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Short communication (Pre-print)

### **Short-term releases of CO<sub>2</sub> from newly mixed biochar and calcareous soil**

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#### **Abstract**

The work aims to quantify native organic C mobilized in one calcareous soil in the 21 days after addition of biochar at a range of high to very high doses. The experiment was carried out in unplanted microcosms and CO<sub>2</sub> flux was used as a measure of net mineralization. A rapid methodological approach, which does not require <sup>13</sup>C as a tracer, was used to assess any priming effects induced by the biochar. The amount of CO<sub>2</sub>-C mobilized was small relative to the amount of biochar C and proportional to the amount of the biochar added. The additional CO<sub>2</sub>-C was similar to the content of the WSOC of the biochar added in each respective dose. No interaction with native soil C, i.e. priming effect, was observed.

*Keywords:* biochar, soil respiration, priming effect, first-order model, water soluble carbon.

#### **Introduction**

Biochar has the potential to be used for applications in many fields, such as agriculture and environmental remediation. Results from meta-analysis show an overall positive influence on soil quality (Biederman and Harpole, 2013). Soil responses to biochar may depend on source material

and pyrolysis conditions that introduce variations in the structure, nutrient content, pH and phenolic contents in the product (Novak et al., 2009). Biochar is also suggested to be a soil conditioner able to mitigate atmospheric increases in CO<sub>2</sub> by leading to a net increase in soil organic matter (Sohi, 2012). A review by Xie et al. (2016) highlighted the potential of biochar to maintain soil quality and sequester carbon. However, following the addition of biochar to the soil, increases in soil respiration have been observed, leading to greater losses of C from the amended soil than those of controls (Zimmerman et al., 2011). This effect is attributed to decomposition of the organic C released from the biochar or stimulation of the mineralization of native organic substances (positive priming effect). The scientific literature also reports a reduction in microbial activity due to the addition of biochar, which leads to a decrease in soil respiration (negative priming effect) (Jones et al., 2011) or the lack of variations in the CO<sub>2</sub> efflux (Novak et al., 2010).

The study investigates the releases of CO<sub>2</sub> from mixed biochar and calcareous soil in a short-term incubation.

## **Materials and methods**

The soil was collected from the top 5-15 cm at the research centre of the Department of Agriculture, Food and Environment of the University of Pisa, Italy. The biochar was produced by burning pruning residues of fruit trees by slow pyrolysis at 550 °C. The main characteristics of soil and biochar were determined using standard methods (Table 1).

Total organic C (TOC) was determined by induction furnace 900 CS, ELTRA.

The water soluble organic carbon (WSOC) was determined with a C analyzer for liquid samples (Hach QbD1200).

In 250-ml microcosms, biochar was added in order to obtain the application doses of 0, 2, 4, 6, 8 and 10% of the weight of the incubating soil. The CO<sub>2</sub> evolution was monitored daily for a 21-d incubation period. The CO<sub>2</sub> evolved was trapped in NaOH solution and the alkali excess was titrated with HCl after precipitation of carbonates with a 2N solution of BaCl<sub>2</sub>.

The C mineralization kinetics were determined by Graph Pad Prism 5 (2007) following a first-order model [ $C_m = C_0 (1 - e^{-kt})$ ], where  $C_m$  is the cumulative value of mineralized C during  $t$  (days),  $C_0$  is the potentially mineralizable carbon, and  $k$  is the rate constant of C mineralization.

## **Results and discussion**

The soil amended with biochar released little greater amounts of carbon in the form of CO<sub>2</sub> than the control (Figure 1). Data of C mineralization were analyzed as a series of parallel curves and results indicated that the same basic curve with slightly modified parameters fits all data. Therefore, the significance of intercepts and slopes can be evaluated simultaneously. Figure 2 shows a linear increase in CO<sub>2</sub> production with the increased addition of biochar C. The regression line describing the cumulative C loss from biochar-soil systems as a function of different loading rates of biochar C enabled us to estimate, without resorting to the use of isotopically labeled biochar, the influence of the material on the native organic C decomposition in the soil. This procedure consists in comparing the value of the intercept of the regression line (Figure 2) with the y-axis, corresponding to CO<sub>2</sub>-C produced at 0 dose of TOC application, with the CO<sub>2</sub>-C actually measured in the control (Levi-Minzi et al., 1989). The value of the priming ratio, obtained by dividing the CO<sub>2</sub>-C value of 51.2 mg 100g<sup>-1</sup> estimated by the intercept of the line with the y axis for the value of CO<sub>2</sub>-C of 51.9 mg 100g<sup>-1</sup> actually measured in the control, was very close to 1 (0.99), indicating a lack of priming effect. This result is in agreement with findings reported by Kuzyakov et al. (2009), Novak et al. (2010) and Zimmerman et al. (2011). The CO<sub>2</sub>-C values of the mixtures were corrected to eliminate the cumulative values of CO<sub>2</sub>-C released from the control soil (Table 2). When the proportions, expressed as percentages, of the biochar C that were mineralized were compared, data from the treated soils showed that the amount of biochar C that was mineralized represented only about 0.1% of the TOC present in biochar. However, since around 50% of the soluble C of biochar was mineralized over the 21-d incubation period (Table 2), it was assumed that the additional CO<sub>2</sub> was derived from a small labile fraction of biochar. Therefore, a parallel curves analysis was performed using WSOC as a parameter instead of TOC. The value of the intercept of the regression line ( $Y=51.2+0.4943x$ ,  $R^2=0.99$ ) was the same as that obtained with TOC as a parameter. Since WSOC was not fully mineralized and given the clear trend towards the stabilization of CO<sub>2</sub> production which suggests a significant block of mineralization, it could be deduced that the remaining portion of soluble compounds may have been incorporated into native C or microbial biomass, or may precipitate (Smith et al., 2010).

