

**The Impact of Soil pH on the Carbon and Nitrogen Retention Capacity and Cumulative
CO₂ Emissions in Microbially Activated Biochar-Amended Soil**

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Abstract

While industry and fossil fuels have greatly contributed to greenhouse gas emissions, the agricultural sector is another major driver, accounting for roughly 21-37% of human-related emissions (Mbow et al., 2022). The purpose of this study was to examine how soil pH influenced CO₂ emissions and carbon and nitrogen retention in microbially activated biochar-amended soils. It was hypothesized that an alkaline pH would demonstrate greater carbon and nitrogen retention and lower cumulative CO₂ emissions (ppm) over 21 days as opposed to acidic or neutral soils, because alkaline conditions are expected to favor microbial colonization of biochar pores and reduced carbon mineralization rates (Wang et al., 2023). To test this hypothesis, biochar-inoculum mixture was added to soil-filled jars divided into various pH levels. CO₂ emissions were measured on days 1, 4, 7, 14, and 21. After 21 days, total organic carbon (TOC) and total organic nitrogen (TN) were measured with a soil testing kit. The ANOVA and post-hoc Tukey results supported the hypothesis: the alkaline group had the lowest CO₂ emissions, while the neutral group had the highest ($F(3, 116) = 42.01, p < .001$). TOC and TN values showed that alkaline conditions experienced the highest nutrient retention ($\chi^2(9, 120) = 72.37, p < .001$). There is sufficient evidence to suggest that there is a difference in CO₂ emissions based on soil pH. The results propose that microbially-activated biochar be instituted in agricultural settings to abate CO₂ emissions and improve carbon and nitrogen retention.

Introduction

The balance of gases in the Earth's atmosphere is important in regulating its climate. Greenhouse gases, such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), trap heat in the atmosphere, contributing to the greenhouse effect (United States Environmental Protection Agency [US EPA], 2025). These emissions have become a significant contributor to global warming, as increased accumulation intensifies the impacts of climate change (Tian et al., 2015). Human activity is the largest contributor of greenhouse gas emissions, primarily through the burning of fossil fuels (US EPA, 2025). Out of all human-caused emissions, the food system, which includes the agricultural sector, contributes roughly 21-37% (Mbow et al., 2022). Agriculture's significant contribution to CO₂ emissions can be attributed to soil degradation and ineffective land or crop management (Atilano-Camino et al., 2022), raising concerns as soils are critical for supporting ecosystems, nutrient cycling, retaining water, and sustaining biodiversity (Bach et al., 2020). Soil dynamics depend on a delicate balance between physical, chemical, and biological factors that work together to maintain ecosystem conditions and stability (Dasgupta & Brahma Prakash, 2021). There are numerous factors at play in soil chemistry, but one of those, pH, has a vital role in sustaining soil health and gas exchange (Hou et al., 2022).

According to the National Oceanic and Atmospheric Administration's Pacific Marine Environmental Laboratory (n.d.), the potential of hydrogen, more commonly referred to as pH, is the concentration of hydrogen ions (H⁺) and is measured logarithmically on a scale from 0-14. Every one-unit change represents approximately a ten times difference in ion concentration. When the hydrogen ion concentration rises, the pH lowers, increasing acidity. In contrast, a decline in hydrogen ion concentration raises the pH, decreasing acidity, and increasing alkalinity. In relation to soil, pH is a central chemical component that regulates microbial community

composition and gene activity, altering carbon cycling outcomes (Hou et al., 2022). Studies have shown that certain bacterial groups, such as Proteobacteria and Firmicutes, tend to increase in population under alkaline conditions, while others like Patescibacteria experience a decline, indicating that pH shifts can reshape microbial populations, regulating soil functions (Xiong et al., 2024). These changes also occur at the genetic level, where shifts in microbial community composition affect the abundance and expression of functional genes, such as the Particulate Methane Monooxygenase Alpha (pmoA) gene, which is involved in methane oxidation and carbon processing (Zhang et al., 2024). While soil pH influences microbial activity, it also varies across ecosystems and plays an important role in shaping plant communities and their nutrients.

