Biochar impact on the estimation of the colorimetric-based enzymatic assays of soil

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Running Title: Biochar impact on enzymatic assays of soil

Abstract

This study was carried out in order to assess the influence of biochar applications on the estimation of colorimetric-based enzymatic assays and to verify the effectiveness of the most common methods. Since most methods used to determine enzymatic activities in the soil are based on colorimetry, biochar may absorb substrates and/or colored products thereby distorting the analytical result. Biochar was added to two soils, with different textures and cation exchangeable capacities, at a rate of 2% (w/w), and seven enzyme activities were determined following standard methods. The biochar amendment lowered the spectrophotometer reading of the activity of FDAase and dehydrogenase in the sandy soil. In the three enzymatic activities based on p-nitrophenol production (β-glucosidase, phosphatase and arylsulphatase), the addition of biochar did not change the enzyme assays. The biochar led to an overestimation in terms of the protease and urease activities in the sandy soil. In the clay loamy soil, biochar did not change the response of any of the enzyme activities tested. A biochar dose of up to 2% only guarantees the effectiveness of the most common spectrophotometric methods for not excessively sandy soils.

Keywords: biochar, soil improver, enzymatic assays, spectrophotometry

Introduction

Biochar is a carbon-rich solid material derived from the pyrolysis of agricultural and forest residual biomass. Soil is frequently amended with biochar due to its properties, including soil conditioning, enhanced fertility, sorption of pollutants and hormones, and C sequestration (Rinklebe *et al*., 2016). However, there are conflicting data on the enzyme activities as a consequence of biochar addition (Mierzwa-Hersztek *et al*., 2016), perhaps because of the imprecision of the analytical methods. Since almost all methods for the determination of soil enzymatic activities are based on colorimetry using a spectrophotometer, the biochar may partially absorb the color, thus distorting the results. Swaine *et al.* (2013) reported that the amount of biochar can have a significant effect on substrate and product

concentrations during an enzymatic soil analysis, which led to a decrease in color. They hypothesized that biochar may increase the solid phase absorption of assay chemicals by acting as an absorbent itself or by altering the strength of sorbate interactions with native soil components (organic material and clay minerals) through indirect effects on soil chemistry.

This study was carried in order to assess the influence of biochar amendment on the colorimetry-based enzymatic assay.

Materials and methods

Two soils, differing above all in texture and cation exchangeable capacity (Table 1), were collected from the top 5–15 cm at the research center 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 soils and biochar were passed through a <2 mm sieve, and both the physical and chemical characterizations were carried out following standard procedures (Table 1). Biochar was added to soils at a rate of 2% (w/w), which is the highest dose commonly used in agricultural applications. The samples were carefully mixed, watered at the 60% maximum water holding capacity, closed with parafilm to permit a gaseous exchange, and refrigerated at 4 °C for the analyses.

Enzyme activities were determined by spectrophotometry on the control and mixtures following the standard methods (Table 2). The results were expressed as the mean of five replicates. The differences between non-amended and biochar-amended soils were analyzed using one-way analysis of variance (ANOVA). Significantly different means were separated at the 0.05 probability level by Tukey's test.

Results and discussion

The results show that biochar lowered the estimation of FDAase and dehydrogenase activities in the sandy soil mixture, but not in the clay loamy soil (Fig. 1). This is probably because the absorbent components of biochar in the clay loamy soil were linked to the clayey fraction and therefore were

