



1 **Biochar reduces early-stage mineralization rates of plant residues** 2 **more in coarse than fine-texture soils – an artificial soil approach.**

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11 **Abstract.** Quantifying the impact of biochar on carbon persistence across soil textures is complex, owing to the variability in
12 soil conditions. Using artificial soils with precise textural and mineral composition, we could disentangle the effects of
13 biochar from the effects of soil particle size. We can show that biochar application significantly reduces early-stage carbon
14 mineralization rates of plant residues in various soil textures (from 5 to 41% clay) but more significantly in sandy soils. This
15 finding suggests that biochar can compensate for the lack of clay in promoting C persistence in soil systems. This short
16 report significantly contributes to our understanding of soil texture and biochar application interactions.

17 **1 Introduction**

18 Biochar application in agriculture has been recognized to enhance carbon sequestration and improve soil quality
19 (Lehmann, 2007). In this regard, soil texture may play a fundamental role in the overall effectiveness of biochar in
20 promoting carbon persistence, mainly due to influences on soil structural properties (Wang et al., 2017). However, the
21 mechanisms behind the interaction between biochar and soil texture in promoting C storage are not fully understood due to
22 the high heterogeneity in soil properties and climate conditions of natural soils. As a result, the findings in the literature on
23 this topic can vary depending on the specific experimental conditions used (Gross et al., 2021). Therefore, more systematic
24 analyses of these factors are necessary to understand how biochar can affect overall C dynamics in soil.

25 In this sense, using artificial soil with known particle size and mineral composition provides an excellent base for
26 understanding the mechanisms behind soil processes (Pronk et al., 2012). These artificial soils can be mixed to mimic the
27 composition of typical arable soils of the temperate region, while their individual properties, like soil texture, can be freely
28 adjusted (Bucka et al., 2021).



29 This short communication explored the interactions between soil texture and biochar application in the early-stage
30 soil organic matter mineralization using artificial soils with precise mineral and textural composition in a controlled
31 microcosm. We hypothesized that biochar could reduce organic soil mineralization, especially in coarser textured soils.

32 2. Material and methods

33 2.1 Artificial soils preparation: texture range

34 For producing the different textures of artificial soils, we have used quartz grains of varying particle sizes
35 (Euroquarz, Laußnitz, Germany, and Quarzwerke, Frechen, Germany). We added goethite, illite, and bentonite (Aspanger,
36 Aspang, Austria) to create soil-like reactive surfaces in the clay-size fraction. The C-content of the artificial soils was
37 considered negligible due to the insignificant C concentration of the ingredients. The soil mixtures were prepared according
38 to the proportions presented in Table 1. The texture classes were defined as: 1) Loamy Sand, 2) Sandy Loam, 3) Loam, 4)
39 Clay Loam, and 5) Silty Clay.

41 **Table 1:** Composition of the artificial soils.

Fraction	Ingredients	Soil 1 Loamy Sand	Soil 2 Sandy Loam	Soil 3 Loam	Soil 4 Clay Loam	Soil 5 Silty Clay
		----- Proportion (mass %) -----				
Sand	Quartz Sand	81	61	45	21	8
Silt	Quartz Silt	14	29	39	48	51
Clay	Quartz Clay	4.45	8.9	14.24	27.59	36.49
	Goethite	0.05	0.1	0.16	0.31	0.41
	Illite (MICA SFG 75)	0.25	0.5	0.8	1.55	2.05
	Bentonite	0.25	0.5	0.8	1.55	2.05
	Total	100	100	100	100	100

42
43 The artificial soils were prepared by mixing the ingredients in a dry state from coarser to finer scale particles. As
44 each component was added, 10 manual turns were executed to combine them. After all the ingredients were mixed, the
45 containers were added to a horizontal shaker and shaken overnight at 140 rpm. After the overnight shaking period, each soil
46 mix container was manually turned 30 times.



47 **2.2 Incubation Experiment and Treatments**

48 The experimental setup was a 5 x 2 factorial design testing 5 different textures of artificial soils with and without
49 biochar application with three replicates. We used 20 g of artificial soil per experimental unit in 120 ml glass flasks for the
50 incubation experiment. We added ball-milled air-dried clover biomass (*Trifolium sp.*) (C:N ratio of 18) as an organic matter
51 source to all samples at a rate of 27 mg C g⁻¹ soil to mimic a natural background OC content of arable topsoils. We have used
52 dissolved organic matter extracted from a local crop field as an inoculum of soil microorganisms to the artificial soils at a
53 rate of 0.06 ml g soil⁻¹, according to (Pronk et al., 2012). The artificial soils were incubated under 60% of the maximum
54 water hold capacity to ensure microbial activity (Supplementary Figure 1). The water used to add the inoculum was
55 accounted for in the amount of water added. Biochar was produced from Norwegian Spruce under a temperature of 700° C
56 and added to the soil at a rate of 50 mg of biochar g⁻¹ soil. The basic biochar properties are described in the Supplementary
57 Table 1.

