ 14.4 a
MC	546 $\pm$ 40 a	3.85 $\pm$ 0.23 a	39.9 $\pm$ 5.0 a	73 $\pm$ 14.4 a
SS	458 $\pm$ 40 ab	3.56 $\pm$ 0.23 a	41.5 $\pm$ 5.0 a	92 $\pm$ 14.4 a
BC+UN	361 $\pm$ 40 bc	1.85 $\pm$ 0.23 c	30.1 $\pm$ 5.0 a	83 $\pm$ 14.4 a
BC+MC	399 $\pm$ 40 bc	1.80 $\pm$ 0.23 c	45.9 $\pm$ 5.0 a	122 $\pm$ 14.4 a
BC+SS	431 $\pm$ 40 abc	1.92 $\pm$ 0.23 c	39.0 $\pm$ 5.0 a	92 $\pm$ 14.4 a

Different letters indicate statistically significant differences according to Fisher's LSD test at the 0.05 level.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.