More than 92% of the whole potentially mineralizable C (C<sub>0</sub>) of mixtures had already been mineralized during the short-term incubation (Table 3). Thus, it may be assumed that most of the carbonaceous material remained in the soil as a recalcitrant C pool.

## Conclusions

The additional CO<sub>2</sub> released from the soil after adding the biochar was small, relative to background respiration of non-amended soil, and it was proportional to the amounts of TOC and WSOC added with the material. The value of the priming ratio close to 1 indicates that the enhanced CO<sub>2</sub>-C was not due to a greater carbon release of native soil organic matter. The amounts of water soluble organic carbon added with biochar accounted for the additional amounts of C mineralized to CO<sub>2</sub>-C during incubation.

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Captions of tables:

Table 1. Soil and biochar properties.

Table 2. Cumulative amount of CO<sub>2</sub>-C from soil systems and water soluble organic carbon (WSOC) added with biochar at the different loading rates.

Table 3. Parameter estimates according to the first-order model for C mineralization of biochar in soil systems.

Table 1.

	Soil	Biochar
Sand %	73.3	
Silt %	12.2	
Clay %	14.5	
pH	8.2	10.2
Inorganic C %	7.7	12.7
Organic C %	1.42	86.0
Total N mg·g <sup>-1</sup>	1.30	4.80
C to N ratio	10.9	179
Available P μg·g <sup>-1</sup>	40	443
Exchangeable K μg·g <sup>-1</sup>	350	12500
Cation exchangeable capacity Cmol <sup>(+)</sup> ·kg <sup>-1</sup>	12.1	29.0
Maximum Water Holding Capacity %	40	260
Water Soluble Organic C mg·g <sup>-1</sup>	0.17	2.2
NO <sub>3</sub> - N μg·g <sup>-1</sup>	165	440
Total phenolic compounds μg·g <sup>-1</sup>	38	260
Bulk density g·cm <sup>-3</sup>	1.62	0.52

Table 2.

Doses	<sup>a</sup> C <sub>m</sub> mg.100g <sup>-1</sup>	<sup>b</sup> C <sub>m</sub>	<sup>c</sup> C <sub>m</sub> % TOC	C added with biochar g.100g <sup>-1</sup>	WSOC added with biochar mg.100g <sup>-1</sup>
Control	51.9	-	-	-	-
2%	53.2	1.3	0.08	1.7	4.4
4%	55.8	3.9	0.11	3.4	8.9
6%	58.2	6.3	0.12	5.2	13.3
8%	59.4	7.5	0.11	6.9	17.8
10%	62.4	10.5	0.12	8.6	22.2

<sup>a</sup>Cumulative amount of CO<sub>2</sub>-C from biochar-soil mixtures.

<sup>b</sup>Cumulative amount of CO<sub>2</sub>-C from biochar.

<sup>c</sup>Cumulative amount of CO<sub>2</sub>-C from biochar as percentage of TOC.



Table 3.

Doses	C <sub>0</sub> from mixtures mg.100g <sup>-1</sup>	k day <sup>-1</sup>	R <sup>2</sup>	C <sub>m</sub> from mixtures % C <sub>0</sub>
Control	55.0	0.14	0.98	94.5
2%	56.0	0.15	0.97	95.1
4%	58.3	0.15	0.97	95.7
6%	59.9	0.15	0.98	97.2
8%	61.2	0.15	0.98	97.2
10%	67.3	0.13	0.97	92.7

C<sub>0</sub> = potentially mineralizable organic C (amount present at t = 0).

K = rate constant.

R<sup>2</sup> = coefficient of determination.

Captions of figures:

Figure 1. C loss during 21 days incubation in biochar-soil systems at different loading rates.

Figure 2. Cumulative evolution of CO<sub>2</sub>-C from soil systems as a function of different loading rates of biochar.



