

Carbon dioxide emissions from semi-arid soils amended with biochar alone or combined with mineral and organic fertilizers

José M. Fernández^a, M. Aurora Nieto^{a,b}, Esther G. López-de-Sá^a, Gabriel Gascó^c, Ana Méndez^b, César Plaza^{a,*}

^a Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Serrano 115 bis, 28006 Madrid, Spain

^b Departamento de Ingeniería de Materiales, E.T.S.I. Minas, Universidad Politécnica de Madrid, Ríos Rosas 21, 28003 Madrid, Spain

^c Departamento de Edafología, E.T.S.I. Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, 28004 Madrid, Spain

A B S T R A C T

Semi-arid soils cover a significant area of Earth's land surface and typically contain large amounts of inorganic C. Determining the effects of biochar additions on CO₂ emissions from semi-arid soils is therefore essential for evaluating the potential of biochar as a climate change mitigation strategy. Here, we measured the CO₂ that evolved from semi-arid calcareous soils amended with biochar at rates of 0 and 20 t ha⁻¹ in a full factorial combination with three different fertilizers (mineral fertilizer, municipal solid waste compost, and sewage sludge) applied at four rates (equivalent to 0, 75, 150, and 225 kg potentially available N ha⁻¹) during 182 days of aerobic incubation. A double exponential model, which describes cumulative CO₂ emissions from two active soil C compartments with different turnover rates (one relatively stable and the other more labile), was found to fit very well all the experimental datasets. In general, the organic fertilizers increased the size and decomposition rate of the stable and labile soil C pools. In contrast, biochar addition had no effects on any of the double exponential model parameters and did not interact with the effects ascribed to the type and rate of fertilizer. After 182 days of incubation, soil organic and microbial biomass C contents tended to increase with increasing the application rates of organic fertilizer, especially of compost, whereas increasing the rate of mineral fertilizer tended to suppress microbial biomass. Biochar was found to increase both organic and inorganic C contents in soil and not to interact with the effects of type and rate of fertilizer on C fractions. As a whole, our results suggest that the use of biochar as enhancer of semi-arid soils, either alone or combined with mineral and organic fertilizers, is unlikely to increase abiotic and biotic soil CO₂ emissions.

1. Introduction

Biochar, the C-rich solid material obtained from biomass through a pyrolysis process, has recently gained much attention due to its potential to enhance soil quality and mitigate climate change (Lehmann, 2007; Lehmann and Joseph, 2009). Biochar decomposition rates have been shown to depend on several factors, such as pyrolysis temperature, feedstock, particle size, soil type and moisture regime, and presence of more labile organic substrates, which may promote mineralization by microbial co-metabolism (Kuzyakov et al., 2009; Nguyen and

Lehmann, 2009; Sigua et al., 2014). Nonetheless, due to its structure, biochar C is considered to be very stable and can be stored in soils for hundreds to thousands of years (Swift, 2001; Kuzyakov et al., 2009), albeit some studies point to lower turnover times, in the range of several decades, under certain conditions (Hilscher et al., 2009; Zimmermann et al., 2012).

Soil C is one of the largest active C reservoirs on Earth, and as such it plays a paramount role in the global C cycle and climate change (Falkowski et al., 2000). In particular, soils are estimated to hold from 2157 to 2293 Gt of organic and inorganic C in the upper 100 cm (Batjes, 1996), about three times the amount of C stored in the atmosphere (Falkowski et al., 2000). Consequently, not only the stability of biochar but also its effects on soil C dynamics need to be understood

* Corresponding author. Tel.: +34 91 745 2500x950191.
E-mail address: c.plaza@ica.csic.es (C. Plaza).

to evaluate potential of biochar for enhancing C sequestration in agricultural ecosystems.

Recent studies have shown increased mineralization rates of native soil organic C in some biochar-amended soils, which may reduce not only the capacity of biochar as a C sequestration strategy but also its value as a soil amendment, due to the profound influence of soil organic matter on soil quality and fertility (Johnston et al., 2009). In particular, Wardle et al. (2008) found that biochar stimulated loss of boreal forest humus over a 10-year period under field conditions. Similarly, Luo et al. (2011) showed positive priming effects on native soil organic C after the application of biochar to a clay-loam soil in an incubation experiment. Other studies in the literature, however, report no effects or negative priming effects of biochar on soil organic matter (Liang et al., 2010).

To date, most of the studies dealing with the effects of biochar on native soil C have been conducted on soils with no or virtually no inorganic C. Consequently, these studies are focused on the mineralization of native soil organic C and assume that CO₂ emissions are mostly due to microbial respiration. Soils are estimated to hold from 695 to 748 Gt of inorganic C in the upper 100 cm (Batjes, 1996), mainly as calcium carbonate in drylands (i.e., arid, semi-arid, and dry subhumid areas), which cover more than 40% of Earth's land surface (Reynolds et al., 2007; Lal, 2008). Therefore, further information on the effects of biochar on the typically calcareous soils of these regions is needed to fully understand the potential of biochar as a global greenhouse gas mitigation strategy. Similarly, compared to the effects of biochar added alone or combined with simple organic substrates (e.g., glucose) or plant materials on native soil organic C, much less work has been done to investigate the effects of biochar combined with mineral and organic fertilizers. This information is also highly relevant, since biochar itself is not a significant source of plant nutrients and applying it with other materials, such as synthetic fertilizers or compost, is highly recommended to increase crop yields (Major, 2010).

