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Influence of Acidified Biochar on CO_2–C Efflux and Micronutrient Availability in an Alkaline Sandy Soil

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Abstract: Biochar, an alkaline carbonaceous substance resulting from the thermal pyrolysis of biomass, reportedly enhances the micronutrient availability in acidic soils with little or no effect on alkaline soils. In this study, biochars were produced from poultry manure (PM) at 350 °C and 550 °C (BC350 and BC550 respectively). The acidified biochars (ABC350 and ABC550, respectively) were incorporated into an alkaline sandy soil, and their effects on the soil micronutrients (Cu, Fe, Mn and Zn) availability, and CO_2–C efflux were investigated in a 30-day incubation study. The treatments (PM, BC350, BC550, ABC350, and ABC550) were administered in triplicate to 100 g soil at 0%, 1%, and 3% (*w*/*w*). Relative to the poultry manure treatment, acidification drastically reduced the pH of BC350 and BC550 by 3.13 and 4.28 units, respectively, and increased the micronutrient availability of the studied soil. Furthermore, the biochars (both non-acidified and acidified) reduced the CO_2 emission compared to that of the poultry manure treatment. After 1% treatment with BC550 and ABC550, the CO_2 emissions from the soil were 89.6% and 91.4% lower, respectively, than in the 1% poultry manure treatment. In summary, acidified biochar improved the micronutrient availability in alkaline soil, and when produced at higher temperature, can mitigate the CO_2 emissions of soil carbon sequestration.

Keywords: biochar; alkaline sandy soil; acidified biochar; CO_2–C efflux; micronutrients

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1. Introduction

Biochar is a carbonaceous substance resulting from biomass pyrolysis under low- or no-oxygen conditions. In recent times, biochar has been widely used as a soil additive, and its applicability to C sequestration by mitigating CO_2–C emissions has also been seriously considered [1]. Biochar derived from biomass can potentially sequester carbon without contributing to climate change. As a soil additive, it might improve the soil quality for the enhanced growth and development of crops [2]. Studies have shown that biochar can improve the chemical, physical and biological properties of soil [3]. Besides carbon sequestration [4], biochar-incorporated soil improves soil fertility [4,5] by improving the crop water and efficiency of plant nutrient uptake [6], and by retaining the nutrients required by plants [7]. The crop yield is then increased [8]. An estimated 1.8–9.5 Pg of annually released carbon dioxide is reportedly sequestered by biochar [9]. Nevertheless, biochar stability in the soil is necessary for long-term carbon sequestering [10]. Depending on its interaction with the microorganisms and organic matter in the soil, biochar can either sink or source carbon [11].

Greenhouse gases, such as CO_2 release to the atmosphere have a profound effect on global warming. A possible way of mitigating greenhouse gas emission especially CO_2 is the use of biochar which has the ability to capture C [12]. According to Weng et al. (2017), the rhizodeposits which is absorbed by biochar and enzymes in the soil hinder the activities of soil microorganism responsible for the degradation of C, hence mitigate CO_2 emission [13]. Abagandura et al. (2019) reported a reduction in CO_2 emission following

the addition of biochar and manure at the rate of 10 Mg ha^{-1} [14]. However, according to their study, soil texture played a role in the extent of CO_2 mitigation following biochar application as CO_2 was mitigated in sandy loam soil while CO_2 was not mitigated in clay loam soil. Also, Yang et al. (2020) investigated the influence of biochar on CO_2 emission in a two-year field experiment, biochar was found to reduce CO_2 emission by 18–25% and 19–41% in the first and second growing seasons, respectively [15]. Furthermore, cumulative CO_2 emissions were reduced by 20% and 24% following the addition of banana peel biochar at 1% and 2%, respectively to the soil [16].

In alkaline soils, fixation of micronutrients (with the exception of molybdenum) lowers the availability of micronutrients for plants. Introducing biochar as a soil additive can improve the micronutrient availability, but biochar studies on micronutrient availability have obtained conflicting results. In one study, biochar derived from poultry manure (PM) increased the availability of Cu, Zn, and Mn, but reduced the amount of plant-available Fe [17]. Contrarily, after incorporating *Conocarpus* wood-waste biochar in calcareous soil and incubating the sample for 90 days, El-Naggar et al. (2015) reported a decrease in all plant-available micronutrients (Fe, Zn, and Mn) except Cu.