Different ecosystems exhibit distinct soil pH profiles due to variations in climate, vegetation, and parent material (Xia et al., 2024). Forest soils are typically acidic due to organic matter accumulation and leaching, while grassland soils tend to be closer to neutral (Zhang et al., 2025). Soils primarily used for agriculture vary on multiple factors: fertilizer usage, irrigation technique and frequency, crop rotation, and whether the pH has been adjusted for optimal yields through human intervention (Qi et al., 2021). Soil pH not only reflects ecosystem conditions but also shapes them, as it controls nutrient availability for plants and microbes. pH also influences microbial activity in the rhizosphere, the biologically and chemically active area directly surrounding a plant's roots, affecting nutrient cycling (Halder & Sengupta, 2015). As soil ecosystems rely on plants and microbes, microbial communities and microbial inocula are essential for supporting soil health and productivity.

Soils contain immense biodiversity; over 25% of all known species live in the soil, where they help their ecosystem by conducting critical functions (Bach et al., 2020). Currently, the farming industry widely utilizes chemical fertilizers that hinder microorganism health, but an

alternative, biofertilizers, promotes ecosystem success while also being less harmful (Shahwar et al., 2023). Biofertilizers, also known as microbial inoculum, are living microorganisms added to soils to boost nutrient flow and soil fertility (Atilano-Camino et al., 2022). These microorganisms include multiple bacterial genera and fungi. There are beneficial bacteria, such as *Rhizobium* and *Azospirillum*, that promote plant growth and nitrogen fixation. These are a few of the microorganisms that can be utilized as microbial inocula. Their presence in a plant's roots or surrounding soil varies based on the pH, environmental conditions, and nutrient availability (Shahwar et al., 2023). These introduced microbes are vital to soil carbon cycling, and their efficiency can determine whether carbon is stored. However, microbial activity can also result in gas release.

The release of gases, such as CO₂, during organic matter decomposition, is one process that microorganisms perform in soil respiration, making it a central component of the carbon cycle (Atilano-Camino et al., 2022; Bach et al., 2020). While gases can be released from the soil, they can also be retained, and there are two main factors that can be used to determine soil health and emission outcomes: total organic carbon (TOC) and total nitrogen (TN). TOC represents the amount of carbon in soil (Hou et al., 2022). Healthy soils can essentially serve as carbon sinks by storing organic carbon, supporting long-term greenhouse gas stability. TN serves as another measure of soil fertility since nitrogen availability, which is influenced by the nitrogen cycle, affects plant growth and microbial activity (Qi et al., 2021). These interactions underscore the importance of soil amendments that are able to maintain long-term soil health.

Among the numerous soil amendments that have been used, biochar has demonstrated high effectiveness at carbon sequestration (Atilano-Camino et al., 2022). Biochar is a charcoal-like substance produced through the decomposition of biomass and organic waste that

is rich in organic carbon. When soil is amended with biochar, a complex system is developed between it and its surrounding environment, driving the reduction of gases like nitrogen by managing denitrification, the process that converts nitrites and nitrates into gaseous forms (Kalu et al., 2022). In addition to its gas sequestration capabilities, biochar also serves as an effective adsorber of toxins and heavy metals (Atilano-Camino et al., 2022). Biochar is highly porous, providing a large surface area that enhances water retention and nutrient absorption. Arguably, one of the key benefits of biochar is that its pores allow for microbial colonization, increasing biodiversity through increased microbial activity (Qi et al., 2021). The beneficial effects of biochar depend on feedstock type and even production conditions during pyrolysis, which is the anaerobic breakdown of raw materials (Atilano-Camino et al., 2022). For instance, biochar derived from wheat and rice straw led to a noticeable increase in key bacterial groups, while grass-derived biochar reduced CO₂ emissions (Qi et al., 2021). When pyrolyzed at higher temperatures, biochar mitigated N₂O emissions but not CO₂ (Atilano-Camino et al., 2022).