The objectives of this research were to (a) investigate the effects of biochar application on CO₂ emissions from semi-arid calcareous soils unamended or amended with synthetic and organic fertilizers (mineral fertilizer, municipal solid waste compost and sewage sludge), (b) evaluate the effects of biochar and its interactions with the type and rate of fertilizer on soil organic and inorganic C contents, and (c) determine changes in soil microbial biomass C, inasmuch as microorganisms are critical for soil C cycling processes.

2. Materials and methods

2.1. Soil, biochar, and mineral and organic fertilizers

A surface sample of soil (0–15 cm) was collected in September 2012 from a Xerofluvent annually cropped with cereal in the Spanish National Research Council (CSIC) La Poveda research station, located southeast of Madrid, central Spain (40°19'N, 3°29'W, 534 m above sea level). This site has a semiarid climate, with a mean annual temperature of 14 °C and an average annual rainfall of 437 mm. In the laboratory, the soil sample was air dried at room temperature, gently crushed, passed through a 2-mm sieve, and thoroughly homogenized. A portion of the 2-mm-sieved soil sample was ground for chemical analysis.

The biochar used in this work was produced in a pyrolysis plant from holm oak (*Quercus ilex* L.) chips through a slow pyrolysis process at 600 °C. The mineral fertilizer was a commercial synthetic fertilizer with a N–P–K value of 8–15–15. The municipal solid waste compost was produced using a conventional windrow composting system at a waste treatment plant. The sewage sludge was the granular end product obtained by heat drying urban wastewater solids at about 75 °C at a wastewater treatment facility. To ensure homogeneity, all the materials were air-dried at room temperature and ground to pass a 0.5-mm sieve.

The properties of the soil and the amendments were determined in triplicate by conventional methods. Briefly, pH was measured on suspensions of 1:2.5 sample:water (Sparks, 1996). Electrical conductivity

was measured on water extracts obtained at a sample-to-water ratio of 1:5 (Sparks, 1996). Total C and N contents were determined by dry combustion. Total organic C content was determined by dry combustion after acid fumigation and inorganic C content was calculated as the difference between total C and organic C (Harris et al., 2001). The available P content of the soil was analyzed by continuous flow colorimetry after extraction with calcium magnesium carbonate buffer (Burriel and Hernando, 1950). Available K, Ca, Mg, and Na were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after extraction with ammonium acetate (Sparks, 1996). The total P, K, Ca, Mg, and Na contents of the amendments were determined by ICP-AES after the digestion with nitric and perchloric acid (Faithfull, 2002).

2.2. Incubation experiment

Three factors, namely biochar addition, type of fertilizer, and rate of fertilizer, were tested in a full factorial experimental design with three replicates. In particular, two levels of biochar, not applied or applied at a rate of 20 t ha⁻¹, were combined with mineral fertilizer, municipal solid waste compost, or sewage sludge applied at rates equivalent to 0, 75, 150, and 225 kg potentially available N ha⁻¹. The rate of biochar used in this study can be considered medium and moderate based on the literature (Jeffery et al., 2011), whereas N rates meet common annual crop needs.

The treatments with the fertilizer rates targeted (in t ha⁻¹) were set up for CO₂ evolution measurements by thoroughly mixing 50 g of 2-mm sieved soil (bulk density of 1.15 g cm⁻³) with the appropriate quantity of amendment in a 50-mL plastic glass. For calculations, we assumed that potentially available N in organic fertilizers equals to 30% of organic N, 50% of ammonium N, and 100% of nitrate N. Deionized water was added to 40% of the soil water-holding capacity, which was found to be optimal moisture conditions for microbial activity in the soil used here. The plastic glasses were placed in 1-L jars together with 10 mL of deionized water to maintain humidity and a glass vial containing 20 mL of 1 M NaOH as CO₂ trap. Three blanks without soil were prepared. The jars were hermetically sealed, randomly placed in a chamber at 25 °C, and incubated in the dark for 182 days. The NaOH vials were replaced after 1, 3, 7, 14, 28, 60, and 120 days. The CO₂ that evolved and trapped in the NaOH vials was determined by automatic titration with HCl.

The treatments were also set up using 100 g of soil in open 100-mL plastic containers to determine soil organic, inorganic, and microbial biomass C contents after 182 days of incubation under the same temperature and moisture conditions as for the CO₂ evolution measurements. Total organic and inorganic C contents were determined on air-dried, ground soil samples by acid fumigation and dry combustion (Harris et al., 2001). Soil microbial biomass C was determined on fresh soil samples by fumigation–extraction (Vance et al., 1987).