Most biochars, especially those produced at higher temperatures, are alkaline. The biochar pH increases with increasing temperature of pyrolysis [18–20]. As a soil additive, biochar increases the pH of acidic soils [21–23] and some alkaline soils [24]. Owing to its inherently alkaline nature, biochar does not always alter the pH of alkaline soil [7,25]. Acidification can bring the pH of biochar into the acidic region, thereby reducing the pH of alkaline soil when incorporated as a soil additive. If acidified biochar can reduce the pH of alkaline soil to near-neutral, it could overcome the micronutrient fixation that challenges plant growth in alkaline or calcareous soils. Hence, the current research investigates the effects of acidified biochar from PM on (i) CO_2–C efflux, (ii) changes in some chemical properties of alkaline soil, and (iii) availability of micronutrients (Cu, Fe, Mn, and Zn) in alkaline soil.

2. Materials and Methods

2.1. Feedstocks, Production of Biochar, and Acidification

The biochar was produced by pyrolyzing PM feedstock at 350 °C or 550 °C. The produced biochar was ground, sieved through a 53-µm mesh, and stored in an airtight container. The biochars produced at 350 °C and 550 °C were labeled as BC350 and BC550, respectively (where "BC" denotes biochar, and the number is the pyrolysis temperature). The biochar was then acidified by shaking in 0.5 N HCl for 45 min. Here the biochar: liquid ratio was 1:10. The suspension was stood for 24 h, then filtered through Whatman 42 filter paper. The biochar collected on the filter paper was oven-dried at 65 °C for 48 h and then stored in an airtight container. The acidified biochars produced at 350 °C and 550 °C were tagged as ABC350 and ABC550, respectively.

2.2. Soil, Poultry Manure, and Biochar Characterization

The experimental soil was gathered from the agricultural farm at King Saud University located in the kingdom of Saudi Arabia. In preparation for analyzing their physical and chemical properties, the collected samples were dried in air, crushed, and filtered through a 2-mm sieve. The physicochemical properties of the soil were characterized by standard methods [26], and the texture was characterized using the hydrometer technique [27]. The textural group of the soil was determined from the soil textural triangle described by the United States Department of Agriculture [28]. The soil pH and electrical conductivity (EC) were measured with a pH and EC meter, respectively, in a soil:water mixture of 1.0:2.5. The soil organic matter (SOM) was analyzed [29], and the calcium carbonate ($CaCO_3$) in the experimental soil was measured with a calcimeter. The concentrations of the available forms of micronutrients (Fe, Mn, Zn, Cu), were determined in an inductively coupled plasma (ICP) (Perkin Elmer Optima 4300 DV ICP-OES, USA) with ammonium bicarbonate diethylenetriaminepentaacetic acid (AB-DTPA) as the extracting solution [30]. Flame

photometer was used to measure K while P was determined using the color method in a spectrophotometer after being extracted by AB-DTPA solution [31]. To determine the total contents of micronutrients in the soil sample, the sample was digested following the Hossner method [32] and the solution was read with ICP. The saturation percentage of the experimental soil was determined by measuring a known weight of soil, saturating the sample with water, and re-weighing after saturation. The soil was placed in the oven and dried at 105 °C until its weight remained constant. The percentage saturation was then calculated by Equation (1):

$$SP = \frac{loss\ in\ weight}{oven - dried\ soil\ weight} \times 100\% \quad (1)$$

Proximate Analysis of Biochar

The biochars were subjected to a proximate analysis of their yields, moisture contents, volatile matters, and ash contents. The proximate analysis method followed the ASTM E872-82 standard [33]. To obtain the biochar yield, the biochar weight was divided by the biomass weight. The moisture content was measured by heating the biochar at 105 °C for 24 h. The volatile matter was measured by heating the materials (in covered crucibles) at 450 °C for 30 min, and the ash content was measured by heating the produced biochars (in open crucibles) at 750 °C for 30 min. The difference between 100% and the summed percentages of moisture content, ash content, and volatile matters computed the resident matter (representing the fixed carbon). All the measured soil and biochar properties were computed in Table 1.

Table 1. Properties of Soil and Biochars.

	Sand	**Silt**	**Clay**	**Textural Class**	**pH (1:2.5)**	**EC (dS m^{-1})**	**SOM**	**$CaCO_3$**	**Cu**	**Fe**	**Mn**	**Zn**
		%					**%**		**AB-DTPA (mg kg^{-1})**			
Soil	95.62	1.25	3.13	Sandy soil	8.20	0.15	0.39	18.00	0.00	0.56	0.00	0.00
	pH (1:10)	EC (ds m^{-1})	Yield	Moisture	Volatile matter	Ash	Fixed carbon	Total P	Total Cu	Total Fe	Total Mn	Total Zn
			%						%			
PM	7.54	8.20	-	8.94	47.05	22.71	21.30	2746.38	14.80	1439.00	249.80	272.03
BC350	8.83	2.77	53.27	0.98	16.19	42.28	40.55	2765.45	3.20	1587.00	404.55	404.08
BC550	10.97	1.11	38.96	0.44	3.12	37.88	58.55	3660.35	1.45	1972.25	611.00	569.75
ABC350	5.70	6.50	-	1.12	27.19	50.08	21.61	2666.90	8.00	2364.00	356.20	602.00
ABC550	6.69	0.75	-	1.17	4.22	49.09	45.52	3588.82	3.23	2874.50	761.50	911.25

