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Assessing the Modulatory Effects of Biochar on Soil Health Status in Response to Pesticide Application

Giovanna B. Melas [1,2], Oriol Ortiz [3,†], Amira M. Roshdy [4], Mohamed Y. Hendawi [5], Dimitrios Triantakonstantis [6,*] and Sameh Shaddad [7]

[1] Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia, Facultat de Biociències, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain; gianna_melas@yahoo.it
[2] CREAF (Centre de Recerca Ecologica i Aplicacions Forestals), 08193 Cerdanyola del Vallès, Spain
[3] Departemento di Ciencia Agrarias y del Medio Natural, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, 22071 Huesca, Spain
[4] Microbial Chemistry Department, Biotechnology Research Institute National Research Centre, 33 Bohouth Street, Dokki, Giza 12622, Egypt; amiraroshdy82@yahoo.com
[5] Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; mhendawi@gmail.com
[6] Department of Sustainable Agriculture, University of Patras, 2 Seferi Str., GR30100 Agrinio, Greece
[7] Soil Science Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; smshaddad@agri.zu.edu.eg
* Correspondence: trdimitrios@upatras.gr
† The author has passed away.

Abstract: Considering the global competition to increase food productivity due to the increasing population growth, the use of chemical pesticides has become the quick solution, but by increasing awareness about the serious dangers of wasteful chemicals in various areas of life, it has become necessary to move immediately, albeit gradually, towards safe biological treatments. From this point of view, the use of biochar is one of the trends in reducing soil pollution with chemical pesticides. Therefore, the main objectives of this work are (i) to assess if the application of three pesticides based on imidacloprid, methyl thiophanate, and glyphosate has detectable adverse consequences on soil organisms' activity and (ii) to evaluate if the addition of biochar modifies the effects of these chemicals. An agricultural soil was amended with different doses of biochar. The treated soil received realistic amounts of currently used pesticides. Samples were stored at 21 °C and 50% WHC (water holding capacity) for a period of 28 days under dark conditions. Oxygen consumption was measured for 12 consecutive hours after the addition of 2.5 g glucose kg^{-1} as a stimulant for soil organisms. Biomass C was estimated from the difference between the amount of C in 0.5 M K_2SO_4 extracts of $CHCl_3$ fumigated soil and the extractable C in non-fumigated samples. Specific respiration was computed as the amount of O_2 consumed per unit of Biomass Carbon. The results of this work proved that the tested biochar could modulate the effects produced by the agrochemicals on soil biomass.

Keywords: biochar; pesticides; soil biosystem; soil biodiversity; soil micro- and macro-organisms; soil vitality; climate change; agrochemical contamination; soil quality; mitigating effect

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1. Introduction

Pesticides are compounds used to eliminate or reduce the harmful effects of unwanted weeds, insects, or fungi. Due to the growth of the world population and the increasing need for greater quantities of food, man has resorted to the synthesis of many chemical pesticides to speed up and strengthen their effect. Synthesized pesticides are classified chemically into four types: carbamate, organophosphate, organochlorine, and pyrethroid pesticides. Undoubtedly, the spread of synthetic pesticides is closely related to the spread of many diseases, such as blood diseases, tumors, lung and skin diseases, autoimmunity, and congenital malformations. Pesticides biodegrade in the soil in two ways: by their interaction with soil organisms or by the organisms' metabolism. However, its metabolism as a carbon source for energy has many risks to the organisms that metabolize pesticides as it lacks many important elements for the functions of the organism and strongly affects growth and reproduction [1]. In this regard, studies have indicated the role of biochar in reducing the bioavailability of pesticides for microbial decomposition [2]. Some pesticides, such as chlorinated derivatives, are very toxic and persistent compounds in the environment; for this reason, most of them were banned decades ago, but they can still be found in the soil [3].

Biochar is a stable, porous, carbonate-based solid that is produced through the thermochemical transformation of biomass in the presence of a limited amount of oxygen. It is characterized by its high carbon content, high cation exchange capacity, porosity, stability, abundance of surface aggregates, low cost, environmental friendliness, and soil amendment. It can be used alone or as an additive to improve soil efficiency and avoid some environmental pollutants. It is effective for soil bioremediation and reducing heat emission and climate change [4].