2.3. Data analysis

A double exponential model (Jenkinson, 1977) was fitted to the cumulative amount of CO₂ that evolved during the incubation by non-linear regression, using the Levenberg–Marquardt algorithm and the sum of the squared residuals as the loss function. In this model, two C compartments, one relatively stable with slow turnover rate and the other more labile with higher turnover, decompose and emit CO₂ according to the following equation:

$$\text{CO}_2\text{-C} = C_s(1 - e^{-k_s t}) + C_l(1 - e^{-k_l t})$$

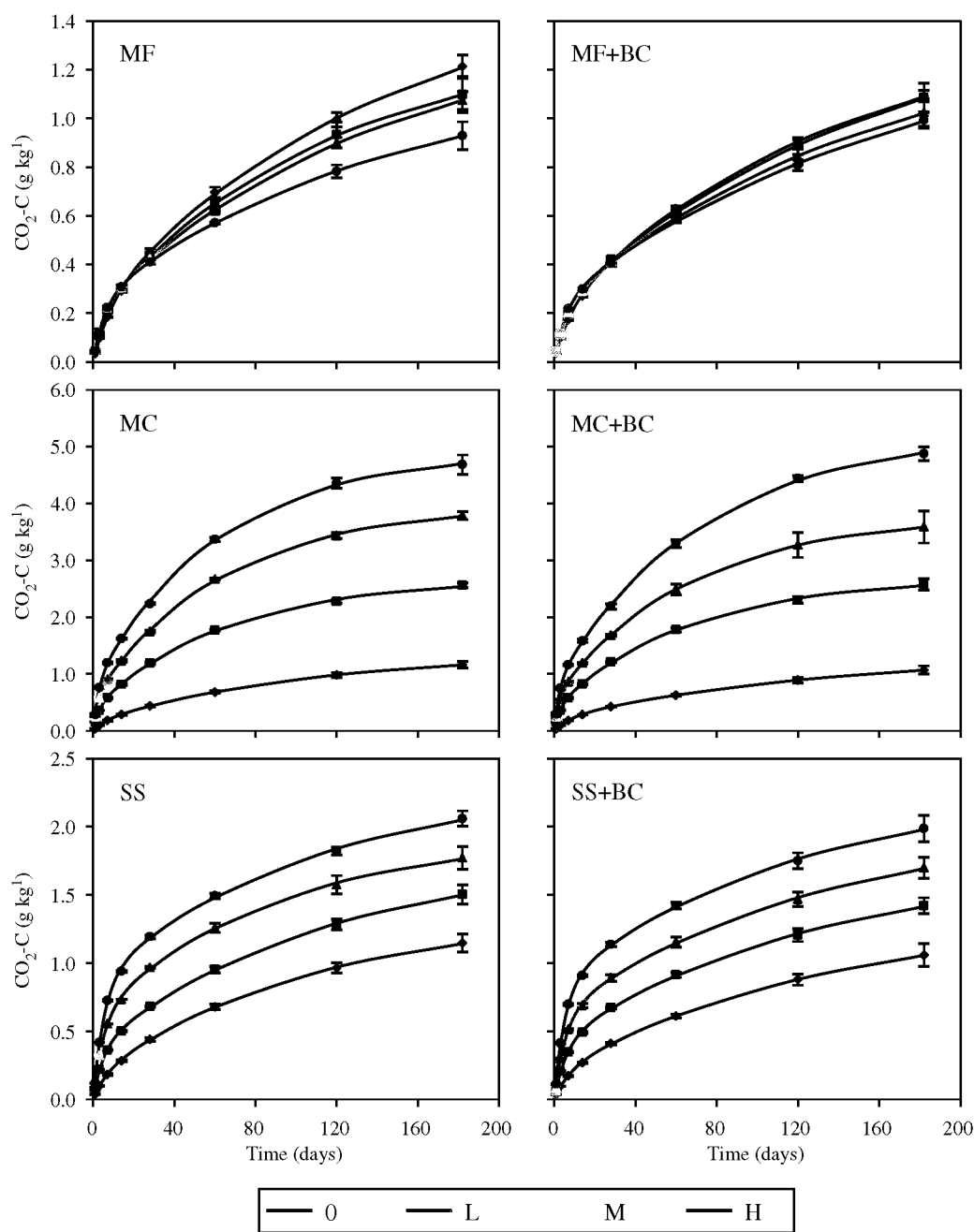
where CO₂-C is the cumulative C emitted as CO₂, C_s and C_l are the size of the stable and labile C compartments, respectively, k_s and k_l are their corresponding CO₂ emission rates, and t is time. In previous experiments with unamended and organically-amended soils, we tested to

Table 1Chemical properties (mean \pm standard error of three laboratory replicates) of the soil, biochar, municipal solid waste compost, and sewage sludge used in this work.