Previous literature shows that long-term cultivation of perennial grasses improved soil organic carbon (SOC) storage by altering factors of soil chemistry, such as pH, iron (Fe), and aluminum (Al) oxides, while also enhancing microbial diversity (Hou et al., 2022). Furthermore, research shows that in topsoil, soil pH strongly regulates microbial communities, while deeper layers are more influenced by carbon fractions (Tian et al., 2021). Biochar can improve both soil layers by regulating the pH and raising the TOC by adding recalcitrant carbon, carbon fractions that are resistant to decomposition, which is found within biochar. As pH is measured on a logarithmic scale, small changes are magnified, thereby strongly impacting soil chemical properties (Xiong et al., 2024). Biochar can serve as a buffer to minimize these effects, stabilizing the soil environment. In addition, saline-alkaline soils, characterized by high pH and

content of soluble salts, tend to moderate active carbon loss (Zhang et al., 2023). These findings exemplify the close relationship between soil conditions and carbon behavior, highlighting the need for methods that stabilize carbon and limit emissions.

The purpose of this study was to examine how soil pH influences carbon dioxide (CO₂) release and carbon and nitrogen retention, measured by CO₂ emissions (ppm) and total soil organic carbon and nitrogen in soils amended with microbially activated biochar. The experiment aimed to understand how varying pH conditions affect biochar's ability to minimize carbon loss. Although biochar, microbial inocula, and pH have been studied independently, their synergistic effects remain unexplored. It was hypothesized that soil amended with microbially activated biochar at an alkaline pH (~8.5) would demonstrate greater carbon and nitrogen retention and lower cumulative CO₂ emissions over 21 days compared to soil at an acidic (~5.5) or neutral (7) pH, because slightly alkaline conditions favored microbial colonization of biochar pores and reduced carbon mineralization rates (Wang et al., 2023). To test this hypothesis, soil was placed into 120 jars and divided into four treatment groups: control (no inoculum), neutral, acidic, and alkaline biochar-amended soils. Each jar contained soil and was mixed with a biochar-microbial inoculum solution. Based on the experimental group, the soil pH was adjusted using dilute solutions of hydrochloric acid (HCl) or sodium hydroxide (NaOH). CO₂ emissions were measured on days 1, 4, 7, 14, and 21 using a PASPORT carbon dioxide gas sensor and the PASCO Capstone software. At the end of the 21 day period, soil samples were analyzed for TOC and TN with a soil testing kit to determine how each treatment influenced nutrient retention.

Materials

8 oz airtight plastic jars
(Walmart)
Peat-Free potting mix
(Back To The Roots)

Biochar (Buy Activated
Biochar)
Microbial inoculant
(TeraGanix)

PASPORT Carbon Dioxide
Gas Sensor

Computer with at least Windows 10 or MacOS v 11	Digital scale	Aluminum foil
PASCO Capstone Software	Distilled water	Plastic wrap
Soil pH probe	1 mL micropipette	Plastic spoons
Soil Testing Kit (Soil1)	100 mL graduated cylinder	Black Sharpie
0.1 M Hydrochloric acid (Flinn Scientific)	1000 mL beaker	Masking tape
0.1 M Sodium hydroxide (Flinn Scientific)	2000 mL beaker	Fume hood
	Funnel	Protective goggles
	Stirring rod	Gloves
	Parafilm	
	Spoons	

Methods

One hundred and twenty 8-oz glass jars with lids were prepared for the experiment. As shown in Figure 1, four treatment groups (control, neutral, acidic, alkaline), each replicated 30 times, adding up to 120 times total. Approximately 60 grams of soil was massed on a digital scale and placed into one jar. After putting the soil into the jar, a piece of plastic wrap was placed on the jar, and the lid was then tightly shut. The process was repeated 120 times. On the work surface, the jars were separated and organized into sets of 30 based on the respective experimental group, with each area being labeled using masking tape and a Sharpie.