2.3. Incubation Experiment

The influences of acidified biochar on the CO_2–C efflux and micronutrient availability were investigated in a 30-day incubation study. One hundred grams of the prepared alkaline sandy soil were placed in 250-mL glass vessels. For simplicity, the treatments with 0%, 1%, and 3% (w/w) of PM, BC350, BC550, ABC350, and ABC550 were denoted by the treatment type followed by the added amount in parentheses: PM(1%), PM(3%), BC350(1%), BC350(3%), BC550(1%), BC550(3%), ABC350(1%), ABC350(3%), ABC550(1%), ABC550(3%). All treatments were homogeneously applied to the glass vessels containing the experimental soil. The untreated soil (0% w/w treatment) was the control (CK) sample. All treatments were replicated 18 times where each sampling period had 3 replicates of each treatment. Deionized water was added to the treated and untreated soils to a field capacity of 80%. Each treatment was incubated at 30 °C. At 0, 1, 3, 7, 15, and 30 days, three replicates of each treatment were collected from the incubator, and their plant-available micronutrients (Mn, Fe, Cu, and Zn), EC, and pH were analyzed. The CO_2–C efflux was captured and measured at 1, 3, 7, 10, 15, 20, 25, and 30 days. To determine the CO_2–C efflux, the evolved CO_2–C was collected into small vials containing 5 mL of 1.0 M NaOH solution.

The NaOH solution in the vials was replaced at each sampling interval. The soil chemical properties were measured as described earlier. The excess CO_2 evolved and trapped was titrated against 0.1 M HCl after adding a few drops of $BaCl_2$ solution. The CO_2–C efflux rate (in mg C g^{-1} soil day^{-1}) and the cumulative CO_2–C efflux (in g kg^{-1} soil) were then calculated [34,35].

2.4. Statistical Analysis

The collected data were subjected to analysis of variance using Statistica software. The means of the treatments were separated using the least significant difference (LSD) at the 5% probability level.

3. Results and Discussion

3.1. Effect of Acidified Biochar and Incubation Periods on the pH and EC Dynamics

Table 2 lists the pH changes in the soils after applying different treatments (PM, BC, and ABC) at different amounts at each incubation time. At the beginning of the incubation experiment (Day 0), the pH values of the soils treated with ABC350(1%), ABC350(3%), ABC550(1%), and ABC550(3%) were significantly lower than the control pH ($p > 0.05$). The PM(1%) and PM(3%) treatments also significantly decreased the soil pH from the control pH ($p > 0.05$), but the BC350(1%), BC350(3%), BC550(1%), and BC550(3%) treatments significantly increased the soil pH ($p > 0.05$). The ABC350(3%) treatment yielded the greatest pH decrease (6.38 versus 8.21 in the control). The pH reduction in the ABC-treated soil can be explained by the reduced pH of ABC following acidification. When BC350 and BC550 were acidified to ABC350 and ABC550, respectively, the pH reductions were 8.83 to 5.70 and 10.97 to 6.69, respectively (Table 1).

Table 2. Effect of the treatments (PM, biochar, and acidified biochar) on soil pH.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	3	7	15	30	
1	CK	0.0	8.21 e	8.06 c	8.07 f	8.26 f	8.15 h	8.22 f	0.145
2	PM	1	7.62 f	7.16 e	8.34 cd	8.51 e	8.54 e	8.71 d	0.084
3		3	7.42 g	6.92 f	8.52 b	8.56 de	8.66 c	8.66 d	0.184
4	BC350	1	8.42 d	8.00 c	8.38 c	8.72 c	8.42 g	8.73 d	0.063
5		3	8.48 c	8.07 c	8.56 b	8.93 b	8.76 b	9.15 b	0.087
6	BC550	1	9.02 b	8.78 b	8.47 bc	8.76 c	8.45 fg	8.80 c	0.061
7		3	9.48 a	9.26 a	8.97 a	9.28 a	9.19 a	9.40 a	0.039
8	ABC350	1	6.99 h	6.92 f	8.20 e	8.54 de	8.47 f	8.56 e	0.103
9		3	6.38 i	6.25 g	8.23 de	8.67 cd	8.61 d	8.52 e	0.116
10	ABC550	1	7.57 f	7.41 d	8.01 fg	7.94 g	7.95 i	8.25 f	0.186
11		3	7.38 g	7.20 e	7.92 g	7.60 h	7.81 j	8.03 g	0.132
	LSD		0.065	0.097	0.145	0.181	0.054	0.071	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