Property	Soil	Biochar	Mineral fertilizer	Compost	Sewage sludge
pH	8.80 \pm 0.03	10.06 \pm 0.01	4.03 \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	111.9 \pm 1.3	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	80.9 \pm 1.8	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	2.0 \pm 0.2	70.0 \pm 1.9	3.1 \pm 0.2	19.2 \pm 0.8
K (g kg ⁻¹)	0.21 \pm 0.02	9.1 \pm 0.7	123.4 \pm 5.0	6.9 \pm 0.2	3.6 \pm 0.1
Ca (g kg ⁻¹)	2.93 \pm 0.14	52.4 \pm 4.0	48.4 \pm 2.5	38.2 \pm 1.6	30.3 \pm 0.8
Mg (g kg ⁻¹)	0.25 \pm 0.02	3.5 \pm 0.3	3.0 \pm 0.0	3.5 \pm 0.2	6.2 \pm 0.1
Na (g kg ⁻¹)	0.069 \pm 0.006	0.3 \pm 0.0	3.5 \pm 0.1	4.5 \pm 0.2	0.7 \pm 0.0

EC, electrical conductivity.

P, K, Ca, Mg, and Na refer to available P, K, Ca, Mg, and Na contents of soil and total P, K, Ca, Mg, and Na contents of biochar, mineral fertilizer, compost, and sewage sludge.

**Fig. 1.** Measured (symbols) and model-predicted (lines) cumulative CO₂ emissions from soils amended with mineral fertilizer (MF), municipal solid waste compost (MC), and sewage sludge (SS) at rates equivalent to 0, 75, 150, and 225 t potentially available N ha⁻¹ (0, L, M, and H, respectively), without or with biochar at 20 t ha⁻¹ (BC), during 182 days of incubation. Error bars represent standard errors of the means (n = 3).

six different kinetic models and the double exponential model was found to be the most appropriate to describe C mineralization kinetics for all the treatments (Fernández et al., 2007).

Dependent variables were subjected to three-way analysis of variance. Effects were deemed significant when $P < 0.05$. All the statistical analyses were performed with IBM SPSS Statistics, version 21 (Somers, NY).

3. Results and discussion

3.1. Initial properties of soil, biochar, and fertilizers

Table 1 gives details of the initial properties of the soil and the amendments used in this work. The soil has an alkaline pH, low electrical conductivity, low contents of total organic C, total N, and available P, and medium contents of exchangeable K and Mg (Horneck et al., 2011). Exchangeable Ca percentage is 83%, whereas exchangeable Na percentage is only 1.7%, typical of calcareous and not sodic soils (Horneck et al., 2011). Approximately 37% of the total soil C is inorganic. Sanz-Cobena et al. (2008) found 3.4% of calcium carbonate in a soil of similar origin collected from the same study area used here. High calcium carbonate contents and alkaline pH are common characteristics of soils in arid and semi-arid regions (Lal, 2008).

With respect to the biochar and the organic fertilizers, the mineral fertilizer, whose properties accord with the composition declared by the manufacturer, exhibits the highest electrical conductivity and N, P, and K contents, the lowest pH and Mg contents, and intermediate Ca and Na contents. Approximately 97% of the total C in the biochar and the organic fertilizers is organic C. Compared to the municipal solid waste compost and the sewage sludge, the biochar has much higher organic C content and C/N ratio, higher pH and K and Ca contents, similar or lower electrical conductivity and Mg content, lower P and Na contents, and much smaller total N content. With respect to the sewage sludge, the municipal solid waste compost features higher electrical conductivity, C/N ratio, and K and Na contents, slightly higher total organic C and Ca contents, slightly lower pH, smaller Mg content, and much lower N and P contents. It is noteworthy that, in general, soil pH

Table 3

Analysis of variance for sizes and decomposition rates of stable and labile soil C pools (C_s , k_s , C_i , and k_i , respectively) estimated by a double exponential model as affected by biochar (0 and 20 t ha⁻¹), type of fertilizer (mineral fertilizer, municipal solid waste compost, and sewage sludge), and rate of fertilizer (equivalent to 0, 75, 150, and 225 t potentially available N ha⁻¹).

Source of variation	C_s	k_s	C_i	k_i
Biochar (B)	0.783	0.146	0.691	0.537
Type of fertilizer (F)	<0.001	<0.001	<0.001	<0.001
Rate of fertilizer (R)	<0.001	0.135	<0.001	0.055
B × F	0.991	0.967	0.956	0.996
B × R	0.253	0.997	0.978	0.981
F × R	<0.001	0.128	<0.001	0.577
B × F × R	0.671	0.979	0.998	1.000

tends to decrease with increasing incubation time and rate of mineral and organic fertilizer (data not shown). After 182 days of incubation, the average soil pH for the treatments without biochar is 8.0, whereas for those with biochar is 8.2.

3.2. Soil CO₂ evolution

First of all, it is important to mention that the physical disruption (sampling, air-drying, and sieving) to which soils are subjected prior to incubation experiments may result in a release and loss of easily degradable organic C. Further, incubation conditions of moisture, aeration, and temperature differ intrinsically from real field conditions. Nonetheless, laboratory methods involving incubation of soil or soil-amendment mixtures under controlled conditions are widely recognized to supply valuable information about CO₂ evolution and organic matter dynamics (Levi-Minzi et al., 1990; Kirschbaum, 2000).

