Residual effect of biochar on microbial biomass and enzyme activities of soil cultivated with grape varieties: a two-year field assessment

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Abstract
Biochar has received increasing attention as a soil conditioner, being known for its ability to confer numerous benefits to the soil. Derived from the thermal decomposition of organic materials under low levels of oxygen, biochar concentrates essential nutrients and carbon while having low carbon dioxide emissions. Our hypothesis is that biochar applied as a top dressing can improve soil microbial attributes even after two years in a soil cultivated with different grape varieties. To test this hypothesis, we conducted a field experiment using different doses of cashew wood biochar applied to two grape varieties and evaluated its residual effect on microbial biomass and soil enzymatic activities. The design used was a randomized block design, in a 4 x 2 factorial scheme, with five replications: four doses of biochar (0, 5, 10 and 15 ton ha⁻¹) and two grape varieties (Cabernet sauvignon and Malbec). Soil collection was carried out at a depth of 0-20 cm, to evaluate total organic carbon, microbial biomass carbon and soil enzymatic activities. Our findings revealed that the effect of grape varieties on soil biological properties was more pronounced than the biochar dose, indicating that there is a small residual effect of biochar doses after 2 years of application. Overall, our study provides important insights into the residual effect of biochar on soil microbial attributes and is considered an ecologically sustainable alternative, as it solves the problem of waste reuse and provides a disposal that brings benefits to soil properties.

Keywords: Black carbon; Vitis vinifera L.; Environmentally friendly; microbial biomass carbon.

Efeito residual do biochar na biomassa microbiana e nas atividades enzimáticas do solo cultivado com variedades de uva: uma avaliação de campo de dois anos

Resumo
Biochar tem recebido atenção crescente como condicionador, sendo conhecido por sua capacidade de conferir inúmeras vantagens ao solo. Derivado da decomposição térmica de materiais orgânicos sob baixos níveis de oxigênio, o biochar concentra nutrientes essenciais e carbono, ao mesmo tempo que apresenta baixas emissões de dióxido de carbono. Nossa hipótese é que o biochar aplicado como cobertura pode melhorar os atributos microbianos do solo mesmo depois de dois anos em um solo cultivado com diferentes variedades de uva. Para testar esta hipótese, conduzimos um experimento de campo utilizando diferentes doses de bio carbônio de madeira de caju aplicado em duas variedades de uva e avaliamos seu efeito residual na biomassa microbiana e nas atividades enzimáticas do solo. O delineamento utilizado foi o de blocos casualizado, em esquema fatorial de 4 x 2, com cinco repetições: quatro doses de biochar (0, 5, 10 e 15 ton ha⁻¹) e duas variedades de uva (Cabernet sauvignon e Malbec). A coleta do solo foi realizada na profundidade de 0-20 cm, para avaliação do carbono orgânico total, carbono da biomassa microbiana e atividades enzimáticas do solo. Nossas descobertas revelaram que o efeito das variedades de uva nas propriedades biológicas do solo foi mais pronunciado do que o efeito das doses de biochar.
Introduction

In recent years, biochar has been considered an important issue in environmental studies (Zeng et al., 2024) due to it is a tool for the management of waste. In this sense, the Brazilian agroindustry generates approximately 200 million tons of organic waste, of which only a portion is recycled. A substantial amount of this waste remains underutilized, such as sludge, soursop (Annona muricata L.), coffee, bean husk, poultry litter, and viticulture (Zeng et al., 2024; Medeiros et al., 2023; Mota et al., 2023; França et al., 2023). These residues presenting an opportunity to generate energy or produce value-added products like biochar, bringing various benefits to agriculture (Nematian et al., 2021; Patel et al., 2023; Medeiros et al., 2023).

Biochar is a carbon-rich, highly porous material produced by the thermochemical conversion of biomass through the pyrolysis of organic materials (Mota et al., 2023; França et al., 2023). Usually, biochar is described as soil amendment due to its potential to improve soil physical, chemical, and biological properties (Wang et al., 2024; Blenis et al., 2023; Medeiros et al., 2023) such as improve microbial biomass and activity, alters soil pH and nutrient content (Azadi et al., 2021; Zhou et al., 2023; Wang et al., 2023; Medeiros et al., 2023). However, there are few studies in the literature that evaluate the impact of broadcast-applied biochar on soil properties, and even fewer that examine its effects on biological properties in long term field experiments. Several studies have validated the use of biochar for increase crop productivity in tropical soils (Martins Filho et al., 2021; Medeiros et al., 2023; Da França et al., 2023). These increases results from the supply of fertility, nutrient retention, and carbon sequestration (Sakhiya et al., 2021; Medeiros et al., 2021; Hamidzadeh et al., 2023).