Further, biochar has been shown to aid in stabilizing and restoring soil organic matter levels [5]. Several authors have evaluated the adsorbent characteristics of biochar and the possibility of using it as a way to block the toxic effects of certain substances or pollutants in the soil [6]. Thus, biochar can be useful in the remediation of pesticide-contaminated soil because of its capacity to interact with some functional groups of these agrochemicals [7]. Moreover, biochar makes the xenobiotics present in soils and sediments less available to organisms and hinders their off-site transport into receiving environments [1].

Currently, 220,000 tons of agrochemicals, basically fungicides, herbicides, insecticides, and growth regulators, have been released into the European environment since 2012 [3]. It must be hypothesized that if a pesticide can reach the soil surface, it could be partially immobilized by the biochar present in it, reducing its mobility/activity and the possible adverse effects on soil microbiota. The interaction of biochar to break down pesticides depends on its chemical and physical properties, in addition to the biodiversity of soil micro- and macro-organisms with their metabolites, to obtain the best response against pollutants [2].

Therefore, the main objectives of this work are (i) to assess if the application of three pesticides based on imidacloprid, methyl thiophanate, and glyphosate has detectable adverse effects on soil biodiversity and (ii) to evaluate if the addition of biochar modifies the toxicity or adverse effects of these chemicals in soil. The selection of these three pesticides is due to their diverse mechanisms of action, which represent various classes of pesticides and allow for the study of a broader range of potential impacts on soil biodiversity. These pesticides are also widely used worldwide.

2. Materials and Methods

2.1. Soil Sampling

The surface Ap horizon (0–30 cm) of an agricultural soil (*Fluventic Haploxerept*, SSS 2010) from the experimental farm of Torre Marimon (Catalonia, NE Spain) was selected to be amended in the greenhouse with 0, 1.9, and 11.5 g kg^{-1} of biochar (Catalonia, Spain), which roughly correspond to agronomic contributions of 0, 5 and 30 Mg ha^{-1}. The samples amended with these three different dosages of biochar received realistic amounts of currently used pesticides. The soil was sieved to 2 mm and brought to 40% of its water holding capacity (WHC) before the addition of biochar and pesticides. Table 1 shows the main physical and chemical parameters of the unamended soil used in this study.

Table 1. Main characteristics of the unamended soil used in this work.

Parameter	Units	Value
Clay (<0.002 mm)	g kg^{-1}	174
Fine silt (0.002–0.02 mm)	g kg^{-1}	125
Coarse silt (0.02–0.05 mm)	g kg^{-1}	105
Sand (0.05–2 mm)	g kg^{-1}	596
pH (H_2O) 1:2.5 *w:v*	——————	8.3
E.C. 25 °C (1:5 *w:v*)	dS m^{-1}	0.21
Organic matter (dichromate oxidation)	g kg^{-1}	16.0
$CaCO_3$ equiv.	g kg^{-1}	60.0
N (Kjeldahl)	g kg^{-1}	0.8
P (Olsen)	mg kg^{-1}	27.0
K (NH_4Ac extract)	mg kg^{-1}	159
Ca (NH_4Ac extract)	mg kg^{-1}	5557
Mg (NH_4Ac extract)	mg kg^{-1}	233
Na (NH_4Ac extract)	mg kg^{-1}	62
Cd (acid digestion)	mg kg^{-1}	<0.5
Cu (acid digestion)	mg kg^{-1}	17
Ni (acid digestion)	mg kg^{-1}	7
Pb (acid digestion)	mg kg^{-1}	25
Zn (acid digestion)	mg kg^{-1}	65
Cr (acid digestion)	mg kg^{-1}	10
Hg (acid digestion)	µg kg^{-1}	<40

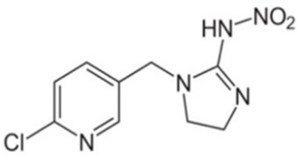
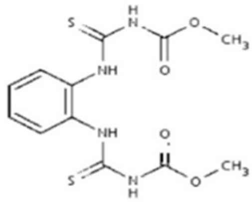
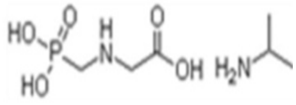
2.2. Biochar Preparation

The biochar was obtained by the slow pyrolysis of pine wood biomass and supplied by the "Grupo de Ingeniería Química y Ambiental del Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad" of the Universidad de León (León, Spain). Pine chips were charred for approximately 15 min at a pyrolysis temperature ranging from 500 °C to 550 °C. The obtained biochar had a concentration of 862 g kg^{-1} of total carbon (elemental C).