For all treatments, soil CO₂ emissions peak on the first day of the incubation and decreased with time. During the entire incubation period, cumulative CO₂ emissions (Fig. 1) are larger for the soils amended with municipal solid waste compost than for the soils amended with sewage sludge and with mineral fertilizer, which exhibit the smallest emissions. The soils treated with sewage sludge, however, emit more CO₂ per unit of C added than the soils amended with municipal solid waste compost.

Table 2

Sizes and decomposition rates of stable and labile C pools estimated by a double exponential model (C_s , k_s , C_i , and k_i , respectively; mean ± standard error) in soils amended with mineral fertilizer (MF), municipal solid waste compost (MC), and sewage sludge (SS) at rates equivalent to 0, 75, 150, and 225 t potentially available N ha⁻¹ (0, L, M, and H, respectively), without or with biochar at 20 t ha⁻¹ (BC), and percentage of C ($C_s + C_i$) that developed on the total C in the various soils after 182 days.

Soil	C_s (g CO ₂ -C kg ⁻¹ soil)	k_s (day ⁻¹)	C_i (g CO ₂ -C kg ⁻¹ soil)	k_i (day ⁻¹)	RMSE	R ²	($C_s + C_i$) / soil total C (%)
MF-0	1.375 ± 0.134	0.007 ± 0.002	0.212 ± 0.070	0.114 ± 0.049	0.035	0.994	10.0
MF-L	1.155 ± 0.117	0.009 ± 0.003	0.181 ± 0.058	0.218 ± 0.128	0.052	0.983	8.2
MF-M	1.182 ± 0.078	0.007 ± 0.001	0.197 ± 0.025	0.233 ± 0.057	0.025	0.996	8.4
MF-H	0.950 ± 0.131	0.007 ± 0.002	0.242 ± 0.044	0.177 ± 0.055	0.035	0.988	7.5
MC-0	1.240 ± 0.083	0.009 ± 0.002	0.148 ± 0.060	0.179 ± 0.117	0.044	0.990	8.8
MC-L	2.305 ± 0.035	0.015 ± 0.001	0.386 ± 0.036	0.372 ± 0.074	0.039	0.998	16.3
MC-M	3.416 ± 0.049	0.016 ± 0.001	0.561 ± 0.047	0.455 ± 0.089	0.058	0.998	22.4
MC-H	4.144 ± 0.097	0.016 ± 0.001	0.780 ± 0.103	0.400 ± 0.113	0.114	0.996	26.1
SS-0	1.204 ± 0.087	0.009 ± 0.002	0.163 ± 0.069	0.157 ± 0.100	0.045	0.989	8.5
SS-L	1.425 ± 0.105	0.009 ± 0.002	0.366 ± 0.056	0.207 ± 0.056	0.048	0.992	11.1
SS-M	1.344 ± 0.085	0.011 ± 0.003	0.601 ± 0.087	0.193 ± 0.045	0.064	0.989	11.7
SS-H	1.473 ± 0.086	0.009 ± 0.002	0.852 ± 0.059	0.183 ± 0.021	0.044	0.996	13.4
BC + MF-0	1.219 ± 0.062	0.007 ± 0.001	0.188 ± 0.032	0.130 ± 0.031	0.018	0.998	6.0
BC + MF-L	1.276 ± 0.183	0.006 ± 0.002	0.207 ± 0.049	0.169 ± 0.068	0.039	0.990	6.2
BC + MF-M	1.160 ± 0.168	0.007 ± 0.002	0.209 ± 0.048	0.180 ± 0.072	0.040	0.988	5.9
BC + MF-H	1.160 ± 0.151	0.006 ± 0.002	0.238 ± 0.031	0.170 ± 0.037	0.025	0.995	6.1
BC + MC-0	1.166 ± 0.178	0.007 ± 0.003	0.214 ± 0.089	0.125 ± 0.072	0.049	0.984	6.1
BC + MC-L	2.315 ± 0.061	0.015 ± 0.002	0.391 ± 0.065	0.351 ± 0.121	0.068	0.995	11.4
BC + MC-M	3.217 ± 0.178	0.015 ± 0.003	0.577 ± 0.174	0.411 ± 0.276	0.203	0.976	15.5
BC + MC-H	4.479 ± 0.087	0.014 ± 0.001	0.788 ± 0.076	0.407 ± 0.089	0.091	0.997	20.0
BC + SS-0	1.169 ± 0.175	0.007 ± 0.003	0.189 ± 0.089	0.132 ± 0.088	0.052	0.982	5.8
BC + SS-L	1.326 ± 0.126	0.008 ± 0.002	0.406 ± 0.061	0.165 ± 0.040	0.044	0.992	7.4
BC + SS-M	1.423 ± 0.164	0.008 ± 0.003	0.603 ± 0.077	0.178 ± 0.038	0.059	0.989	8.2
BC + SS-H	1.470 ± 0.155	0.009 ± 0.003	0.821 ± 0.084	0.188 ± 0.032	0.066	0.990	9.6

RMSE, root mean square error; R², coefficient of determination.