Figure 1. *Experimental Design Diagram*

Independent Variable (IV) Soil pH/Inocula (6 mL inocula : 3 g biochar)				
Levels of IV (All levels contain biochar)	Neutral (~7) with no inocula <u>Control</u>	Acidic (~5.5) Inocula (2:1 v/w)	Neutral (~7) Inocula (2:1 v/w)	Alkaline (~8.5) Inocula (2:1 v/w)
Number of Repeated Trials	30	30	30	30

Dependent Variable
Cumulative CO ₂ emissions over 21 days (parts per million - ppm) - samples taken on days 1, 4, 7, 14, and 21
TOC (total organic carbon) and TN (total nitrogen) - (pounds per acre - lb/acre)
Control Group
Biochar-amended soil with no inocula, maintained at a neutral pH
Constants
Temperature (during biochar-inoculum mixture incubation and in the lab), biochar (type and quantity), CO ₂ gas sensor, soil pH probe, container type/size, plastic wrap seal

The liquid microbial inoculant, which contained *Lactobacillus casei*, was diluted in distilled water at a ratio of 1 mL inoculant per 10 mL distilled water, creating approximately 720 mL of solution. 654.5 mL of distilled water and 65.5 mL of inoculum were measured using a graduated cylinder, added to a 1000 mL beaker, stirred using a stirring rod, and sealed with Parafilm to be refrigerated until biochar was added. Two 2 L beakers were obtained, each being filled with 320 mL of the solution. Around 3 g of biochar was required per jar, summing to 360 g across 120 jars. This corresponded to a total volume of 2.12 L, with 1.06 L being placed in each beaker. All handling of biochar was performed under a fume hood while wearing goggles, gloves, and a mask to minimize dust exposure. The biochar-inoculum mixture was mixed until it became sludgy, loosely covered with aluminum foil, and then incubated for 24 hours at 23-24°C. After incubation, ~1 tablespoon of the biochar-inoculum mixture was thoroughly mixed into the soil in each jar using a spoon. Control jars at a neutral pH received a biochar-distilled water mixture (530 mL of biochar with 180 mL distilled water) which was prepared in a 1000 mL beaker. The solution was mixed and added to the soil in a similar manner. As the control was at a neutral pH, data collected from it were only used in comparison to the inoculated neutral pH group and not the acidic or alkaline groups.

Soil pH was altered according to the treatment groups. For acidic treatments, approximately 1 mL of 0.1 M hydrochloric acid (HCl) was added to each jar, while in alkaline

treatments, 1 mL of 0.1 M sodium hydroxide (NaOH) was added. The soil was mixed thoroughly with different spoons and the pH of each jar was confirmed with a soil pH probe. Neutral and control treatments were not chemically altered, with distilled water having been added previously to maintain comparable moisture levels.

Carbon dioxide (CO₂) emissions (ppm) were measured on days 1, 4, 7, 14, and 21 using a PASPORT carbon dioxide sensor and the PASCO Capstone software. These days were chosen because CO₂ release from soils does not occur linearly; microbial activity often causes an early spike in emissions that slows or shifts, so measurements at both short and longer intervals helped capture those fluctuations (Wang et al., 2023). Due to time constraints, CO₂ measurements were collected over one to two school days, which may have influenced data due to disproportionate exposure time. The probe was inserted into each of the 120 jars individually for approximately 40-60 seconds to obtain readings. Each treatment group was represented by 30 repeated measurements every sampling day, ensuring replication across all conditions. All measurements were recorded based on the collection day.

At the conclusion of the 21-day experiment, soil from each jar was sampled for TOC and TN analysis using the Soil1 testing kit. The kit-provided reagent was diluted in a 1 mL reagent to 10 mL water ratio. Approximately 5 g of soil from each jar was added to the reagent solution and shaken for 30-60 seconds to fully mix the soil and reagent. The mixture was allowed to settle for a few minutes, after which the clarity of the solution was compared to the kit's color key to determine categorical TOC and TN ranges in lb/acre. The kit provided four ranges for TOC and TN values: very poor quality (TOC: 0-400, TN: 0-12), poor quality (TOC: 400-800, TN: 12-26), good quality (TOC: 800-1600, TN: 26-40), and excellent quality (TOC: >1600, TN: >40). This process was repeated for all 120 jars, with each jar ultimately being assigned to one category.