2.3. Pesticide Selection

The pesticides were selected from the list established for use in Europe according to the Directive 91/414/EEC. The application rates of the three pesticides have been chosen from the doses suggested by the manufacturers (Table 2), assuming that all the products could be distributed and concentrated in the first millimeters of the soil surface, thus considering that the amount of treated soil was 2600 kg ha^{-1}. The three pesticides have been incorporated into the soil at doses 50% higher than those recommended by the manufacturer.

Table 2. Recommended and provided doses of the three selected pesticides on the experimental biochar-amended soil.

	Insecticide	Fungicide	Herbicide
Commercial name	Confidor, Bayer	Pelt, Bayer	Logrado, Mass
Active principle	Active Imidacloprid principle	Thiophanate methyl	Glyphosate, Mono Isopropylamine salt solution
Recommended dose	0.65 L ha^{-1} [potatoes] (0.25 mL kg^{-1})	1.7 L ha^{-1} [cereal] (0.65 mL kg^{-1})	4.5 L ha^{-1} [general use] (1.73 mL kg^{-1})
Provided dose	0.38 mL kg^{-1}	0.98 mL kg^{-1}	2.60 mL kg^{-1}
Molecular structure	 $C_9H_{10}ClN_5O_2$	 $C_{12}H_{14}N_4O_4S_2$	 $C_6H_{17}N_2O_5P$
CAS number	138261-41-3	23564-05-8	38641-94-0
Octanol:water partition coefficient (log K_{ow})	0.57	1.4	−3.2
Water solubility	0.61 g L^{-1} at 20 °C	24.6 mg L^{-1} at 25 °C	12 g L^{-1} at 25 °C
Reported half-life in soil	40–124 d [8]	<60 d [9]	2–197 d [10]

2.4. Pesticides Application

A distilled water solution/emulsion of the pesticides was applied by irrigating the surface of the soil placed in 5 cm depth trays to reach the desired concentration of these agrochemicals. Then, the soil was mixed to ensure the homogeneous distribution of the products. Three replicates of each treatment were separately prepared, and treated soils were transferred to polyethylene containers, analyzed, and stored at 21 °C and 50% WHC for a period of 28 days under dark conditions.

2.5. Microbial Estimation

2.5.1. Microbial Biomass C Estimation

Substrate-induced respiration [11] was measured after 6 and 12 h, and after 28 days of the addition of the pesticides. Oxygen consumption [12] was measured for 12 consecutive hours after 2.5 g glucose kg^{-1} was added as a microbial activator. Microbial biomass C was estimated from the difference between the amount of C in 0.5 M K_2SO_4 extracts of $CHCl_3$ fumigated soil and the extractable C in non-fumigated samples [13]. These measurements were made at 12 h and 28 d after the addition of the pesticides.

2.5.2. Microbial O_2 Consumption Estimation

Specific respiration was calculated as the amount of O_2 consumed per unit of microbial C. The specific respiration at 6 h was calculated from the ratio between O_2 consumption measured at 6 h and the measure of microbial biomass at 12 h.

The experimental combinations between soil, biochar, and selected pesticides are shown in Table 3.

Table 3. Experimental design to evaluate the possible modulation effect of the biochar towards the three pesticides added to the soil.

Biochar Dose in Soil (g kg^{-1})	Insecticide (Confidor) (mL kg^{-1})	Fungicide (Pelt) (mL kg^{-1})	Herbicide (Logrado) (mL kg^{-1})	Code
0	0	0	0	B0 I–F–H–
1.9	0	0	0	B1 I–F–H–
11.5	0	0	0	B2 I–F–H–
0	0.38	0	0	B0 I + F–H–
1.9	0.38	0	0	B1 I + F–H–
1 1.5	0.38	0	0	B2 I + F–H–
0	0	0.98	0	B0 I–F + H–
1.9	0	0.98	0	B1 I–F + H–
11.5	0	0.98	0	B2 I–F–H+
0	0	0	2.60	B0 I–F–H+
1.9	0	0	2.60	B1 I–F–H+
11.5	0	0	2.60	B2 I–F–H+

2.6. Statistics

Statistical analyses were carried out using Statview software 5.0. The effects of the addition of biochar on the microbiological properties have been tested by a one-way ANOVA, comparing data of three doses of biochar-treated soil (three levels) with control soil (soil not treated with pesticides or biochar). The effects of the addition of the selected pesticides in the soil, amended or not with biochar, have been analyzed for each agrochemical by two-way ANOVA biochar (3 doses) and pesticide (yes/no).