For the soils organically fertilized with these materials, cumulative CO₂ emissions tend to increase with increasing the amendment rate, whereas the contrary is true for the soils treated with the mineral fertilizer. These trends seem not to be affected by biochar addition.

Management practices that increase organic inputs, including amendment with municipal solid waste compost and sewage sludge, have been widely reported to increase soil microbial respiration and CO₂ emissions (Dick, 1992; Fernández et al., 2007). The larger volume of CO₂ that evolved in the soils amended with municipal solid waste compost compared to those treated with sewage sludge can be directly attributed to higher amount of organic matter incorporated with the former. In agreement with previous studies on similar systems (Fernández et al., 2007, 2012; Franco-Otero et al., 2012), the larger CO₂ emissions per unit of C added to the soils amended with sewage sludge, however, suggest that the organic matter in the latter is less stable. Unlike other waste treatments such as heat drying, composting is known to promote the biological degradation and stabilization of fresh organic matter (Epstein, 1996; Plaza et al., 2005).

Table 2 shows the best-fit parameters generated by nonlinear regression analysis of the experimental data of cumulative CO₂ evolution

using the double exponential model. The small standard errors, small root mean square errors (RMSE), and large coefficients of determination (R²) indicate that the model fits very well with the observed data.

The analysis of variance for the double exponential model parameters (Table 3) indicates that the type of fertilizer affects significantly the size and decomposition rate of the stable and labile soil C compartments. In particular, the size and rate of the stable C pool (C_s and k_s, respectively) and the rate of the labile pool (k_l) are larger for the soils amended with municipal solid waste compost than for the soils amended with sewage sludge, which in turn feature slightly larger values than the soils treated with mineral fertilizer. In contrast, the soils amended with sewage sludge, followed by the soils treated with municipal solid waste compost, exhibit the largest sizes of labile pool (C_l) (Table 2). The rate of fertilizer and the interactions between type and rate of fertilizer are significant for C_s and C_l, but not for k_s and k_l (Table 3). In particular, C_s and C_l tend to increase with increasing the amount of municipal solid waste compost and sewage sludge applied, but not with increasing the rate of mineral fertilizer (Table 2).

Biochar has no significant effects on the double exponential model parameters and does not interact with the type and rate of fertilizer.