Results

Across the 21-day period, CO₂ emissions differed among the treatment groups, with the constantly lower emissions in alkaline conditions and higher emissions in neutral conditions. The mean emissions for the alkaline group ($M = 27914$) were the lowest, while the mean emissions for the neutral group ($M = 43856$) were the highest (see Table 1). The mean for the acidic group was 34564 ppm and the mean for the control group was 32291 ppm. On Day 21, the mean emissions for all groups but the acidic ($M = 37463$) decreased compared to previous days. All mean values reported in Table 1 were calculated from the full datasets in Tables A1-A5. These tables provide the raw CO₂ measurements for each trial within every treatment group. The mean, standard deviation, and range for each treatment group are provided in Tables B1-B5. These tables show the control group ($SD = 6119$) varied more than the neutral group ($SD = 5007$). The acidic group ($SD = 4652$) had the smallest average standard deviation, indicating low variability.

Table 1.

Descriptive Statistics Summary Table of Mean Cumulative CO₂ Emissions (ppm) in Four Treatment Groups Over a 21 Day Period

Treatment	Data Collection Day					Overall
	Day 1	Day 4	Day 7	Day 14	Day 21	
Control	27091	29967	34653	35346	34399	32291
Acidic	29774	33212	35433	36487	37463	34564
Neutral	43095	43589	44563	44789	43245	43856
Alkaline	23953	27722	29987	29614	28294	27914

Table 1 shows the mean concentrations of carbon dioxide (CO₂) emissions in parts per million (ppm) for each pH

group (Control, Acidic, Neutral, Alkaline) on days 1, 4, 7, 14, and 21.

Table 2 shows the distribution of categorized TOC and TN levels across the four treatment groups (control, acidic, alkaline, and neutral), separated into the outcome categories of very poor, poor, good, and excellent soil quality. The neutral group had the highest frequency of very poor outcomes, while the alkaline group had the highest number of excellent outcomes and the fewest very poor outcomes. The neutral group showed the least excellent outcomes.

Table 2.

Contingency Table for Total Organic Carbon (TOC) and Total Nitrogen (TN) Categories

TOC/TN Category	Experimental Group				Row Total
	Control	Acidic	Alkaline	Neutral	
Very poor	2	3	0	18	23
Poor	3	14	3	8	28
Good	17	8	12	3	40
Excellent	8	5	15	1	29
Column Total	30	30	30	30	120

Table 2 shows the frequencies of TOC and TN (measured in lb/acre) outcome categories (very poor, poor, good, excellent) across the control, acidic, alkaline, and neutral treatment groups.

In Tables C1-C5, the CO₂ emission difference between the control, alkaline, acidic, and neutral groups during the testing period was analyzed for statistical significance using a one-way analysis of variance (ANOVA) test at an α -value of 0.05 (95% confidence interval). The null hypothesis stated that there would be no significant difference in cumulative CO₂ emissions among soils amended with microbially activated biochar at acidic, neutral, or alkaline pH levels over the 21-day period. The alternative hypothesis stated that soils amended with microbially

activated biochar at an alkaline pH would exhibit lower cumulative CO₂ emissions and enhanced carbon-nitrogen retention over 21 days compared to soil at acidic or neutral pH levels. For day 1, the inferential results were $F(3, 116) = 83.42, p < .001$. For day 4, the inferential results were $F(3, 116) = 53.07, p < .001$. For day 7, the inferential results were $F(3, 116) = 39.63, p < .001$. For day 14, the inferential results were $F(3, 116) = 40.25, p < .001$. For day 21, the inferential results were $F(3, 116) = 42.01, p < .001$. As the p-value for all five tests were less than the α -value of 0.05, there is sufficient evidence to reject the null hypothesis, indicating that the difference in CO₂ emissions among the experimental groups are significant. These results indicate that alkaline soil pH may reduce CO₂ loss, therefore improving carbon-nitrogen retention in microbially activated biochar-amended soils used in an agricultural setting.