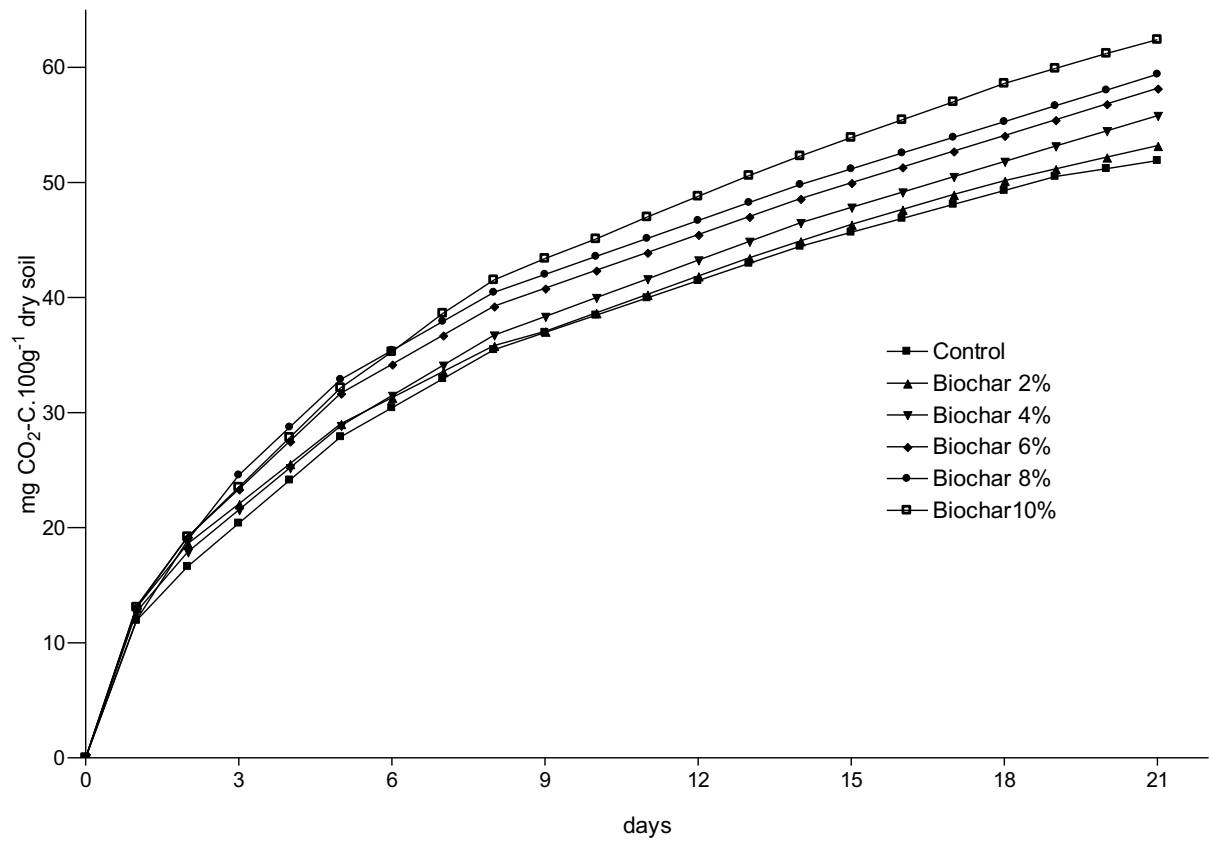
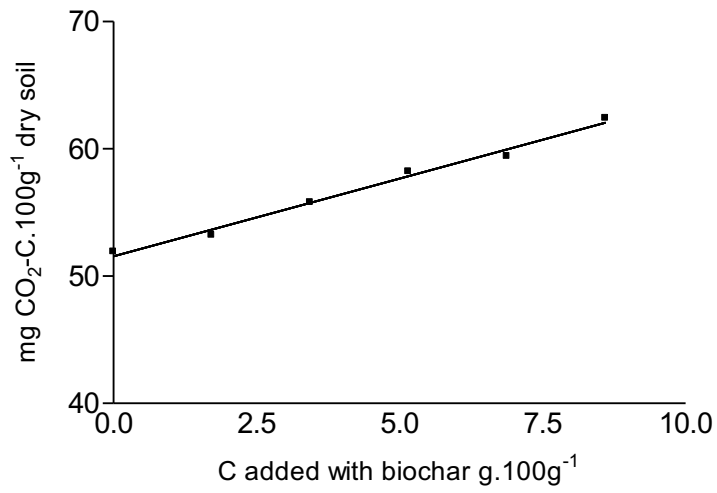


Figure 1



$$Y = 51.2 \pm 0.4844 + 0.0013 X \quad (r^2=0.99)$$

Figure 2