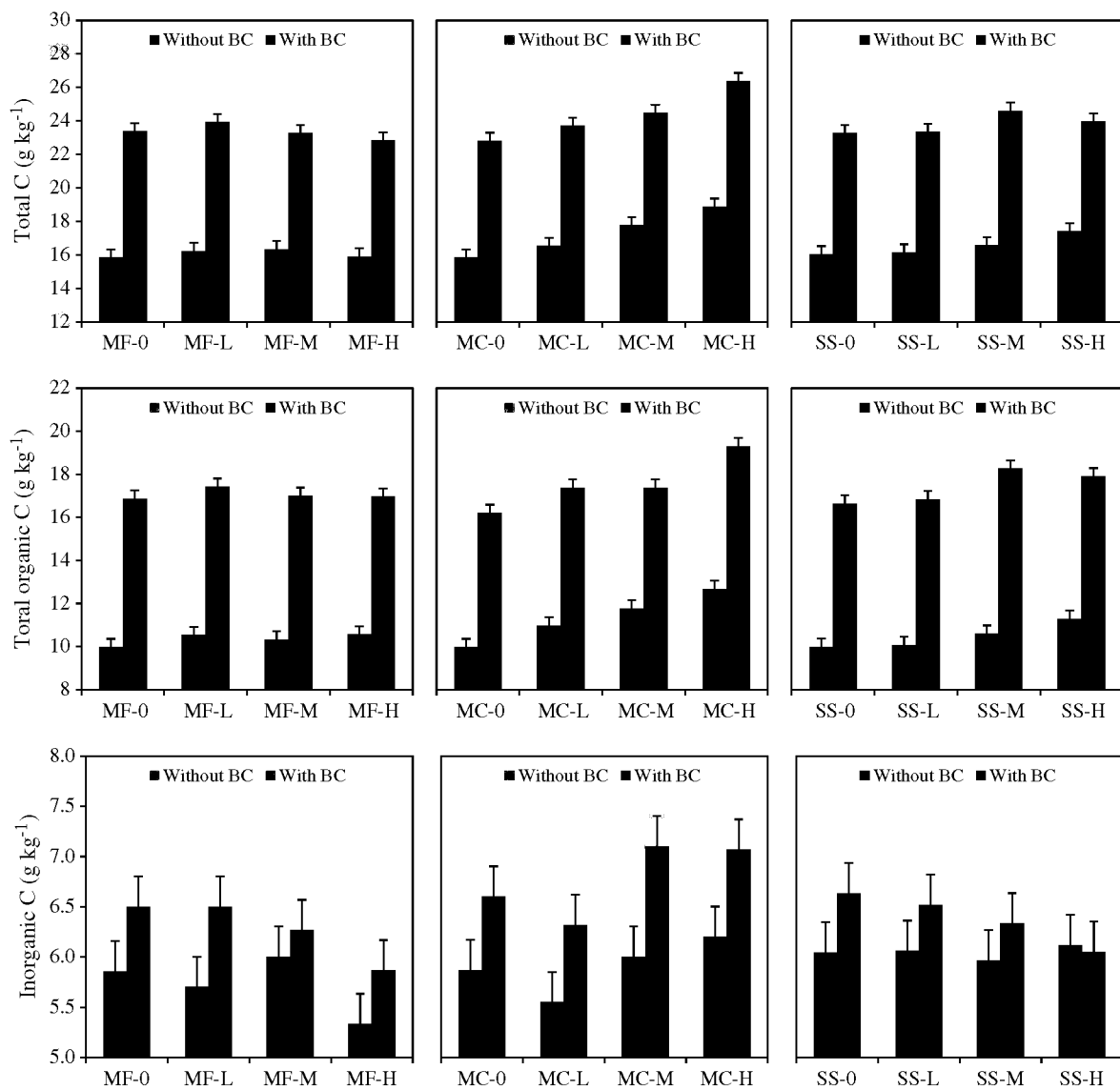


Fig. 2. Contents of total, organic, and inorganic C content in soils amended with mineral fertilizer (MF), municipal solid waste compost (MC), and sewage sludge (SS) at rates equivalent to 0, 75, 150, and 225 t potentially available N ha⁻¹ (0, L, M, and H, respectively), without or with biochar at 20 t ha⁻¹ (BC), after 182 days of incubation. Error bars represent pooled standard error (n = 3).

These results are consistent with those reported by Liang et al. (2010) in suggesting that biochar does not stimulate the mineralization of added organic matter. On the contrary, these authors found that the presence of black C enhances the incorporation and stabilization of exogenous organic matter into aggregate and organo-mineral fractions. Here the percentage of C that developed on the total C in the various tests after 182 days is found to be lower for the treatments with biochar than for the corresponding treatments without biochar.

3.3. Soil C fractions

The analysis of variance for the soil C fractions reveals that total and organic C contents are significantly affected ($P < 0.05$) by the biochar application, the type and rate of fertilizer applied, and the interactions between type and rate of fertilizer, whereas inorganic C content is only affected by biochar. In particular, both total and organic C contents in soil increase with increasing the rate of sewage sludge and especially municipal solid waste compost, whereas increasing the rate of mineral fertilizer appears not to have any effect (Fig. 2). These results may be directly related to the amount and stability of the organic C applied with the different fertilizers (Fernández et al., 2007).

Biochar application increases significantly both the organic and inorganic C fractions ($P < 0.05$) without interacting with the other amendments (Fig. 2). This result is consistent with the CO_2 evolution measurements reported here and agrees with other studies in strongly suggesting that biochar is relatively inert in the time scale of this incubation (Swift, 2001; Kuzyakov et al., 2009). It is especially noteworthy that the average increase in inorganic C found in the soils amended with biochar with respect to the unamended soils ($654 \pm 41 \text{ mg kg}^{-1}$) is markedly larger than the amount of inorganic C added ($170 \pm 19 \text{ g kg}^{-1}$). This enhanced inorganic C content, which

may contribute to C sequestration, might be attributed to the relatively large pH and/or sorption capacity of biochar (Cornelissen et al., 2013).

Soil microbial biomass C content and microbial biomass C to total organic C ratio are significantly affected ($P < 0.05$) by biochar addition, type of fertilizer, and the interactions between each of these two factors with rate of fertilizer. Whereas increasing the rate of mineral fertilizer tends to cause a slight decrease in soil microbial C content, the organic fertilizers, especially the municipal solid waste compost, have a positive impact (Fig. 3). Previous incubation and field studies suggest that mineral N additions may suppress microbial biomass C by a number of mechanisms, which include an increase in osmotic potential in soil solution, decrease in soil pH and Al mobilization, decrease in C availability due to inhibition of key enzymes for organic matter decomposition, and formation of recalcitrant N organic compounds (Treseder, 2008). Many studies show that the composition and application rate of organic amendments are important drivers of soil microbial biomass C (Kallenbach and Grandy, 2011). The larger microbial biomass found in the soils amended with municipal solid waste compost compared to those treated with sewage sludge may be related to the larger amount of C added with the compost and the faster decomposition and exhaustion of the organic matter added with the sludge, which is typically richer in labile organic C fractions (Fernández et al., 2012).