There are different trends in changes in soil pH as affected by treatments application. The pH of the ABC-treated soils increased with incubation time in most cases. At the end of the incubation period (Day 30), the pH of the soils treated with all additives except ABC550(3%) exceeded the pH of the control soil. The pH increase is feasibly explained by the buffering capacity of the soil. A similar result was reported by Hartley et al. (2016). They found that biochar produced from woody materials raised the pH from that of untreated soil [36]. Oo et al. (2018) also recorded a pH increase in biochar-amended soil after a 71-day incubation period [37]. Likewise, the biochar produced from *Conocarpus* increased the soil pH when applied at 1%, 3%, and 5% application rates. At the highest application rate (5%), the increase was most significant (0.16–0.17 units) [38].

Table 3 reports the EC dynamics in the treated and untreated soils at each incubation time. Relative to the untreated soil, the treatments significantly increased the soil EC throughout the incubation period. The exceptions were ABC550(1%) at all incubation

times, and ABC550(3%) on Days 1 and 30. At the end of the incubation period, the soil treated with 3% PM exhibited the maximum EC increase (823% higher than that of the control soil). A similar soil EC after biochar addition was reported by Al-Wabel et al. (2015). This outcome might result from the accretion of soluble salts present in ashes [7].

Table 3. Effect of the treatments on EC (dS m^{-1}) dynamics in soil at different incubation times.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	3	7	15	30	
1	CK	0	0.15 g	0.18 fg	0.14 h	0.12 h	0.14 h	0.13 f	0.022
2	PM	1	0.45 d	0.40 c	0.41 f	0.50 e	0.46 de	0.43 d	0.073
3		3	0.77 b	0.80 b	1.00 b	1.15 b	1.18 a	1.20 a	0.249
4	BC350	1	0.34 e	0.41 c	0.59 d	0.53 de	0.52 c	0.44 d	0.039
5		3	0.85 a	0.88 a	1.38 a	1.25 a	1.16 a	1.04 b	0.078
6	BC550	1	0.28 e	0.32 d	0.37 f	0.36 f	0.45 e	0.39 d	0.039
7		3	0.64 c	0.77 b	0.93 c	0.86 c	0.98 b	0.91 c	0.045
8	ABC350	1	0.25 f	0.23 e	0.28 g	0.27 g	0.28 f	0.25 e	0.014
9		3	0.33 e	0.29 d	0.51 e	0.59 d	0.50 cde	0.47 d	0.039
10	ABC550	1	0.20 fg	0.16 g	0.18 h	0.15 h	0.17 gh	0.14 f	0.021
11		3	0.24 f	0.21 ef	0.24 g	0.23 g	0.20 g	0.22 ef	0.039
	LSD		0.070	0.056	0.061	0.080	0.061	0.136	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

3.2. CO_2–C Emissions

The CO_2–C efflux rates and cumulative CO_2–C amounts in the treated soils throughout the incubation period are shown in Figures 1 and 2, respectively.