The association between soil pH level and TOC/TN category was analyzed using a chi-square test of independence (Table C6). The test was conducted with an α -value of 0.05, and a total sample size of 120. The results indicated significant association between soil pH and TOC/TN category, $\chi^2(9, N = 120) = 72.37, p < .001$.

The distribution of CO₂ emissions across the four treatment groups (control, neutral, alkaline, acidic) is shown in Figures D1-D5. The alkaline group showed the lowest median carbon dioxide emissions, whereas the neutral group showed the highest concentration. The spread of the data in the control group was the highest, suggesting higher variability in CO₂ emissions within that treatment group. Few outliers were observed, demonstrating that the distributions were mostly consistent in each treatment. These outliers can be seen in Tables A1, A2, and A4, as well as in the corresponding boxplots: Figures D1, D2, and D4.

Table 3 summarizes the post-hoc Tukey results for all pairwise comparisons between the treatment groups for each day. As the null hypothesis was rejected, a post-hoc test was conducted

to determine which groups were statistically significant from one another. The alkaline group, which was hypothesized to have the lowest mean CO₂, was statistically different from all other groups on every testing period except for the alkaline vs control pairwise comparison on days 1 and 4. However, despite the post-hoc results, it is important to note that the neutral group is the only experimental group that can be compared to the control group as both were maintained under similar pH conditions.

Table 3.

Post-Hoc Summary Table of Pairwise Comparisons of CO₂ Emissions Between Treatments Across Days 1, 4, 7, 14, and 21

Pairwise Comparison	Statistically Different
Acidic vs Alkaline	Yes (1, 4, 7, 14, 21)
Acidic vs Control	No (1, 4, 7, 14, 21)
Acidic vs Neutral	Yes (1, 4, 7, 14, 21)
Alkaline vs Control	Yes (7, 14, 21); No (1, 4)
Alkaline vs Neutral	Yes (1, 4, 7, 14, 21)
Control vs Neutral	Yes (1, 4, 7, 14, 21)

In Table 3, following each relationship (with a yes or no), with yes indicating the relationship was statistically significant and no indicating the relationship was not statistically significant, the parentheses indicate the testing day associated with that pairwise comparison (1, 4, 7, 14, or 21).

Discussion

The purpose of this research study was to investigate the influence of soil pH on carbon dioxide emissions as well as carbon and nitrogen retention capacity in biochar-amended soils

with microbial inocula. The hypothesis that the alkaline group would release the least CO₂ and retain the most carbon and nitrogen was supported. This trend was observable across all data tables and figures, as the alkaline group consistently produced the lowest CO₂ emissions and had the highest frequency of trials in the good and excellent TOC and TN categories. As a result, it was concluded that an alkaline pH with microbial inocula is effective at both minimizing CO₂ emissions and enhancing carbon-nitrogen sequestration.

The descriptive statistics in Table 1 show that the alkaline treatment had the lowest mean CO₂ concentrations throughout the duration of the study, while the neutral treatment maintained the highest values. The acidic and control treatments were between the alkaline and neutral groups. Greater variability in the control group suggests that without added microbes, the soil system demonstrated less stability, highlighting the influence of microbial activation under specific pH levels. The frequency of TOC and TN outcome categories in Table 2 further exhibit that the alkaline group retained the most carbon and nitrogen, which aligns with the lower CO₂ emissions observed. This trend supports existing literature that alkaline conditions stabilize carbon and reduce mineralization (Hou et al., 2022).

The one-way ANOVA and chi-square analyses demonstrated that soil pH significantly influenced CO₂ emissions and carbon-nitrogen retention across the testing period. Post-hoc comparisons showed that alkaline soil with microbially activated biochar consistently differed from the neutral and acidic treatments, aligning with existing trends of minimized carbon loss in alkaline conditions. These statistical results display that soil pH changes the microbial community of biochar and influences the soil's capacity to retain carbon and nitrogen.

