Biochar Co-Compost: A Promising Soil Amendment to Restrain Greenhouse Gases and Improve Rice Productivity and Soil Fertility

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Abstract: Agriculture is a major source of greenhouse gas (GHG) emissions. Biochar has been recommended as a potential strategy to mitigate GHG emissions and improve soil fertility and crop productivity. However, few studies have investigated the potential of biochar co-compost (BCC) in relation to soil properties, rice productivity, and GHG emissions. Therefore, we examined the potential of BC, compost (CP), and BCC in terms of environmental and agronomic benefits. The study comprised four different treatments: control, biochar, compost, and biochar co-compost. The application of all of the treatments increased the soil pH; however, BC and BCC remained the top performers. The addition of BC and BBC also limited the ammonium nitrogen (NH$_4^+$-N) availability and increased soil organic carbon (SOC), which limited the GHG emissions. Biochar co-compost resulted in fewer carbon dioxide (CO$_2$) emissions, while BC resulted in fewer methane (CH$_4$) emissions, which was comparable with BCC. Moreover, BC caused a marked reduction in nitrous oxide (N$_2$O) emissions that was comparable to BCC. This reduction was attributed to increased soil pH, nosZ, and nirK abundance and a reduction in ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) abundance. The application of different amendments, particularly BCC, favored rice growth and productivity by increasing nutrient availability, soil carbon, and enzymatic activities. Lastly, BCC and BC also increased the abundance and diversity of soil bacteria, which favored plant growth and caused a reduction in GHG emissions. Our results suggest that BCC could be an important practice to recycle organic sources while optimizing climate change and crop productivity.

Keywords: biochar co-compost; greenhouse gases; microbial abundance; nitrification; pH

1. Introduction

Food production nourishes the world; however, it warms the planet by generating approximately one-third of the total greenhouse gas emissions (GHGs [1]). The global average temperature is continuously soaring, and it is projected to increase by 2 ºC by the end of the 21st century [2] due to a substantial increase in global warming and GHG emissions [3]. Agriculture is one of the main sources of GHG emissions, and it accounts for 58%, 51%, and 20% of anthropogenic nitrous oxide (N$_2$O), methane (CH$_4$) emissions, and carbon dioxide (CO$_2$) emissions, respectively [4–7]. Agriculture is the single largest source of N$_2$O emission, and more than 50% of N$_2$O emission is contributed by fertilized soils [8]. Thus, reducing the GHG emissions would reduce global warming and subsequently improve the stratospheric ozone layer’s stability [8].

Rice is a leading cereal crop and a staple food for more than 50% of the world’s population [9]; however, a significant amount of GHGs is emitted from rice fields. For instance, N$_2$O and CH$_4$ emissions from rice fields account for 10% and 20% of N$_2$O and CH$_4$ emissions from the agriculture sector [6,9]. Soils are an important source of carbon
storage, and they store three times more carbon compared to the atmosphere; however, industrialized agriculture is leading to a rapid loss of carbon from soils [10]. This loss of carbon can compromise the capacity of the agro-ecosystem to produce food and combat climate change [12]. The application of organic amendments, like biochar (BC) and compost, is considered an important strategy to improve soil organic carbon (SOC) and mitigate climate change [13,14]. The ability of BC to reduce GHGs has further reinforced their importance in mitigating climate change. Different recent studies have recorded that BC application reduces soil CO$_2$, N$_2$O, and CH$_4$ emissions [4,15,16]. However, the extent and magnitude of the effect of BC on GHG depends on the feedstock type, pyrolysis conditions, the rate of BC application, soil properties, and climatic conditions [17].

Biochar application reduces the soil bulk density; improves soil texture, soil pH, and porosity [18–20]; and modifies soil microbial communities linked to GHG emissions [20–22]. Biochar also reduces soil ammonification and nitrification, which in turn affects GHG emissions from agricultural soils [23,24]. Besides this, BC also directly absorbs N$_2$O and causes a reduction in production and emission rates of N$_2$O [25], while a group of authors reported that BC can increase N$_2$O emissions [26]. This difference could be due to the feedstock type, biochar properties, and soil and climatic conditions [27]. Furthermore, BC also reduces CH$_4$ emissions by increasing the abundance of methanotrophic proteobacteria and decreasing the proportion of methanogenic archaea [28]. Additionally, BC has a large surface area and oxygenated function groups, which adsorb the CH$_4$ and promote its oxidation and, therefore, reduces CH$_4$ emission [29].