Biochar has been used as an indirect tool against plant diseases (Medeiros et al., 2022; Medeiros et al., 2021), modify the dynamics of heavy metals in soil (Chagas et al., 2021), and can be used as tool to accumulate carbon in the soil (Kabir et al., 2023).

Biochar applied to the soil can induce persistent residual effects that modulate soil physicochemical properties and facilitate plant growth over an extended duration (Mian et al., 2021). This includes the elevation and sustenance of heightened levels of total phosphorus, along with its organic, inorganic, and bioavailable fractions, for a minimum of two years after biochar application (Figueiredo et al., 2020). Several studies have demonstrated the residual effect of biochar to the soil by different mechanisms such as enhancement of soil buffering capacity and pH, which in turn increases the availability of silicon (Si), improvement in chemical and microbiological attributes in degraded areas (Oliveira et al., 2023), mitigation of heavy metal effects (Rafi et al., 2022), as well as the maintenance of soil fertility accompanied by an increase in crop productivity for species such as corn (Figueiredo et al., 2020) and wheat (Mian et al., 2021).

Particularly, the specific properties of the biochar and its interaction with the soil type determine the magnitude and duration of its residual effects (Moreno et al., 2022; Oliveira et al., 2023). However, the literature is incipient in elucidating the residual effect of biochar on the soil microbial attributes in long-term field experiments and it’s important to optimize its application for sustainable agricultural practices. Thus, in this study, we hypothesized that the application of biochar would change the biological properties in soil cultivated with grapes after two years of biochar application. To address this hypothesis, we assessed the absolute and specific enzyme activities of a soil that, two years ago, received different doses of biochar and was cultivated with grape varieties.

Material and methods

Production and characterization of biochar from cashew wood

Biochar was generated using cashew wood (Anacardium occidentale) wastes through the pyrolysis process (530 °C for 10–12 h under


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oxygen-limited conditions. The properties of biochar are shown in Table 1.

**Soil chemical characterization**

Soil pH was determined in H₂O (1:2.5). Soil macronutrients (phosphorus [P] and potassium [K⁺]) were extracted through the ion exchange resin and pH in CaCl₂ solution (0.01 mol L⁻¹). Soil micronutrients (Aluminum [Al³⁺], calcium [Ca²⁺], magnesium [Mg²⁺]) were extracted using KCl solution (1 mol L⁻¹), while sodium (Na⁺) was extracted by Mehlich-1 solution, while H+Al³⁺ was extracted with calcium acetate [(CH₃COO)₂Ca.H₂O] (0.5 mol L⁻¹, pH 7). The contents of micronutrients Cu, Fe, Mn, Zn were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), while B was extracted with hot water (Table 2).

**Table 1.** Chemical properties of biochar from cashew wood (*Anacardium occidentale*).

<table>
<thead>
<tr>
<th>pH</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>SB</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>B</th>
<th>Zn</th>
<th>CTC</th>
<th>C</th>
<th>N</th>
<th>C/N</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2.5</td>
<td>1.20</td>
<td>6.42</td>
<td>157.40</td>
<td>10.4</td>
<td>61.3</td>
<td>403.5</td>
<td>6.6</td>
<td>87.3</td>
<td>7.12</td>
<td>0.4</td>
<td>8.4</td>
<td>1.9</td>
<td>2.4</td>
<td>0.9</td>
<td>12.7</td>
<td>229.1</td>
</tr>
</tbody>
</table>

CEC = cation exchange capacity; OM = organic matter

**Table 2.** Chemical properties of the soil from the experiment

<table>
<thead>
<tr>
<th>Var.¹</th>
<th>pH</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>CaH₂O</th>
<th>Al</th>
<th>SB</th>
<th>P</th>
<th>Cu</th>
<th>Mn</th>
<th>B</th>
<th>Zn</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2.5</td>
<td>1.20</td>
<td>6.42</td>
<td>157.40</td>
<td>10.4</td>
<td>61.3</td>
<td>403.5</td>
<td>6.6</td>
<td>87.3</td>
<td>7.12</td>
<td>0.4</td>
<td>8.4</td>
<td>1.9</td>
<td>2.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

¹Data from the soil fertility analysis in the area of each grape variety; CS – Cabernet Sauvignon; M – Malbec; sample collected from 0-20 cm depth; pH, Ca, Mg, Na, K, Al, H+Al, P, Cu, Fe, Mn, Zn, SB - Soil Analysis Methods Manual; Embrapa; 2017.