78 unable to exert the adsorbing capacity, and did not interfere with color formation. However, in the
79 sandy soil, which is low in colloidal materials that form bonds with biochar adsorbing fraction, the
80 assay chemicals were adsorbed, thus decreasing the color and lowering the estimation of FDAase and
81 dehydrogenase activities.
82 In the three enzymatic determinations based on p-nitrophenol (pNP) production (β-glucosidase,
83 phosphatase and arylsulfatase), there were no statistical differences between the control and biochar-
84 amended soils (Fig. 2). Paz-Ferreiro *et al.* (2012) found similar results for phosphatase and
85 arylsulfatase, although they did find differences between the control and biochar-amended soils for
86 β-glucosidase, but only at higher doses (4% and 8%) than those of our study. The lack of differences
87 between the control and biochar-amended soils may be due to the fact that pNPs, which are in
88 predominantly phenolate form as negatively-charged molecules with low hydrophobicity ($\log K_{ow}$
89 1.91), are not adsorbed by biochar. This is due to the repulsion between the pNP anions and the
90 negatively charged functional groups of biochar, which may explain the lack of interference of the
91 biochar with color formation.
92 The addition of biochar led to an overestimation of protease and urease activity values in the sandy
93 soil (Fig. 3). Biochar probably releases carbonaceous organic substances which influence the color
94 intensity of the solution used for the spectrophotometer reading (690-700nm). The coloring effect of
95 biochar was evident only in the sandy soil. In the clay loamy soil, the activity of the control is rather
96 high and, therefore, any differences induced by biochar may not be detectable.
97
98 **Conclusions**
99 At the highest dose typically used in agricultural applications (2% w/w), the addition of biochar had
100 no effect on the estimation of the colorimetric-based enzymatic assays of clay loamy soils.
101 For sandy soils, biochar may influence the estimation of some enzyme activities, both decreasing it
102 (FDAase, dehydrogenase) and increasing it (protease, urease).

For doses of up to 2%, the effectiveness of the most common spectrophotometric methods is only ensured for not excessively sandy soils.

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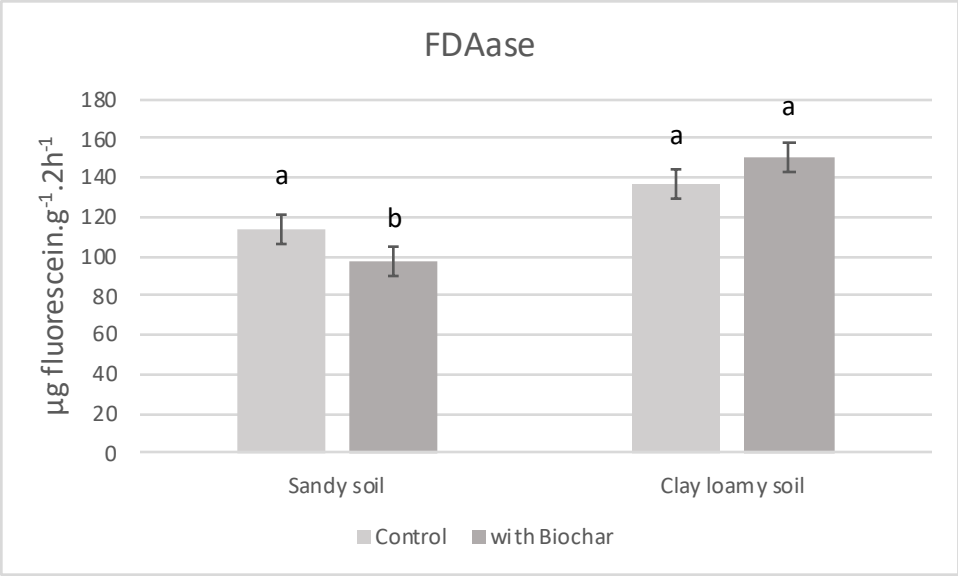
142