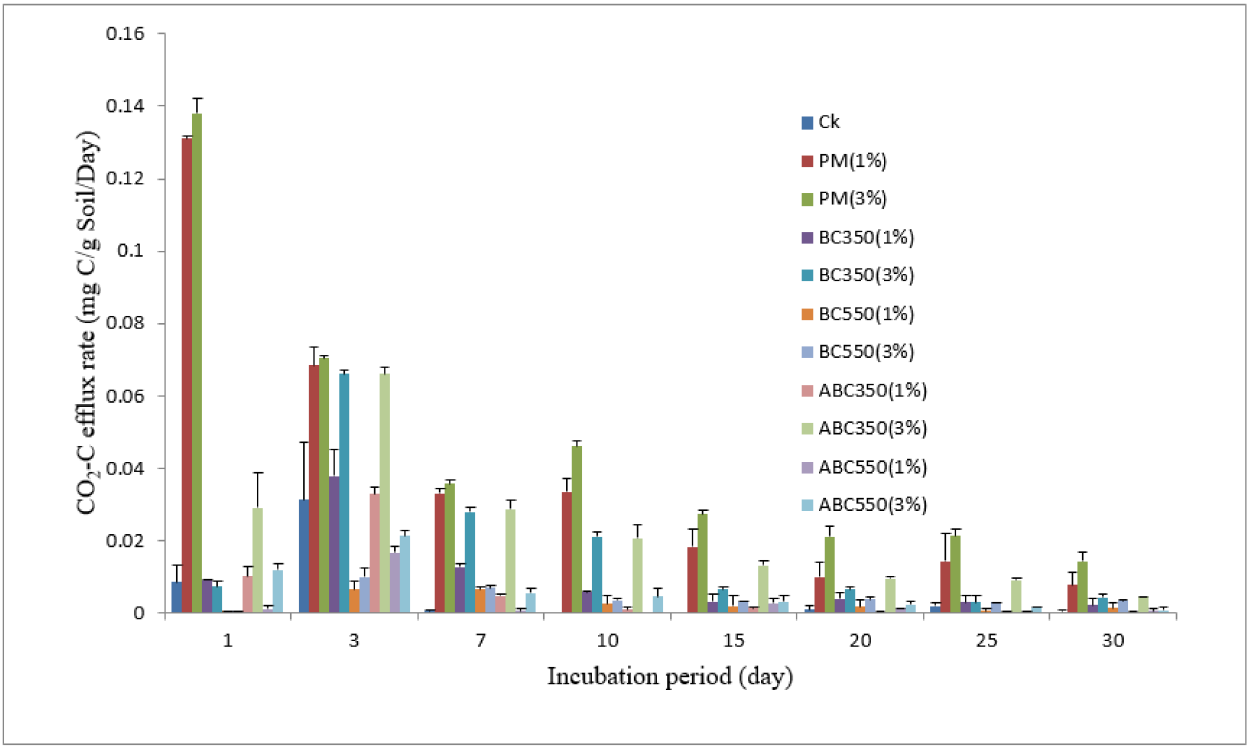

Figure 1. CO_2–C efflux rates (mg C/g soil/day) from soils treated with poultry manure (CK), biochar (prefixed with BC) and acidified biochar (prefixed with ABC) throughout the incubation period.

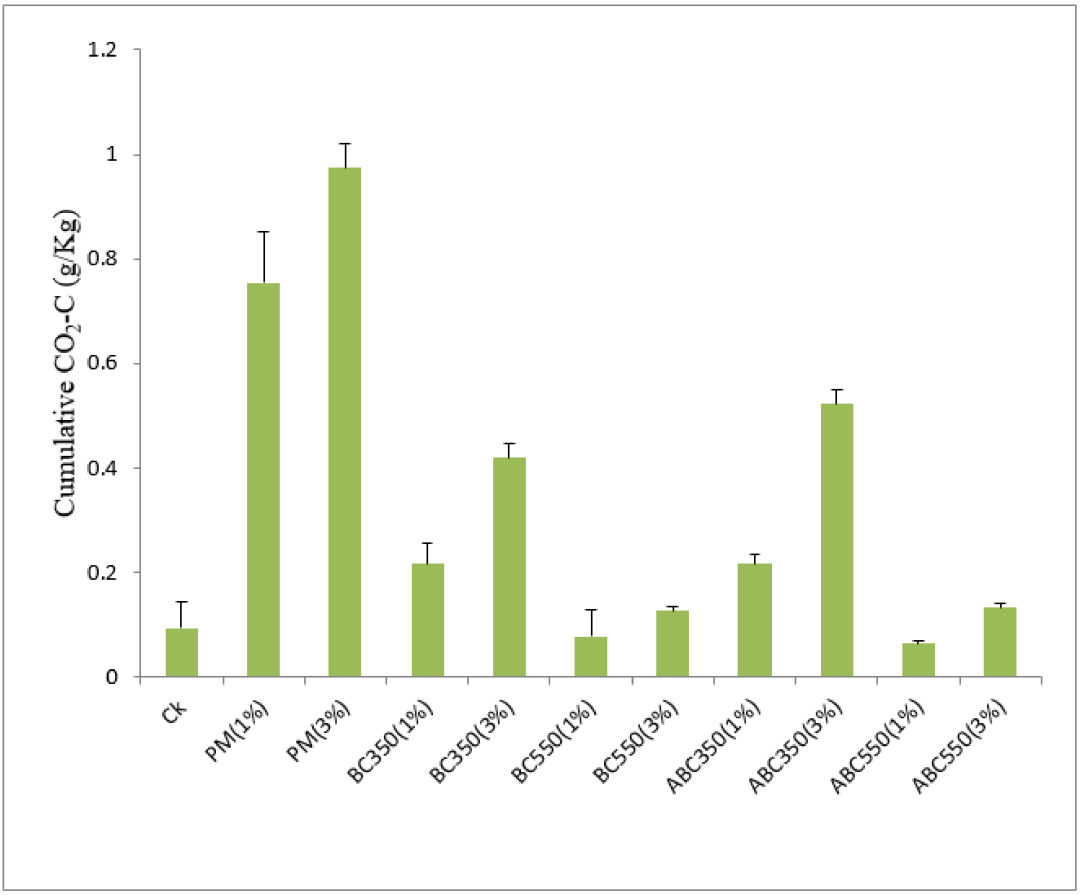

Figure 2. Effect of the treatments on cumulative CO_2–C (g/Kg) efflux from soil.