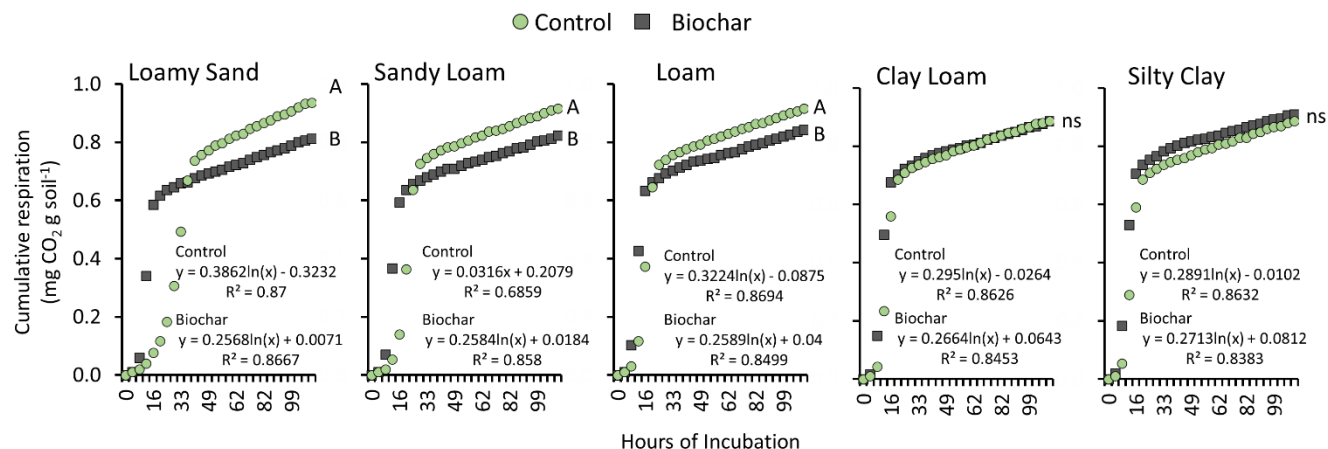
58 **2.3 CO₂ respiration measurements**

59 We measured CO₂ production over 115 hours using an automated incubation system described in detail by Molstad
60 et al. (2007) with modifications in Molstad et al. (2016). The system consists of an autosampler (CTC PAL) connected to an
61 Agilent gas Chromatograph (Model 7890A, Agilent, Santa Clara, CA, USA). The system allows for high-resolution analysis
62 of headspace gas concentrations in airtight 120 ml serum bottles. Corrections were applied to adjust for sampling dilution,
63 leakage, and CO₂ equilibrium state as a function of the material pH and soil solution volume (Appelo and Postma, 2005).

64 **3. Results**

65 **3.1 Early plant mineralization affected by particle size and biochar applications**

66 We observed significant effects of both biochar application and texture on C mineralization and a significant
67 interaction between these variables in the analysis of variance (ANOVA) at p < 0.01. Biochar application significantly
68 reduced C-mineralization rates compared to control in the coarser-textured soils: 1) Loamy Sand, 2) Sandy Loam, and 3)
69 Loam (Figure 1). However, no differences were observed between biochar and control treatments for the finer-textured
70 soils: 4) Clay Loam and 5) Silty Clay (Figure 1).

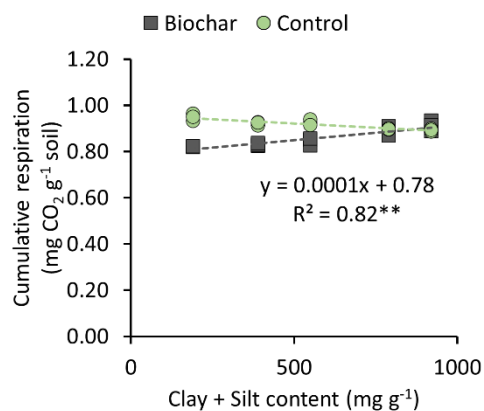


71

72 **Figure 1:** Biochar effect on the CO₂ cumulative respiration in artificial soils with different textures in a 5-day incubation
 73 experiment. For a given soil, different uppercase letters indicate a significant difference in the cumulative respiration
 74 between control and biochar-amended soils by the LSD test at $p < 0.05$. NS = not significant.

75

76 The reduction in C mineralization promoted by biochar in the Loamy sand (Soil 1) was over 6-fold higher than in
 77 the Silt Clay soil (Soil 5). The influence of clay and silt content on C mineralization also differed depending on whether
 78 biochar was applied or not (Figure 2). Control samples' clay and silt content generally decreased C mineralization (Figure 2).