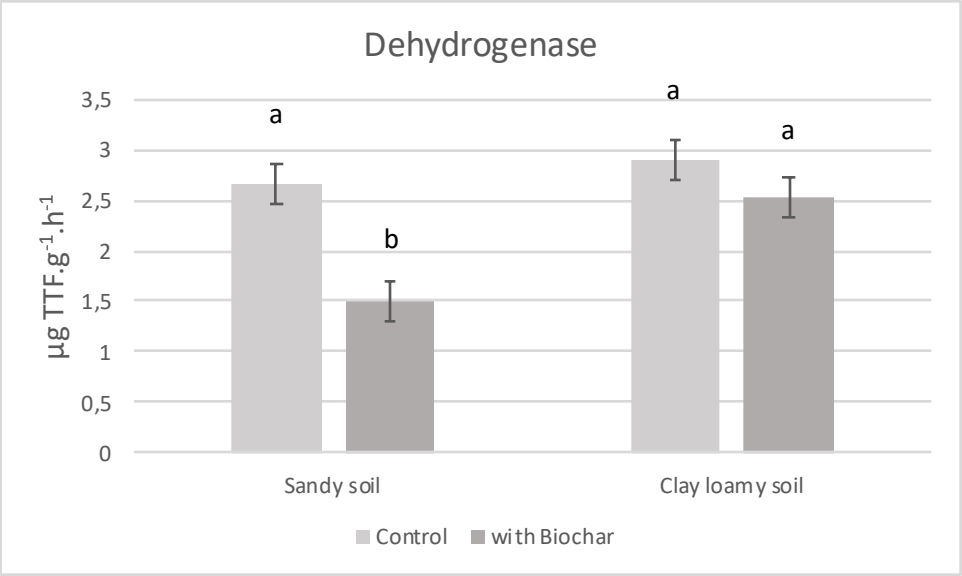
Table 1. Main characteristics of the two soils and the biochar.

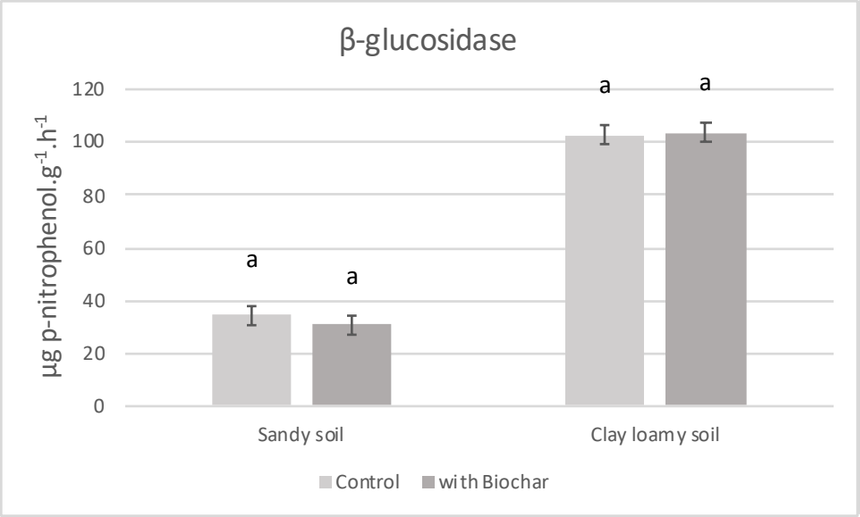
	Sandy soil	Clay loamy soil	Biochar
Sand %	90	27	
Silt %	3	37	
Clay %	7	36	
pH	6.2	7.9	10.2
Organic C %	1.10	1.41	86.0
Total N $mg \cdot g^{-1}$	0.87	1.55	4.80
C to N ratio	12.6	9.1	179
Available P $\mu g \cdot g^{-1}$	13.0	7.6	443
Exchangeable K $\mu g \cdot g^{-1}$	31.5	181	12500
Cation exchangeable capacity $Cmol^{(+)} \cdot kg^{-1}$	8.9	21.4	29.0

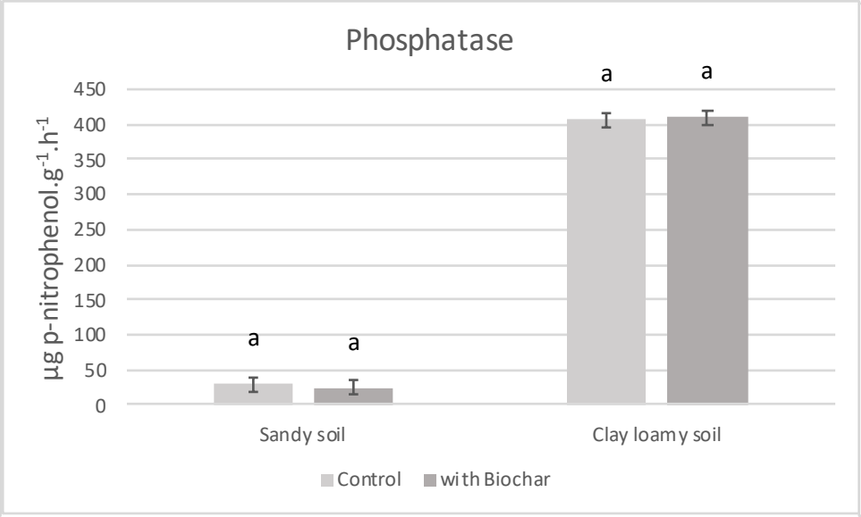
Table 2. Methods for determination of enzyme activities.