Due to different opinions on the impacts of BC in mitigating GHG emissions, the modification of BC has been employed to reduce GHG emissions. Globally, BC is used in combination with different fertilizers and organic amendments (manures and compost) to improve its efficiency in reducing GHG emissions. For instance, BC, in combination with nitrogen, reduced the N$_2$O emissions by 7.57–12.93% [30], while Harrison et al. [31] reported that biochar composting appreciably decreases CH$_4$ emissions. Recently, biochar co-compost (BCC) has emerged as an effective strategy to improve crop productivity and soil fertility and reduce GHG emissions. The application of BCC improves crop yield and soil health [32,33], and the effect of BCC may lead to agronomic advantages compared to other amendments [33]. These advantages may be due to the high retention of feedstock N [33,34] and the adsorption of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) on the BC surface due to the formation of a mixed-charged organo-mineral layer because of the composting process [35,36]. Furthermore, much remains unknown about how agro-ecosystems would respond to BCC application when considering the agronomic, soil, and environmental (GHG) emission impacts. However, there could be some limitations to using BC and BCC; for instance, BC and BCC may carry toxic metals, which can affect plant growth and development [37]. Their benefits can have different effects under different soil and climate conditions, and they do not have positive effects on all soil types [38].

The effect of BC on soil fertility, rice productivity, and GHG emissions is well-documented in the literature. However, in the literature, no study is available regarding the impact of BCC on rice productivity, soil fertility, gene abundance, microbial activities, and GHG emissions. We hypothesized that the impact of BC and BCC on GHG emissions will depend on soil properties, gene abundance, and soil microbial activities. Thus, this study was performed with the following objectives: (i) to compare the impacts of BC, compost, and BCC on GHG emissions; (ii) to test the impact of BC, compost, and BCC on soil properties and rice productivity; and (iii) to assess the effects of BC, compost, and BCC on gene abundance and microbial diversity and abundance.

2. Materials and Methods

2.1. Experimental Details

The present incubation and pot studies were conducted to determine the effect of BC, compost, and BCC on rice crop performance soil properties, and fluxes of GHG emissions.
The studies were performed at Jiangxi Agricultural University (28°46′ N, 115°55′ E) in Nanchang, China. Soil samples for the incubation and pot studies were collected from the experimental field (0–20 cm). The study site has a humid subtropical climate with a rainy monsoon climate. The collected soil was dried and sieved to remove all the debris and then used to determine different properties. The soil had a silt loam texture (sand: 25.1%, silt: 57.2%, and clay: 17.3%), with a pH of 5.39, 11.62 g kg\(^{-1}\) of organic carbon, 26.33 and 108.13 of mg kg\(^{-1}\) available phosphorus (P) and potassium (K), total nitrogen (N) of 1.56 g kg\(^{-1}\), and a cation-exchange capacity of 7.38 cmol kg\(^{-1}\). The study comprised different treatments: control, biochar (BC: 2%), compost (2%), and biochar co-compost (BCC: 2%).

2.2. Preparation of Biochar and Biochar Co-Compost

Biochar produced at temperatures of 500–700 °C has shown promising results in improving soil fertility and reducing GHG emissions [39–41]. Therefore, to prepare biochar for this study, maize straws were collected, dried, and pyrolyzed at 600 °C for 8 h to prepare BC. The prepared BC was sieved (2 mm) and tested for different properties following standard procedures. The resulting BC had an alkaline pH (9.90), total carbon content of 640 g kg\(^{-1}\), and nitrogen (N) content of 4.52 g kg\(^{-1}\). The compost was prepared using dairy manure and crop residues. To prepare BCC, dairy manure and crop residues were mixed. The mixture was turned weekly until the final preparation of BCC. Biochar was added at the rate of 6% to prepare BCC, as application rates of 3–9% have shown beneficial results such as increased nutrient retention and reduced GHG emission during composting [42,43].

The compost used in the study had a pH of 7.32, total carbon content of 354 g kg\(^{-1}\), cation-exchange capacity (CEC) of 8.2 cmol kg\(^{-1}\), and N content of 5.33 g kg\(^{-1}\), while BCC had pH of 8.98, total carbon content of 539 g kg\(^{-1}\), CEC of 12.2 cmol kg\(^{-1}\), and N content of 6.98 g kg\(^{-1}\). The pH of all amendments was measured using pH meter (10 (water): 1 (BC, CP, BCC) [44], while N and C concentrations were determined using an elemental analyzer [45]. The biochar, compost, and BCC had a C:N ratio of 114.59, 66.41, and 77.22, respectively.