3. Results

3.1. Biochar Effects

The interference effect of adding biochar to the soil on each of the fungicides, insecticides, and herbicides is illustrated in the three Figures (Figures 1–3). The addition of biochar does not increase the O_2 consumption after 6 h (F = 1.673, p = 0.2548), 12 h (F = 2.467, p = 0.1545), or at 28 days (F = 2.332, p = 0.1781) after its addition. By contrast, the higher dose of biochar causes remarkable effects on the biomass, which increases after 12 h of the addition (F = 65.418, $p < 0.0001$) and decreases 28 days later (F = 60.331, $p < 0.0001$). Therefore, biochar causes a small reduction of the specific respiration activity at 6 and 12 h (F = 12.600, p = 0.0048, F = 52.209, $p < 0.0001$, respectively) but a significant increase at 28 days (F = 45.711, p = 0.002).

3.2. Insecticide Effects

As shown in Figure 1, at 6 h of incubation, O_2 consumption was not affected by the addition of the insecticide Imidacloprid (F = 2.854, p = 0.1118), with no significant interaction with the presence of biochar (F = 2.275, p = 0.1371). By contrast, a moderate inhibitory effect of insecticide appears at 12 h and was greater at 28 days (F = 11.127, p = 0.0042 and F = 75.003, $p < 0.0001$, respectively).

Twelve hours after the addition of insecticide, there was a significant increase in the biomass (F = 39.311, $p < 0.0001$), but only in soils not treated with biochar or amended with its lower dose (F = 23.180, $p < 0.0001$). After 28 days of incubation, the insecticide caused a decrease in the biomass in soil not treated with biochar, while it generated an increase in that amended (F = 49.819, $p < 0.0001$), especially for the lower dose of biochar (F = 145.170, $p < 0.0001$).