On the first day, the soil treated with PM(3%) and PM(1%) exhibited the highest and second-highest CO_2–C efflux rates, respectively, and this trend was maintained throughout the incubation period. Similar results were reported by El-Naggar et al. (2015), who found that PM with *Conocarpus* waste additive emitted the highest CO_2–C amounts among the treatments applied to a calcareous soil. In the present study, the increased CO_2–C emissions from PM-treated soil added at 1% and 3% can be explained by the presence of easily decomposed organic matter, which is readily attacked by soil microorganisms [34,39]. The CO_2–C efflux rate reduced as the incubation proceeded. On Day 1, the maximum CO_2–C efflux rate was recorded in the PM(3%) treated sample with a value of 0.1380 while BC550(1%) and BC550(3%) had the least CO_2–C efflux rate with value of 0.0007 mg C/g soil/day. Also, on Day 30 of the incubation period, PM(3%) and ABC550(1%) treated soil had the maximum and minimum CO_2–C efflux rate with values of 0.0145 and 0.0005 mg C/g soil/day respectively. According to previous studies, the soil respiration rate is enhanced by biochar application at the beginning of the incubation but tends to decrease over the experimental period [40–42]. The high rate of CO_2–C emission at the beginning of our experiment can be explained by the high readily labile fraction of organic carbon, which is readily attacked by soil microorganisms. This fraction apparently decreases at later incubation times [39,43]. The consumption of labile carbon and other nutrients by microorganisms also explains the decreased CO_2 emissions over the incubation period [44].

Furthermore, the cumulative CO_2-C effluxes of the treated and the untreated soils are in the order PM(3%) > PM(1%) > ABC350(3%) > BC350(3%) > BC350(1%) > ABC350(1%) > ABC550(3%) > BC550(3%) > CK > BC550(1%) > ABC550(1%) (Figure 2). Similar to the rate of CO_2-C efflux, soil treated with PM(3%) also exhibited the cumulative CO_2–C efflux was maximized in the soil treated with PM(3%). In the sample, the cumulative CO_2–C efflux was 10.3-fold above the control value, probably because the content of easily degraded

carbon compounds was much higher in the PM [29] than in the untreated and biochar-treated soils. The CO_2-emission effects of the treatments can also be explained by the treatment characteristics. Regardless of acidification, the biochars produced at 350 °C contained more volatile matter than those produced at 550 °C (Table 1); consequently, soils treated with these biochars emitted more CO_2 than soils treated with biochars produced at 550 °C. Similarly, Yuan et al. (2014) reported that soil treated with biochars produced from *Radix isatidis* residue released more CO_2 after pyrolyzing *R. isatidis* at 300 °C than after pyrolyzing at 500 °C and 700 °C. Deng et al. (2019) also observed that in biochar-treated soils, the CO_2 emission rate decreased with increasing pyrolysis temperature (300 °C, 450 °C, 600 °C) of biochars produced from spent mushroom substrate [45]. In the present study, it was deduced that increasing the pyrolysis temperature increased the carbon-sequestering affinity of biochar.

In general, the C substrate contents available to soil microorganisms are increased by adding biochar. Therefore, the biochar additive should aid the organic carbon mineralization [46,47] or stimulate biochar-C oxidization [48,49], thus increasing the subsequent CO_2 release. In soils amended with biochar, if the CO_2–C accumulates slowly and its release rate is low, the biochar is not readily biodegradable and remains in the soil for a longer time than the feedstock, which contains readily available organics [50–52]. Experiments have indicated that biochar mineralizes very slowly, with low CO_2–C emissions [53,54]. From the results of the present study, it was deduced that biochar can potentially sequester soil carbon with high efficacy.

3.3. Influence of Acidified Biochar and Incubation Periods on the Availability and Dynamics of Micronutrients (Cu, Fe, Mn, and Zn)

The availabilities and dynamics of the micronutrients (Cu, Fe, Mn, and Zn) in the soils treated with biochars and PM are shown in Tables 4–7. At the beginning of the incubation period, the available micronutrient contents (except Cu) were higher in all treated soils than in the control. On Day 0 of the incubation, the Cu availability was raised above the control value ($p > 0.05$) only in the soil treated with BC350(3%). At the end of the incubation period (Day 30), the Cu availability was raised above the control value in most of the treated soils, the exceptions being BC550(1%), BC550(3%), ABC350(1%), and ABC550(1%) (Table 4).