**Field experiment design**

In 2021, a field experiment was conducted in a commercial wine grape production area from Brazilian tropical dry sub-humid of Pernambuco State, Brazil (8°56'18"S 36°31'19"W) at 730 m above sea level. The soil is classified as dystrophic Red-Yellow Argisol, with a sandy loam texture. The climate in the regions classified as “(Cs’a)” (Köppen). The average annual temperature remains about 23.2 °C and the average total annual rainfall is 782 mm.

The experiment employed a block-randomized design in a double factorial scheme: biochar doses (0.5, 10 e 20 t ha⁻¹) and grape variety (cabernet sauvignon and malbec), with four replicates. Each plot had five plants spaced 3 m x 1 m, with the biochar applied in a 0.5 m wide strip on both sides of the crop line, totaling a useful area of 4 m². The chosen concentration of biochar for the experiment is consistent with the literature. The management practices commonly employed for grape cultivation in the region were implemented.

The soil was collected at a depth of 0-20 cm, 10 cm away from the plant stem, after two years after biochar application in different doses and different grape variety. In laboratory the soil samples were sieved (2 mm) and homogenized for soil biological analysis.

**Microbial biomass carbon (MBC) and total organic Carbon (TOC)**

Microbial biomass carbon (MBC) was determined according to Mendonça and Matos (2005). Carbon was extracted from the biomass with potassium sulfate (K₂SO₄) at 0.5 mol L⁻¹. To determine MBC, the extract was mixed with distilled water, working solution and sulfuric acid (Tate et al. 1988). After 18 h, absorbance was measured by colorimetry with a wavelength of 495 nm.

The TOC was determined by the wet method in a highly acidic environment. In which the oxidation of organic carbon to CO₂ occurs by

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potassium dichromate ions (K$_2$Cr$_2$O$_7$) and Sulfuric acid (H$_2$SO$_4$).

**Absolute and specific enzyme activities**

Enzyme activities, i.e., β-glucosidase (Beta), urease (Ure), arylsulfatase (Aril) and the phosphatases (acid- aci.Phos and alkaline - alk.Phos), were determined according to standard methods. Briefly, the β-glucosidase (EC 3.2.1.21) activity was measured using p-nitrophenyl β-glucopyranoside as substrate under incubation (1 h, 37°C) in a modified buffer adjusted to pH 6.5. The p-nitrophenol form was determined spectrophotometrically at 410 nm (Eivazi and Tabatabai, 1988). The urease (EC 3.5.1.5) activity was determined using the method of Geber (1988) with urea as substrate under incubation (1h; 37°C). The specific activities of the enzymes were calculated by the ratio between enzyme activity/TOC and enzyme activity/CBM.

**Statistical analyses**

All data were analyzed using the R software v.4.2.3. Analysis of variance (ANOVA) was conducted using a completely randomized design with four treatments including two grape varieties (Cabernet and Malbec) and three replications, totaling a sample universe of 24 plots. Assumptions of normality were tested using the Shapiro-Wilk test and means of variables with significant variances were compared using Tukey’s HSD test. A significance level of 5% (α = 0.05) was adopted for all statistical tests. Principal Component Analysis (PCA) was performed with the aim of exploring correlations between variables and treatment influences, utilizing resources from the R package ‘factoextra’.

**Results**

**Residual effect of biochar on microbial biomass and enzyme activities of soil cultivated with grape varieties**

The biochar applied broadcast in different doses to soil cultivated with grape varieties did not show an effect on absolute and specific enzymatic activities, after two years of application (Table 3).

### Table 3. Statistics of the variance analysis of absolute and specific enzymatic activities of soils that received different doses of biochar two years ago.