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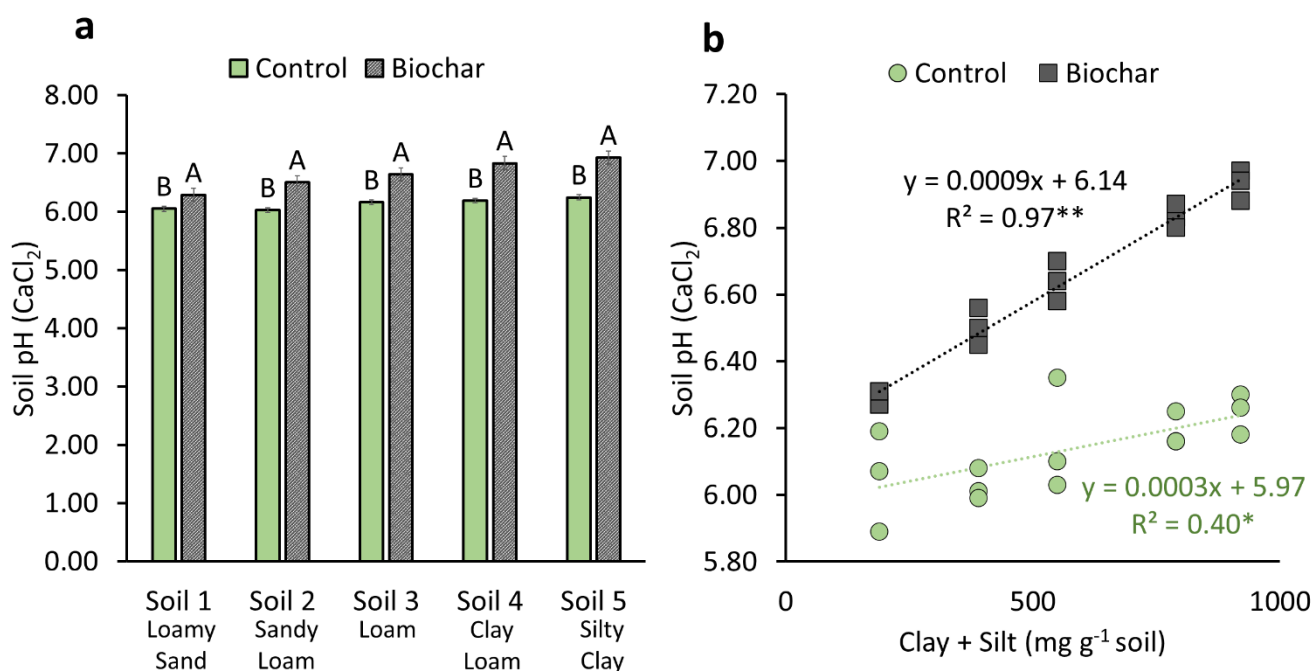
80 **Figure 2:** Influence of clay and silt fraction from artificial soils on the cumulative respiration in soils with and without
 81 biochar in a 5-days incubation experiment. ** $p < 0.01$.

82



83 3.2 Soil pH affected by particle size and biochar application

84 Soil pH (CaCl₂) was significantly influenced by increased soil texture and biochar application (Figure 3). Increases
 85 in pH due to biochar addition were overall higher with increasing clay and silt contents, i.e., 0.68 in the Silt Clay and 0.24 in
 86 the Loamy Sand (Figure 3a). The increase of silt and clay particles alone also promoted increases in soil pH (p<0.05) (Figure
 87 3b). Every mg of clay and silt fraction promoted an increase of 0.0003 in soil pH (CaCl₂). This increase was three times
 88 higher (0.009) when biochar was applied (p<0.01) (Figure 2b).



89
 90 **Figure 3:** Soil pH as affected by the biochar application and soil texture in artificial soils incubated for 5 days. a) Influence
 91 of biochar application on soil pH (CaCl₂) under different textures. For a given soil, different letters indicate significant
 92 differences by the LSD test at p < 0.05. b) Influence of soil texture (clay+silt proportion) on soil pH (CaCl₂) in soils with
 93 and without biochar. ** significant at p < 0.01. * Significant at p < 0.05

94



95 4. Discussion

96 4.1 Interactions of clay + silt content and biochar application in reducing early mineralization of plant residues.