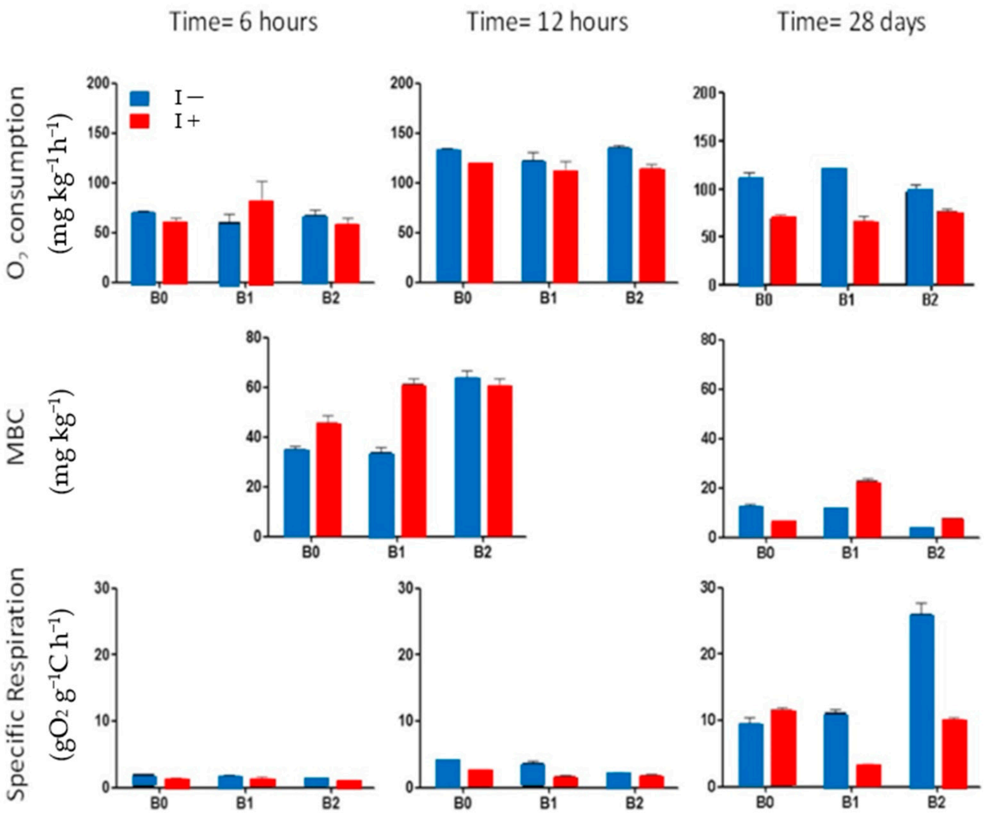


Figure 1. Insecticide effects (treatments I+, I−/F− H−). Average values of O_2 consumption (upper row), microbial biomass-C (middle row), and specific consumption of O_2 (bottom row) along the time since the insecticide was added: 6 h (left column), 12 h (middle column), and 28 days (right column). Within the graphs, blue bars correspond to the soil without insecticide, while red bars indicate the results of the treated soil. B0: soil without biochar; B1 and B2: soil amended with biochar (1.9 and 11.5 g kg^{-1}, respectively).

Specific respiration calculated after 6 h of incubation was very low in samples treated with insecticide (F = 41.958, $p < 0.0001$) and in the respective controls, with a significant interaction with biochar (F = 5.086, $p = 0.0195$). The inhibitory effect of the insecticide persisted at 12 h (F = 111.514, $p < 0.0001$). After 28 d of incubation, a moderate increase of specific respiration was observed for samples not treated with biochar and a big decrease for the others (F = 84.453, $p < 0.0001$) with a clear interaction with biochar (F = 145.170, $p < 0.0001$).

3.3. Fungicide Effects

Figure 2 illustrates the effects of the addition of the fungicide Thiophanate. It caused a slight increase in soil O_2 consumption at 6 h of incubation (F = 5.468, $p = 0.0360$), most visible at 12 h (F = 18.257, $p = 0.0009$) and at 28 days (F = 8.206, $p = 0.0118$), not depending on biochar dose (F = 0.684, $p = 0.5218$; F = 1.823, $p = 0.2005$; F = 2.892, $p = 0.0866$, respectively). Microbial biomass was practically not affected by the incorporation of fungicide 12 h after its addition (F = 2.099, $p = 0.1656$) and presented a sharp increase at 28 days after the treatment (F = 273.177, $p < 0.0001$).

Regarding the specific respiration, no effects were observed during the first 6 or 12 h of incubation, but a strong decrease was found after 28 days (F = 138.549, $p < 0.0001$). This decrement was dependent on the dose of biochar (F = 45.995, $p < 0.0001$), being greater as the highest biochar dose was added.