Table 4. Impact of the treatments on Cu (mg kg^{-1}) dynamics in soil at different incubation times.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	3	7	>15	30	
1	CK	0	0.000 b	0.000 b	0.000 b	0.177 b	0.000 e	0.000 e	0.045
2	PM	1	0.000 b	0.000 b	0.000 b	0.175 b	0.230 a	0.170 b	0.035
3		3	0.082 ab	0.000 b	0.296 a	0.537 a	0.000 e	0.472 a	0.273
4	BC350	1	0.010b	0.027 b	0.022 b	0.011 d	0.068 c	0.133 bc	0.033
5		3	0.203 a	0.110 a	0.036 b	0.079 cd	0.168 b	0.119 bcd	0.241
6	BC550	1	0.107 ab	0.113 a	0.002 b	0.134 bc	0.164 b	0.053 de	0.125
7		3	0.124 ab	0.070 ab	0.035 b	0.129 b	0.215 a	0.065 cde	0.056
8	ABC350	1	0.000 b	0.000 b	0.000 b	0.151 b	0.000 e	0.000 e	0.058
9		3	0.000 b	0.000 b	0.000 b	0.195 b	0.000 e	0.087 cd	0.029
10	ABC550	1	0.000 b	0.000 b	0.000 b	0.132 b	0.009 e	0.021 e	0.028
11		3	0.000 b	0.000 b	0.000 b	0.139 b	0.033 d	0.163 b	0.036
	LSD		0.188	0.085	0.117	0.116	0.020	0.093	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

Table 5. Effect of the treatments on Fe (mg kg^{-1}) dynamics in soil at different incubation times.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	>3	7	15	30	
1	CK	0	0.587 h	0.463 f	0.152 e	0.989	0.977 g	1.998 f	0.295
2	PM	1	1.883 f	1.784 de	1.677 c	2.421 f	3.281 cd	8.491 abc	4.733
3		3	2.763 d	2.042 d	1.548 cd	3.168 e	2.123 f	9.547 ab	16.157
4	BC350	1	0.857 gh	0.710 f	0.969 cd	3.754 d	2.786 e	6.325 cde	0.192
5		3	0.945 g	0.830 f	1.051 cd	4.597 c	3.925 b	7.651 bcd	0.249
6	BC550	1	0.629 h	0.697 f	0.830 de	3.367 e	2.736 e	6.323 c	0.136
7		3	1.151 g	1.198 ef	1.235 cd	4.928 b	4.170 b	8.639 abc	0.522
8	ABC350	1	2.432 e	2.319 cd	1.309 cd	2.355 f	3.178 d	4.135 ef	0.361
9		3	4.175 b	4.128 b	2.479 b	3.329 e	3.562 c	6.017 cde	0.361
10	ABC550	1	3.788 c	2.747 c	2.463 b	3.577 de	8.565 a	4.590 def	0.564
11		3	9.036 a	7.597 a	6.335 a	7.852 a	2.232 f	11.249 a	1.516
	LSD		0.383	0.734	0.934	0.304	0.438	3.773	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

Table 6. Effect of the treatments on Mn (mg kg^{-1}) dynamics in soil at different incubation times.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	3	7	15	30	
1	CK	0	0.000 g	0.000 g	0.000 e	0.000 i	0.374 i	1.109 g	0.260
2	PM	1	5.260 ab	4.902 b	3.876 ab	3.137 e	4.107 d	7.777 cd	1.458
3		3	5.543 a	5.578 a	2.657 c	3.470 de	4.401 d	7.711 cd	0.955
4	BC350	1	2.521 d	2.222 d	2.501 c	8.525 b	6.995 b	8.519 bc	0.435
5		3	2.855 d	2.619 c	2.645 c	9.353 a	9.409 a	13.099 a	0.626
6	BC550	1	0.574 f	0.663 ef	0.744 d	2.286 f	2.703 f	1.641 g	0.143
7		3	0.944 e	0.935 e	0.964 d	3.715d	3.622 e	3.674 e	0.365
8	ABC350	1	4.703 c	4.634 b	3.546 b	4.235c	1.414 g	7.024 d	0.496
9		3	5.061 b	5.569 a	4.159 a	4.520c	2.852 f	9.345 b	0.437
10	ABC550	1	0.000 g	0.000 g	0.466 de	0.719h	4.831 c	1.653 g	0.327
11		3	0.421 f	0.397 f	0.759 d	1.381g	0.891 h	2.629 f	0.229
	LSD		0.428	0.363	0.617	0.429	0.365	1.071	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

Table 7. Effect of the treatments on Zn (mg kg^{-1}) dynamics in soil at different incubation times.