97 The significant interaction between biochar application and soil texture suggests that the extent of biochar's impact
98 on the mineralization of plant residues depends on soil texture. Likewise, the observed effects of soil texture on clover
99 mineralization depended on the biochar application. The differences in the magnitude of early plant mineralization
100 depending on soil texture suggest that biochar had a higher impact on reducing early C-mineralization from clover residues
101 in sandier textures than in clay-rich soils. Biochar has known benefits in enhancing soil properties associated with promoting
102 C protection, such as aggregation (Wang et al., 2017; Juriga et al., 2021), sorption capacity (Siedt et al., 2021), water
103 retention, and porosity (Obia et al., 2016). in natural soils. Such effects may have potentially influenced the decrease of
104 early mineralization of the added clover plant residues since the improvement of soil structure in artificial soils, such as
105 aggregate formation, is already observed within the first days of their development (Pronk et al., 2012). Also, the increase of
106 fine particles in soils is assumed to decrease organic matter decomposability due to the increased opportunity for
107 physicochemical protection (Hassink, 1992; Kravchenko and Guber, 2017), which justifies the influence of clay and silt
108 particles in reducing mineralization in the control soil.

109 Especially in sand-rich soil, where the available mineral surface area (as well as the permanently charged surfaces
110 of clay minerals) is low, biochar can deliver additional surface area for adsorption processes. The biochar effect is probably
111 diminished with increasing clay content as the higher clay mineral surface area and the smaller pores may overrule the
112 biochar effect on the physicochemical protection of OM.

113 Nonetheless, the results on texture controlling soil organic matter mineralization are contrasting in the literature,
114 with results being dependent on soil moisture and other experimental conditions (Li et al., 2020; Li et al., 2022). Our
115 experiment using artificial soils with precise composition and early formation stage helped shed light on texture's and
116 particle size effects on organic matter decomposition. In our experiment, significant correlations were observed between the
117 clay + silt content with the cumulative CO₂ respiration ($p < 0.01$) (Figure 1b). Every mg of silt and clay size particles in the
118 artificial soils reduced clover residue mineralization by 0.00007 mg g⁻¹ soil under a constant moisture level (60% of the
119 water hold capacity). The clover mineralization after a 5-day incubation in the Silt Clay soil (Soil 5) was 0.06 mg CO₂ g⁻¹



120 soil lower than in the Loamy Sand soil (Soil 1) ($p < 0.05$ LSD test). Our results suggest that the increase of clay and silt size
121 particles have a role in reducing early-stage C mineralization of crop residues. This effect was likely related to improved soil
122 physical characteristics, as the water-holding capacity of clay soils was higher than the sandy soils (Supplementary Fig. 1).

123 **4.2 Interactions between clay and biochar in enhancing soil pH, and the consequences of early mineralization of plant** 124 **residues**

125 The pronounced biochar effect on pH is likely due to its high acid buffering capacity. Biochar consists of both
126 alkaline functional groups and mineral ash containing both base cations and secondary carbonates (Fidel et al., 2017),
127 explaining why the biochar and clay + silt content have increased soil pH.

128 The higher pH of the soil solution promotes the dissolution of CO₂ gas, thereby reducing the amount of gas released
129 from soils. This mechanism can result in a measurement artifact of lower-than-actual mineralization rates in pH-enhanced
130 soils (Ma et al., 2013). However, this effect was accounted for in our study, as mineralization rates were corrected for
131 amounts of CO₂ dissolved in solution as a function of solution volume and pH (Appelo and Postma, 2005). Direct sorption of
132 CO₂ into biochar has also been reported alongside N₂O adsorption and can also be considered a mechanism through which
133 biochar can reduce the presence of these gases in the atmosphere (Cornelissen et al., 2013). Our study suggests a higher
134 capacity of biochar in promoting reductions in the early mineralization of clover residues in sandy soils. This potential is
135 diminished as the clay and silt content increase in the soil.

136 **4. Conclusions**

137 We report the results of a screening study using an experimental setup for high-frequency short-term measurements.
138 We have observed significant effects of biochar and soil texture in reducing early mineralization of clover residues in
139 artificial soils and a significant interaction between these factors. Biochar has demonstrated the potential to reduce the early
140 mineralization of plant residues, especially in sandy soils. This effect is diminished with increased clay and silt content in the
141 soil, suggesting that biochar may compensate for the lack of clay in sandy soils by promoting lower mineralization of
142 organic matter. Results on soil organic matter persistence and carbon sequestration must be confirmed using longer-term
143 experiments. However, this first set of results demonstrates the power of using standardized multi-texture artificial soils to



144 study biochar and organic matter interaction in soils. Here we suggest that this artificial soil setup is a valuable platform for
145 understanding mechanisms associated with forming Terra Preta soils.

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195 **Author contribution**

196 TI, SW, FB, EF, and DR designed the experiment. TI and SW carried out the analyses. TI wrote the manuscript with the
197 support of the other authors.

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199 **Competing interests**

200 The authors declare that they have no conflict of interest.