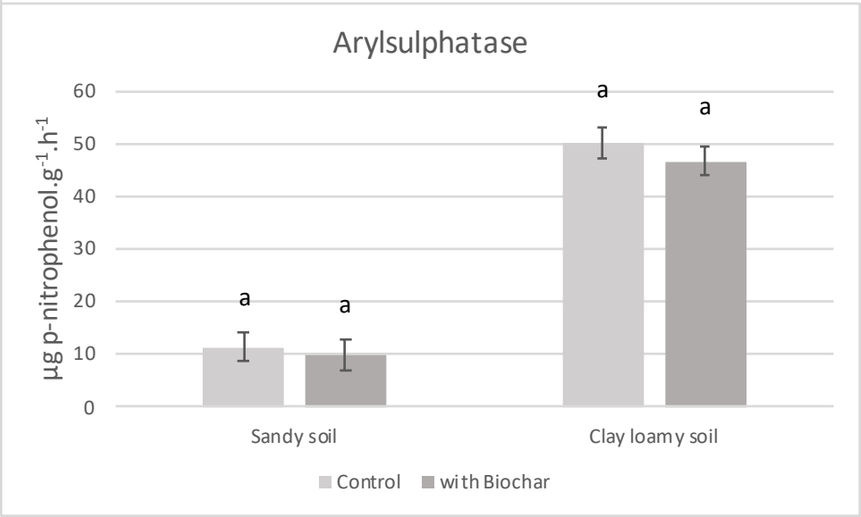
Activity	Reference	Substrate	Product	Wavelength
FDAase	Dick et al. 1996	Fluorescein diacetate	Fluorescein	490
Dehydrogenase	Tabatabai, 1994	2,3,5-Triphenyl tetrazolium chloride	1,3,5-Triphenyl tetrazolium formazan	482
β-glucosydase	Eivazi and Tabatabai, 1988	4-Nitrophenyl β-$_D$-glucopyranoside	*p*-nitrophenol	410
Protesae	Ladd and Butler, 1972	Sodium caseinate	L-Tyrosine	700
Urease	Kandeler and Gerber, 1988	Urea	NH_4^+-N	690
Phosphatase	Eivazi and Tabatabai, 1977	Sodium 4-nitrophenyl phosphate	*p*-nitrophenol	410
Arylsulphatase	Tabatabai and Bremner, 1970	Potassium 4-nitrophenyl sulfate	*p*-nitrophenol	400