<table>
<thead>
<tr>
<th></th>
<th>mean (µg g$^{-1}$ h$^{-1}$)</th>
<th>PD</th>
<th>VC%</th>
<th>F1: variety</th>
<th>F2: treatment</th>
<th>F1vsF2</th>
</tr>
</thead>
<tbody>
<tr>
<td>alk.Phos</td>
<td>36.5</td>
<td>18.1</td>
<td>47</td>
<td>0.069</td>
<td>0.133</td>
<td>0.790</td>
</tr>
<tr>
<td>aci.Phos</td>
<td>25.6</td>
<td>5.8</td>
<td>23</td>
<td>0.492</td>
<td>0.178</td>
<td>0.968</td>
</tr>
<tr>
<td>Beta</td>
<td>55.2</td>
<td>21.0</td>
<td>38</td>
<td>0.864</td>
<td>0.607</td>
<td>0.215</td>
</tr>
<tr>
<td>Aril</td>
<td>64.5</td>
<td>28.7</td>
<td>45</td>
<td>0.061</td>
<td>0.510</td>
<td>0.851</td>
</tr>
<tr>
<td>Ure</td>
<td>6.7</td>
<td>4.1</td>
<td>61</td>
<td>0.507</td>
<td>0.254</td>
<td>0.441</td>
</tr>
<tr>
<td>MBC</td>
<td>269.6</td>
<td>280.1</td>
<td>89</td>
<td><strong>0.020</strong></td>
<td>0.264</td>
<td>0.083</td>
</tr>
<tr>
<td>Corg</td>
<td>31.0</td>
<td>4.6</td>
<td>14</td>
<td>0.100</td>
<td>0.116</td>
<td>0.488</td>
</tr>
<tr>
<td>alk.Phos_TOC</td>
<td>0.0012</td>
<td>0.0005</td>
<td>41</td>
<td>0.100</td>
<td>0.062</td>
<td>0.825</td>
</tr>
<tr>
<td>aci.Phos_TOC</td>
<td>0.0008</td>
<td>0.0002</td>
<td>24</td>
<td>0.534</td>
<td><strong>0.017</strong></td>
<td>0.641</td>
</tr>
<tr>
<td>Beta_TOT</td>
<td>0.0018</td>
<td>0.0007</td>
<td>36</td>
<td>0.653</td>
<td>0.280</td>
<td>0.339</td>
</tr>
<tr>
<td>Aril_TOC</td>
<td>0.0021</td>
<td>0.0010</td>
<td>42</td>
<td><strong>0.008</strong></td>
<td>0.257</td>
<td>0.962</td>
</tr>
<tr>
<td>Ure_TOC</td>
<td>0.0002</td>
<td>0.0001</td>
<td>55</td>
<td>0.252</td>
<td>0.171</td>
<td>0.581</td>
</tr>
<tr>
<td>alk.Phos_MBC</td>
<td>0.381</td>
<td>0.618</td>
<td>154</td>
<td>0.097</td>
<td>0.304</td>
<td>0.357</td>
</tr>
<tr>
<td>aci.Phos_MBC</td>
<td>0.263</td>
<td>0.398</td>
<td>147</td>
<td>0.125</td>
<td>0.519</td>
<td>0.270</td>
</tr>
<tr>
<td>Beta_MBC</td>
<td>0.604</td>
<td>1.053</td>
<td>174</td>
<td>0.155</td>
<td>0.527</td>
<td>0.432</td>
</tr>
<tr>
<td>Aril_MBC</td>
<td>0.596</td>
<td>0.729</td>
<td>122</td>
<td>0.424</td>
<td>0.477</td>
<td>0.298</td>
</tr>
<tr>
<td>Ure_MBC</td>
<td>0.051</td>
<td>0.050</td>
<td>98</td>
<td>0.209</td>
<td>0.873</td>
<td>0.185</td>
</tr>
</tbody>
</table>

p-value = probability of the F test. Bold p values showed significant influence of the factor in ANOVA.

In order to assess the effect of grape variety in soil microbial properties, we compared the variables between cabernet and malbec. The comparison revealed that soils cultivated with Cabernet showed more microbial biomass C and specific arylsulfatase per unit of total organic carbon (Figure 1).

The activity of acid phosphatase per unit of total organic carbon was significantly affected by the doses of biochar applied (Figure 2). Soils that received the highest dose of biochar applied showed the lowest enzymatic activity and was the only treatment that differed from the control treatment.

**Principal component analysis**

The application of biochar to the soil cultivated with the two varieties of grape resulted in distinct clustering patterns compared to the control. However, the effect of grape varieties on soil biological properties was more pronounced than biochar dose, indicating that there is small residual effect of biochar doses after 2 years of biochar application (Figure 3A and B).

**Discussion**

This study was conducted to evaluate the residual effect of biochar applied broadcast in different doses to soil cultivated with grape on absolute and specific enzyme activities, after two years of application. In general, the results revealed no significant interaction between the biochar doses and grape varieties on the biological attributes of the soils. However, evaluating the factor independently, soils cultivated with Cabernet showed a higher amount of microbial biomass carbon and higher activity of arylsulfatase per unit of total organic carbon, compared to Malbec. This indicates that type of grape variety, specifically...
Cabernet, had a distinct impact on microbial activities, as expected due the different root exudate released. This finding is in line with recent studies that showed that different plants lead to diversity in carbon compounds at the rhizosphere that can recruit a diversity of native microbial species (Gupta et al., 2022; Costa et al., 2024; Medeiros et al., 2023). De Medeiros et al. (2023) also observed varying patterns of enzymatic activities in soils associated with different plant species. Their findings highlight the relationship between plant species, microbial recruitment, and soil enzyme activities, demonstrating how different plants can influence the microbial community and enzyme activity in the soil as in the present study.