Microbial biomass C contents in the soils treated with mineral fertilizer are not significantly affected by biochar addition. In contrast, biochar exerts a suppressing effect on the microbial biomass C contents in the soils unamended or amended with low rates of organic fertilizers, which is attenuated with increasing the amendment rate. Most studies in the literature report positive effects of biochar on microbial biomass C contents in soils amended with biochar, mainly due to increased availability of labile organic substrates and micronutrients and provision of a favorable habitat for microbial growth (Lehmann et al., 2011; Luo et al., 2013). Decreased microbial biomass C contents following biochar

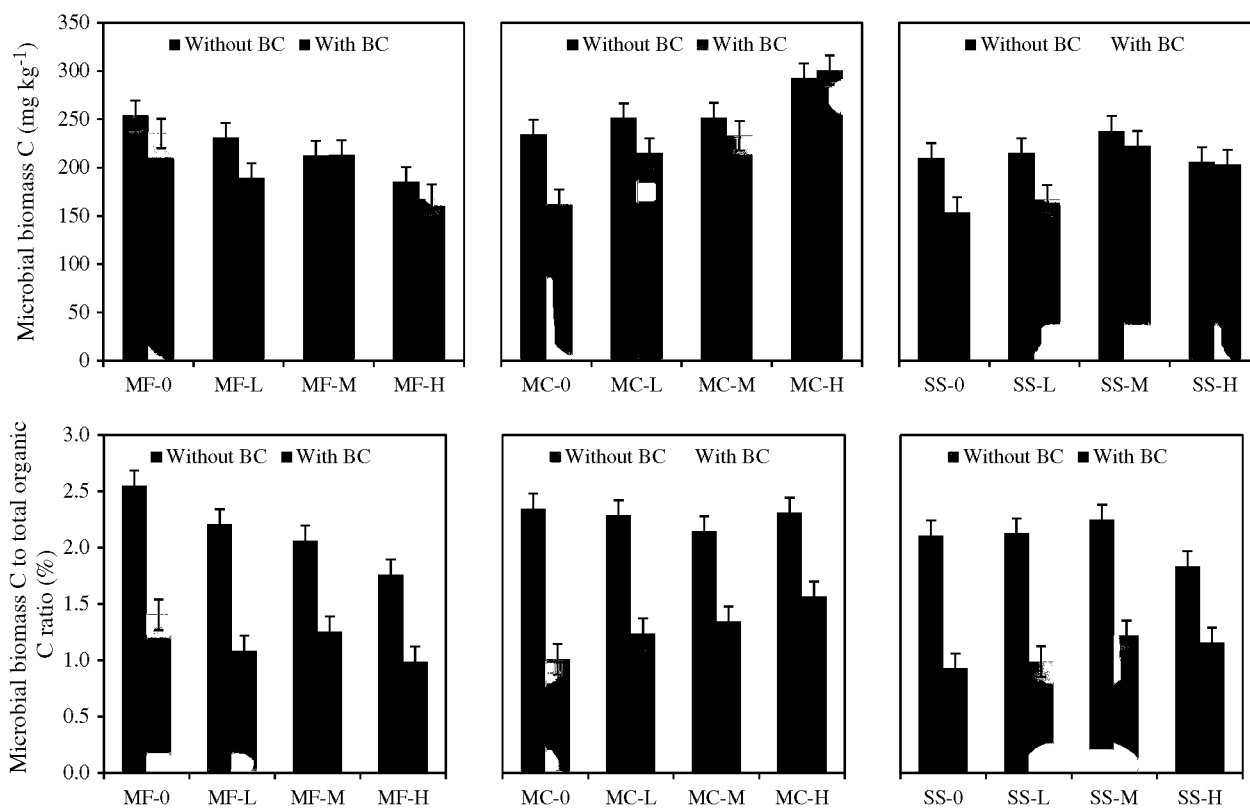


Fig. 3. Microbial biomass C content and microbial biomass C to total organic C ratio of soils amended with mineral fertilizer (MF), municipal solid waste compost (MC), and sewage sludge (SS) at rates equivalent to 0, 75, 150, and 225 t potentially available N ha⁻¹ (0, L, M, and H, respectively), without or with biochar at 20 t ha⁻¹ (BC), after 182 days of incubation. Error bars represent pooled standard error (n = 3).

addition could be attributed to the sorption of organic substrates and key enzymes for organic matter decomposition on the biochar surface, resulting in a decreased C availability for microorganisms, negative effects of increased pH on fungal growth and biomass (Rousk et al., 2009), and incorporation of toxic compounds. Our results suggest that the mechanisms by which biochar may decrease microbial biomass growth can be counterbalanced by the stimulating effect of increasing the addition of decomposable organic substrates with organic fertilizers.

4. Conclusions

The results presented above suggest that biochar application increases organic C contents in soil and does not interact with the effects of mineral and organic fertilizers on soil CO₂ emissions. Consequently, biochar, added either alone or combined with mineral and organic fertilizers, is unlikely to increase abiotic or biotic CO₂ emissions from semiarid agricultural soils, and therefore may have the potential to act as a C sink. Further, our results suggest that biochar, which typically features alkaline pH and large sorption capacity, may change soil acid–base equilibria, resulting in an increase of the inorganic C contents in semiarid soils. This effect may contribute to C sequestration and warrants further research. The findings of the present study should be verified in the field, which is widely recognized to be the ultimate scenario for addressing environmental and agricultural questions.

Conflicts of interest

The authors have declared no conflict of interest.

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