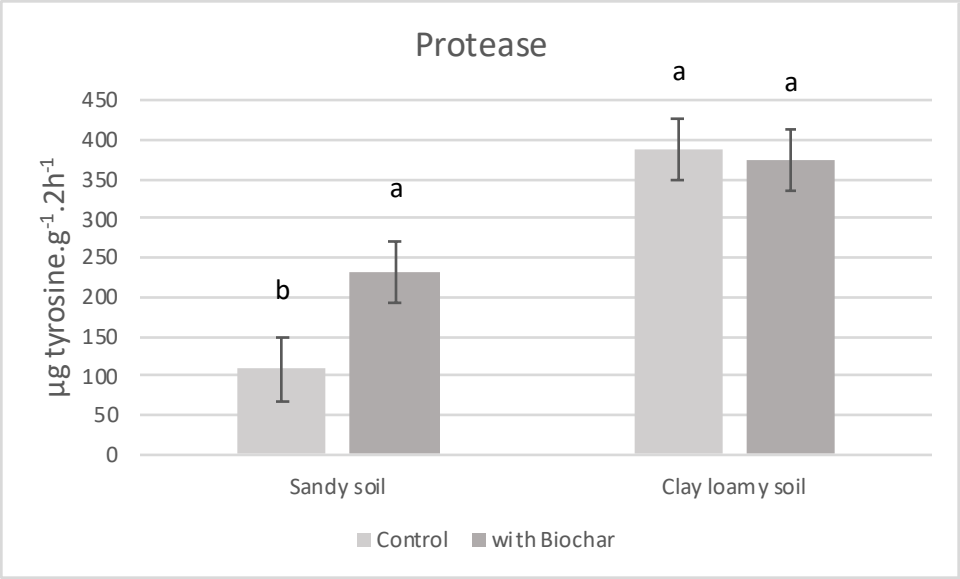

FDAase
µg fluorescein.g-1.2h-1
180
160
140
120
100
80
60
40
20
0
a
b
a
a
Sandy soil
Clay loamy soil
Control
with Biochar

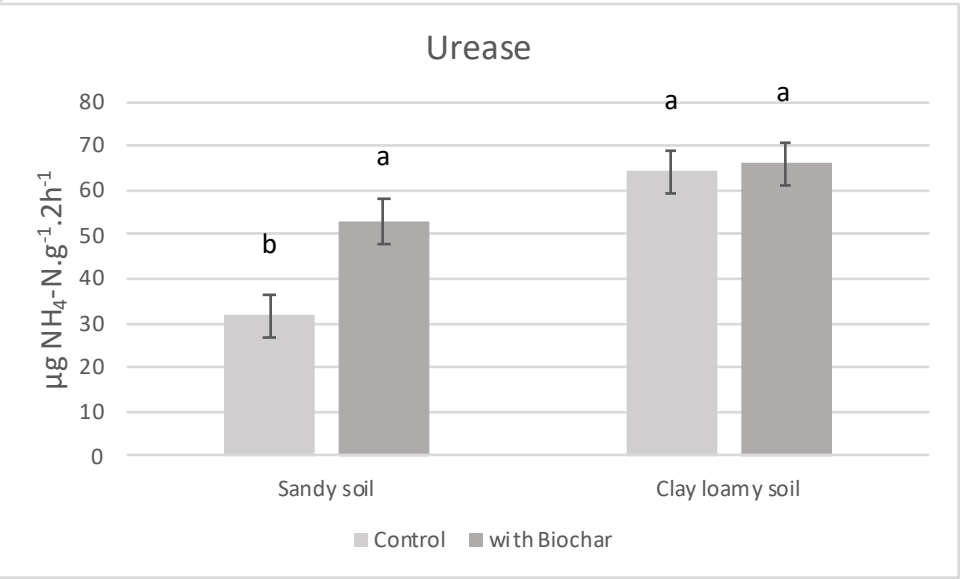

Dehydrogenase
µg TTF.g-1.h-1
3,5
3
2,5
2
1,5
1
0,5
0
a
b
a
a
Sandy soil
Clay loamy soil
Control
with Biochar


β-glucosidase
µg p-nitrophenol.g-1.h-1
120
100
80
60
40
20
0
a
a
a
a
Sandy soil
Clay loamy soil
Control
with Biochar


Phosphatase
µg p-nitrophenol.g-1.h-1
450
400
350
300
250
200
150
100
50
0
a
a
a
a
Sandy soil
Clay loamy soil
Control
with Biochar


Arylsulphatase
µg p-nitrophenol.g-1.h-1
60
50
40
30
20
10
0
a
a
a
a
Sandy soil
Clay loamy soil
Control
with Biochar


Protease
µg tyrosine.g-1.2h-1
450
400
350
300
250
200
150
100
50
0
b
a
a
a
Sandy soil
Clay loamy soil
Control
with Biochar


Urease
µg NH4-N.g-1.2h-1
80
70
60
50
40
30
20
10
0
b
a
a
a
Sandy soil
Clay loamy soil
Control
with Biochar