Order	Treatment	Application Rate (%)	Period of Incubation (d)						LSD
			0	1	3	7	15	30	
1	CK	0	0.000 f	0.000 e	0.000 g	0.032 i	0.000 h	0.000 j	0.033
2	PM	1	0.639 de	0.218 de	0.241 ef	0.753 g	1.954 c	1.183 g	0.534
3		3	3.178 a	1.754 b	1.186 b	2.357 c	0.765 f	3.384 c	0.735
4	BC350	1	0.319 ef	0.259 de	0.438 de	1.607 e	1.000 e	0.683 h	0.184
5		3	0.872 d	0.789 c	1.036 b	4.171 a	3.188 a	3.599 b	0.081
6	BC550	1	0.105 f	0.128 e	0.172 fg	0.433 h	0.597 fg	0.232 i	0.160
7		3	0.293 f	0.520 cd	0.534 cd	1.859 d	1.665 d	1.897 e	0.316
8	ABC350	1	0.279 f	0.520 cd	0.110 f	1.088 f	2.258 b	1.567 f	0.334
9		3	2.426 b	2.485 a	1.804 a	2.685 b	0.465 g	4.961 a	0.388
10	ABC550	1	0.074 f	0.000 e	0.000 g	0.677 g	1.456 d	0.612 h	0.140
11		3	1.339 c	0.713 c	0.718 c	1.547 e	0.541 g	2.691 d	0.269
	LSD		0.411	0.474	0.289	0.218	0.268	0.241	

Different letters indicate significant differences among different treatments according to the least significant difference (LSD) test at $p < 0.05$ where letter a is the most significant difference and j is the least significant difference.

At this time, the Cu availability was maximized in the soil treated with PM(3%) (0.472 mg kg^{-1}, versus 0.00 mg kg^{-1} in the control). The high Cu availability in the soil treated with PM(3%) probably results from the high Cu content in poultry manure (Table 1), which might have mineralized during the incubation period. Furthermore, from Day 0 to Day 30, the available Fe in the CK, PM(1%), PM(3%), BC350(1%), BC350(3%), BC550(1%), BC550(3%), ABC350(1%), ABC350(3%), ABC550(1%), and ABC550(3%) treated soils increased from 0.587, 1.883, 2.763, 0.857, 0.945, 0.629, 1.151, 2.432, 4.175, 3.788, and 9.036 mg kg^{-1} to 1.998, 8.491 18.595, 6.325, 7.651, 6.323, 8.639, 4.135, 6.017, 4.590, and 11.249 mg kg^{-1}, respectively where BC550(3%) and ABC550(1%) recorded the maximum and minimum increase of 7.488 and 0.802 mg kg^{-1} (Table 5).

Like the available Cu, the available Fe content was highest in the soil treated with PM(3%). The soil treated with ABC550(3%) also showed a high Fe availability at the end of the incubation period. This result might be explained by the lower pH of the soil treated with ABC550(3%) than of the untreated soil. It was suggested that biochar mediates the transfer of electrons by acting as an electron shuttle, promoting Fe oxidization on its surfaces [55]. The ferrous ions in solution can be electrostatically attracted to the reactive phenolic and carboxylic functional groups on the char's surface [56].

The trends of the available Mn dynamics differed among the treatments, but the Mn availability in all treatments was higher at the end than at the beginning of the incubation. On Day 30, the available Mn was significantly increased from that of the control value ($p > 0.05$) in all treatments except BC550(1%) and ABC550(1%) (Table 6).

An increase in available Mn after biochar application was also reported in a previous experiment [57]. The available Zn trended similarly to the available Mn. At the end of the incubation period, the available Zn was significantly higher in all treated soils than in the untreated soil ($p > 0.05$), and was maximized in the soil treated with ABC350(3%) (Table 7).

The incremental bioavailabilities of Zn, Cu, and Mn were expected because manure is a known source of nutrients [58]. The increased bioavailability of nutrients following biochar addition has been reported previously [59], and Cu and Zn availability has been improved by PM in previous experiments [60,61]. In fact, nutrient concentration enhancement after incorporating manure and biochar has been widely reported [5,62,63]. Like other organic additives, biochar additive can enhance the functions of soil and conserves nutrients. In this way, biochar behaves as an efficient fertilizer that improves the physicochemical soil properties and concentrates the soluble and/or absorbed nutrients through its charges and surface-area properties [17,64,65].

4. Conclusions

Acidification dramatically reduced the pH of biochar (by 3.13–4.28 units). The pH of acidified biochar was considerably lower than the control pH, but both the acidified and non-acidified biochars reduced the CO_2–C efflux from that of the organic additive (PM). In all treatments, the rate of CO_2–C flux decreased over time. The experimental results confirmed the potential carbon-sequestering ability of biochar derived from PM. At the end of the incubation period, the availabilities of all micronutrients (Cu, Fe, Mn, and Zn) were higher in the treated samples than in the untreated soil. Therefore, PM and biochar can enhance the availability of micronutrients to plants. Biochar is an environmentally friendly alternative to organic additives (such as PM), as it mitigates global warming effects while improving the soil nutrients in alkaline sandy soils. Therefore, its use is recommended to farmers.

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