Biochar's ability to reduce greenhouse gas emissions, attributed to its porosity that increases microbial colonization while minimizing carbon loss, has been extensively studied

(Atilano-Camono et al., 2022). The low CO₂ emissions within the alkaline group corroborate studies explaining how a higher pH improves the survival of helpful bacteria and the efficiency of microbial colonization in biochar pores (Qi et al., 2021). Likewise, Wang et al. (2023) found that microbial amendments in alkaline conditions raise microbial activity but eventually result in a stabilized system, thus lowering CO₂ release. Wang's results match the decrease in emissions in the alkaline group from days 14 to 21 in Table 1. However, this study's results contradict those of Zhang et al. (2023), which found that a rise in pH tends to increase CO₂.

pH greatly influences microbial diversity and enzyme activation. Small pH shifts change the presence of bacterial phyla that control decomposition (Xiong et al., 2024). These pH-driven shifts provide an explanation as for why the neutral group, which is the ideal internal pH for the majority of bacteria, had the highest CO₂ emissions. While alkaline conditions are still suitable, carbon-degrading enzymes might have been slowed, consequently improving carbon retention (Feng et al., 2023).

The TOC and TN results also support existing carbon sequestration studies. Alkaline soils with biochar present store more organic carbon due to chemical stabilization, which causes slower cycles within microbes (Hou et al., 2022). These findings align with previous studies by Kalu et al. (2022) and Shrestha et al. (2023) that describe how biochar interacts with the nitrogen cycle to improve denitrification management.

This study had several limitations which may have influenced the outcome. First, when the biochar-inoculum and biochar-distilled water mixtures were placed into each of the jars, a spoonful, rather than an appropriate metric unit, such as x grams of biochar-inoculum mixture per 60 g of soil. The CO₂ concentrations observed were extremely high compared to soil environments, suggesting that the closed-jar system likely magnified gas accumulation. Gas

collection times varied due to time constraints, meaning certain jars might have had unequal exposure times. Only the neutral group had a control without inocula, which restricted comparisons to the acidic and alkaline groups. The carbon dioxide gas sensor also had limited accuracy opposed to advanced equipment, which may have impacted the result's accuracy. Due to the large cost of professional soil kits, a kit designed for field soil samples was used. Finally, in an attempt to contain gases to the highest degree possible, each jar was covered in a piece of plastic wrap before the lid was shut, which might not have guaranteed a consistent seal.

Future studies should include non-inoculated control groups for each pH condition to allow for more comparisons. Additionally, higher-quality gas measurement instruments would increase accuracy and reliability. Finally, conducting the study in a partially-open environment would likely result in more realistic CO₂ data. Future work could analyze enzyme and microbial activity to fully understand the mechanisms leading to these results.

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Appendices

Data, Statistics, and Figures

Appendix A

Raw CO₂ Emissions on Days 1, 4, 7, 14, and 21

https://docs.google.com/spreadsheets/d/1vtftFdc3w7t_J97VdbxwecQsSnFL2sUofvSY3vWolss/edit?gid=0#gid=0

Appendix B

Mean, Standard Deviation, and Range of CO₂ Emissions on Days 1, 4, 7, 14, and 21

https://docs.google.com/spreadsheets/d/1w5IXpRbkxfPKFaahpckf7Ed1Fpux446leK_iujTQ7p0/edit?gid=0#gid=0

Appendix C

Inferential Statistics for CO₂ Emissions on Days 1, 4, 7, 14, and 21 and Total Organic Carbon (TOC) and Total Organic Carbon (TN)

<https://docs.google.com/spreadsheets/d/1F9rg2p6BSj-lwsdnDb1HvBDSmajf4xm72nfhRBXyG5s/edit?gid=0#gid=0>

Appendix D

Boxplots of CO₂ Emissions by Experimental Group for Days 1, 4, 7, 14, and 21

https://drive.google.com/drive/folders/1xLo09BJCOGrjuXRaC2eBrVM6si7sE9?usp=drive_link