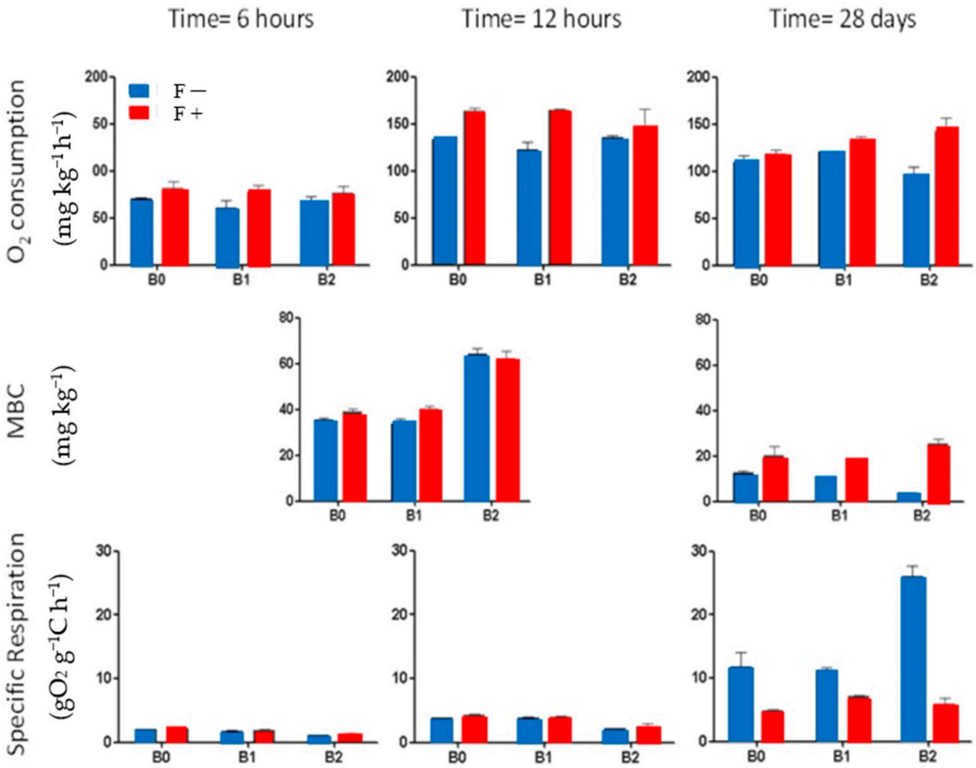


Figure 2. Fungicide effects (treatments I−F+/− H−). Average values of O_2 consumption (upper row), microbial biomass-C (middle row), and specific consumption of O_2 (bottom row) along the time since the fungicide was added: 6 h (left column), 12 h (middle column), and 28 days (right column). Within the graphs, blue bars correspond to the soil without fungicide, while red bars indicate the results of the treated soil. B0: soil without biochar; B1 and B2: soil amended with biochar (1.9 and 11.5 g kg^{-1}, respectively).

3.4. Herbicide Effects

The addition of the herbicide Glyphosate (Figure 3) did not generate a global effect on O_2 consumption at 6 h, 12 h, and 28 d of incubation (F = 0.633, p = 0.4395; F = 0.175, p = 0.6818; and F = 0.707, p = 0.4137, in that order).

Nevertheless, biomass was strongly affected by the addition of the herbicide, displaying an increasing or decreasing trend depending on the interaction with the dose of biochar (F = 7.091, p = 0.0054 and F = 293.637, p < 0.0001, at 12 h and 28 d, respectively). However, the effects of herbicide vary remarkably throughout incubation. At 12 h, the inhibitory effect was detected in the soil treated with the higher dose of biochar, while larger amounts of biomass C were found in the untreated soil. On the contrary, these effects were opposed at 28 d.

The specific respiration was low at short incubation times (6 and 12 h) but still showed marked effects of the addition of the herbicide. At 6 and 12 h (F = 7.456, p = 0.0163 and F = 25.554, p = 0.0002, respectively), the herbicide reduced the specific respiration of the soil not amended with biochar, had no noticeable effect in the soil amended with the lowest dose, and slightly increased on the soil amended with the highest dose (F = 22.086, p = 0.0003). After 28 days of incubation, the effect of the herbicide was clearly reflected in an increase of specific respiration of the unamended soil, but concerning the soil treated with biochar, the addition of the herbicide decreased the specific respiration at the B1 and B2 doses of biochar, respectively (F = 121.643, p < 0.0001).