Our results showed that the temporal impact of biochar applied to soil by broadcast method does not impact microbial biomass and absolute enzymatic activity when comparing different doses. This may be due to the biochar rising to the surface and not interacting with the soil components. Here, there was an impact from the residual effect of the biochar doses after two years of application only on the enzymatic activities of acid phosphatase per unit of total organic carbon. This result indicates that the long-term influence of biochar on soil biology can be specific, affecting some enzymatic activities while leaving others unchanged. Although absolute enzymatic activity is one of the most sensitive indicators in demonstrating changes in soil quality (Costa et al., 2024), several studies have used specific enzymatic activity per unit of microbial biomass or per unit of total organic carbon (Xiao et al., 2021).

The residual effects of biochar on acid phosphatase per unit of total organic carbon suggests that biochar alter the soil environment in a way that specifically affects the cycling of phosphorus. For example, previous studies revealed that the carbonates or oxides in biochar replace P, enhancing P retention and minimizing P losses through leaching and runoff (Yang et al., 2021; Wang et al., 2022; Hu et al., 2023). Also, the porous structure of biochar improves the conditions for soil microorganisms that promote microbiode-driven transformations of soil phosphorus (Wang et al., 2022).

The principal component analysis revealed a clear separation between control and grape varieties that validated the distinct impact of biochar applied to the different grape cultivars on soil characteristics. The type of grape variety plays a crucial role in change the soil environment, potentially due to the unique root exudates and microbial associations specific to each variety that brings different grape-associated microbiota (Chen et al., 2023). These varietal differences seem to have a more pronounced effect on the soil's biological properties, such as microbial activity and enzyme production, compared to the application of biochar.

The small influence of biochar on microbial activity in this research may be due to the method of application of biochar used, since it was applied superficially, without subsequent incorporation. There are different methods of applying biochar according to the crop exploited and the type of management adopted, and it can be applied superficially or incorporated into the soil. However, surface-applied biochar is subject to erosion losses from water and wind, acting as a sterile physical cover with little benefit to the soil (Guo, 2020). Incorporation with the soil is the most efficient method in changing chemical, physical and biological properties, reflecting on soil health (Guo, 2020; Premalatha et al. 2023).

Biochar has been used in agriculture due to its numerous benefits to the soil, such as acting as a soil conditioner, improving the physical, chemical, and biological properties of the soil (Lima et al., 2021; Silva et al., 2021). Moreover, it is used as a tool for the remediation of heavy metals and against plant diseases (Medeiros et al., 2022; Silva et al., 2022). These findings support the idea of that the residual effects of biochar on soil biological properties decrease over two years when applied broadcast, likely because it remains on the surface and fails to interact with deeper soil properties. This method of application restricts biochar's potential to influence key soil functions such as nutrient cycling, water retention, and microbial activity, leading to a minimal residual impact and preventing the full realization of biochar's benefits, including improved soil structure, enhanced microbial habitat, and increased nutrient retention.
Figure 3. Principal Component Analysis (PCA) of residual effect of biochar on microbial biomass and enzyme activities of soil cultivated with grape varieties. A) Biplot of the PCA emphasizing the associations in the space formed by the two main components; (B) Simple and cumulative relative contribution of principal components in explaining variance; (C) Contribution of each variable, ranked from left to right according to decreasing degree of importance. The dashed line indicated the expected global mean contribution, where variables above the line showed above-average importance in terms of explaining environmental variance. C=control, T1= 5, T2=10, and T3=20 t ha⁻¹.

Conclusions

In summary, our study offers new insights into the diminishing residual effects of biochar on soil biological properties over a two-year period following broadcast application. Here, we assessed the absolute and specific enzyme activities of a soil that, two years ago, received different doses of biochar and was cultivated with two grape varieties. Our data revealed that the enzymatic activities were more influenced by grape variety than biochar dose. We showed that the residual effects of biochar on soil biological properties decrease over two years when applied broadcast. Our findings provide a better understanding of residual effects of biochar applied broadcast on enzymatic activities in soil cultivated with...
Cabernet and Malbec grape varieties. Further studies should explore the beneficial potential the use of biochar incorporated into the soil and its residual effect over time.

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Declarations of interest: none

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