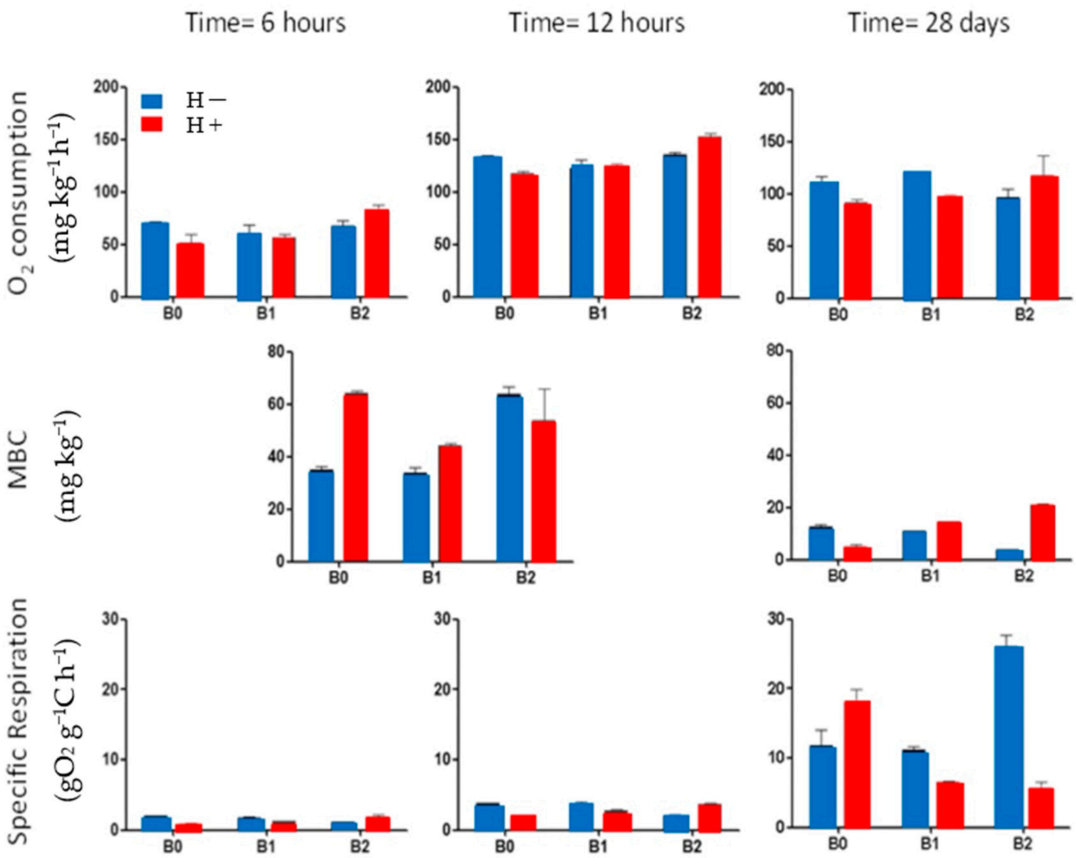


Figure 3. Herbicide effects, treatments I− F− H+/−. Average values of O_2 consumption (upper row), microbial biomass-C (middle row), and specific consumption of O_2 (bottom row) along the time since the herbicide was added: 6 h (left column), 12 h (middle column), and 28 days (right column). Within the graphs, blue bars correspond to the soil without herbicide, while red bars indicate the results of the treated soil. B0: soil without biochar; B1 and B2: soil amended with biochar (1.9 and 11.5 g kg^{-1}, respectively).

4. Discussion

4.1. Influence of Biochar on Soil Microbial Indicators

The addition of biochar represents a carbon source that may be partially available for soil microorganisms [2,14–18], although no noticeable changes in soil O_2 consumption have been found in the present work after this amendment. The mineralization of biochar has been extensively described as a slow process that mainly affects its most labile fraction [19,20], so it could not be easily detected by overall respirometric measurements due to the bigger fluxes of gases (CO_2 and O_2) caused by the mineralization of the native soil organic matter [21], particularly after the addition of glucose. Nevertheless, the addition of the highest dose of biochar caused a notable increase in the amount of microbial biomass-C in the short term. Although remarkable improvement of the soil attributes has been described as a consequence of the addition of biochar [22–24], this change has been produced so quickly, suggesting that some amount of microbial C was colonizing the partially charred biomass, even before its addition to the soil. Indeed, biochar is a porous, nutrient-sorbent, and C-rich material suitable for the colonization of a large variety of microorganisms [25].

Computed in this paper is the ratio between the O_2 consumption and the microbial C, and specific respiration has been applied as an indicator of the C use efficiency of the soil microorganisms. Therefore, the slightly lower values of specific respiration found after 6 and 12 h of the addition of the biochar can be explained by (i) a higher C use efficiency of the soil microorganisms after the addition of biochar [26], or that (ii) the microbial biomass provided with the biochar was more efficient metabolizing C substrate than the soil microbial biomass.

The significant reduction of microbial biomass along the incubation period suggests a progressive depletion of the most labile C sources, which probably led to a selection of microorganisms able to use more stable C sources [27]. Labile organic matter depletion by mineralization and/or protection by biochar sorption [28–31] probably reduce the C use efficiency of soil microorganisms, thus increasing the specific respiration over time.

4.2. Insecticide Effects

The reduction of O_2 consumption 12 h after the addition of the insecticide and over time suggests an adverse effect on the metabolism of the soil microorganisms. Nevertheless, our results did not indicate that the insecticide caused a significant lethal effect on soil microbiota, as the amount of microbial biomass did not change or even increase immediately after the addition of the insecticide. After 28 days of incubation, microbial biomass decreased in soil not treated with biochar while it increased in the others.

Consequently, the specific respiration of the samples containing biochar was lower than that of non-treated ones, suggesting that biochar had a blocking effect on the functional toxicity of the pesticide, but this effect is more evident in the low dose of biochar. Several authors have proposed that biochar could adsorb some pesticides and then partially modulate their adverse effects on soil microbiota [3,32].

4.3. Fungicide Effects

Unlike what was observed in the case of insecticide, the enhancement of O_2 consumption 6 h and 12 h after the addition of the fungicide suggests that the addition of this agrochemical does not produce any adverse effect and can be used as a mineralizable substrate, therefore as an energy source, by soil microorganisms [33]. Moreover, after 28 d of incubation, the consumption of O_2 recovered to normal values; this may be due to the progressive consumption of the chemical added, as suggested by its known half-life in soil [34].

Fungicide does not cause lethal effects on soil microbial biomass (probably dominated by bacteria), which remains stable after 6 and 12 h of incubation and increases after 28 days. This growth indicates that the microbial community found equilibrium in the latest period of incubation, as suggested by the decrease of the specific respiration rate.

The modulatory effect of biochar on the soil response to the addition of the fungicide appears noticeable only at the end of the incubation. At this time, all fungicide-treated soils are showing microbial biomass values greater than their respective controls, but this difference is maximal in the case of soils that received the highest dose of biochar. This is probably due to the capacity of biochar to block labile organic matter [31,35].

4.4. Herbicide Effects

No significant changes in O_2 consumption were observed because of the addition of herbicide during the entire period of soil incubation. On the contrary, an irregular pattern of microbial growth was detected at 12 h, which increased in soil not amended with biochar B0, remained stable in B1, and decreased in B2 treated soil. This result suggests that, in the short term, herbicide modifies the microbial population. The presence of biochar modulates this effect. After 28 days, it is possible to observe the opposite situation of microbial pattern, suggesting the beneficial effects of biochar to enhance microbial biomass. This result clearly demonstrates the ability of biochar to modulate the effects of this herbicide on soil microbial biomass [36,37]. As the immobilization of the pesticide on the biochar surface needs some time to be produced [38], this effect appeared after 28 days of incubation but not in the early stages.

Finally, the results of this work suggest that the expected protective effect of biochar against harmful pesticide actions is not detectable in the early stage of incubation, but it increases over time. High doses of biochar best perform this task. A modulation of the effects produced by the agrochemicals tested due to the presence of pine wood biochar, proceeded by slow pyrolysis, has been proved. Biochar demonstrates significant potential in mitigating pesticide effects on soil microbial communities through its interactions with agrochemicals. It serves as a carbon source for microorganisms and enhances their resilience against insecticides, fungicides, and herbicides. By adsorbing certain pesticides, biochar reduces their toxicity, promoting increased microbial biomass even in the presence of stress from insecticides. It also supports microbial growth using fungicides as energy sources and leads to greater microbial biomass over time with herbicide exposure. This ability to enhance soil microbial health suggests that biochar is a valuable tool for sustainable agriculture, improving soil quality and reducing ecological risks linked to agrochemical use.

5. Conclusions

This study demonstrates that biochar can significantly modulate the adverse effects of commonly used pesticides on soil health, specifically microbial activity and diversity. The application of imidacloprid, methyl thiophanate, and glyphosate revealed deleterious impacts on soil microorganisms; however, the incorporation of biochar offered a mitigating effect. While the initial application of pesticides led to a decrease in microbial biomass and respiratory activity, biochar enhanced microbial resilience over time and supported microbial growth by serving as a valuable carbon source. The findings further demonstrate the capacity of biochar to adsorb and immobilize pesticides, thereby reducing their toxicity within the soil environment and fostering the growth of a robust and healthy microbial community. These findings underscore the potential role of biochar as a sustainable amendment in agricultural practices to improve soil quality and reduce the environmental risks associated with agrochemical use. The research underscores the necessity of integrating biochar into management practices to foster sustainable agriculture while ensuring the protection of vital soil ecosystems.

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