2.3. Soil Incubation Experiment, Gases’ Sampling, and Analysis

The incubation experiment was conducted in a completely randomized design (CRD) with three replicates to determine the impact of different treatments on soil properties, GHG emissions, gene abundance, and microbial activities. The experiment was conducted in 500 mL glass jars, each filled with 100 g of soil. Before starting the incubation experiments, the soil was incubated at 40% water filler pore spaces (WFPS) at 25 °C for 7 days to stimulate the microbial activities [46]. Thereafter, BC, compost, and BCC were added to the jars and mixed thoroughly, and WFPS was increased to 60% (33). The experiment was conducted for 90 days, and gas samples were collected from the head spaces of glass jars after adding the treatments at 0, 1, 2, 3, 7, 10, 13, 17, 20, 25, 30, 35, 40, 45, 51, 58, 65, 72, 79, 86, and 90 days of incubation. We placed an airtight lid with a rubber septum to close the jars for gas sampling. Gas samples were collected twice: immediately after closing the jars (T\(_0\)) and then after 60 min (T\(_{60}\)). The air-tight lid contained a three-way stop-cock syringe for gas sampling. The gas samples were collected in air bags and analyzed for gas concentrations using a gas chromatograph (Agilent 7890B Santa Clara, CA, USA). The gas fluxes were calculated by using the following equation: \(F = p \times V/W \times \Delta C/\Delta t \times 273/(273 + T)\). Here, \(F\) is the indicating rates of gases in µg kg\(^{-1}\) h\(^{-1}\), \(p\) is the density of gases at standard conditions, \(V\) is jar volume (500 mL), \(W\) is the soil weight, \(\Delta C\) is the gas concentration change over 1 h, \(\Delta t\) is the sealing time, and \(T\) is the incubation temperature (25 °C).

2.4. Determination of Soil Properties, Genes, Abundance, and Microbial Activities

To determine soil pH and N dynamics, 1000 mL glass beakers were filled with 600 g of soil, and the same treatments were set up. The glass jars were incubated under the same conditions, and soil samples were collected at different intervals (0, 1, 10, 20, 30, 45, 60, 75,
and 90 days) to determine soil pH and N dynamics. Soil pH was measured with a pH meter using a soil-to-water ratio of 1:5. Nitrate (NO$_3^-$) and ammonium (NH$_4^+$-N) concentrations were determined using the potassium chloride (KCl) extraction method.

Soil organic carbon (SOC) was determined by concentrated sulfuric acid–potassium dichromate external heating method. Available soil phosphorus was determined with sodium bicarbonate extraction (NaHCO$_3$-extractable P) as suggested by the Olsen method [47]. Available potassium contents were determined using ammonium acetate extraction method [48]. For total nitrogen determination, 2 g of soil was digested with 10 mL of concentrated H$_2$SO$_4$ for 2 h at 370 °C. The concentration of total N was determined using Kjeldahl method, as detailed by Bao [49]. Soil urease activity was assessed with sodium hypochlorite-sodium phenate colorimetry assay with urea as the substrate [50]. On the other hand, soil catalase was assessed with a permanganometric assay with hydrogen peroxide as the substrate [51,52]. The activity of urea and catalase was expressed as mg of NH$_4^+$-N g$^{-1}$ 24 h$^{-1}$ and 1 µmol of H$_2$O$_2$ g$^{-1}$ day$^{-1}$, respectively. To determine soil microbial biomass carbon (MBC), fresh soil (20 g) was fumigated with chloroform for 24 h. Then, both fumigated and non-fumigated soils were taken and extracted with 0.5 K$_2$SO$_4$, filtered, and extract was obtained. Thereafter, the concentration of carbon in both fumigated and non-fumigated soil samples was determined by using a carbon analyzer.

Soil samples were collected and immediately brought to the laboratory and stored at −80 °C to determine soil microbial activities. The soil samples were analyzed following standard procedures of Meiji Biomedical Technology Co., Ltd., Shanghai, China, for high-throughput sequencing. A deoxyribonucleic acid (DNA) kit named DNeasy Power Soil Pro Kit (QIAGEN, Germantown, MD, USA) was used to extract the DNA from soil samples, and then both the concentration and purity of DNA in samples were determined with an ultra-micro spectrophotometer. Later, DNA integrity was assessed using 1% agarose gel electrophoresis, and primer-338F (ACTCCTACGGGGAGGCAGCAG) and primer-806R (GGACTACHVGGGTWTCTAAT) were used to amplify 16S rRNA of soil bacteria. Functional genes in soil samples were quantified using DNA extraction and quantitative PCR methods [35]. DNA was extracted with the special kit (Nohe Zhiyuan Science and Technology Co., Ltd., Beijing, China), and genes were quantified to measure the copy number of genes per g of dry soil by normalizing the extraction yield.

2.5. Pot Experiment

A pot experiment was also conducted in a CRD with three replications to determine the impact of the same treatments (BC: 2%, compost: 2%, and BCC: 2%) on rice productivity. Pots with a diameter of 28 cm and length of 35 cm were filled with 8 kg of soil (dry weight), and the same treatments were set up. The experiment was conducted in an open greenhouse with a rain shed to avoid washing with rainwater. Soil from each pot was placed on a plastic sheet, mixed homogeneously with biochar, compost, and BCC, and water was added to attain 100% field capacity. The pots were left for one week to allow the treatments to stabilize; thereafter, five seedlings (30 days old) of rice (variety: Zhongjiazao 17) were transplanted into each pot. The pots were visited daily, and a water depth of 2–3 cm above the soil was maintained throughout the growth period. Weeds growing in the pots were manually uprooted, and no insect/pest attacks or diseases were observed during the study. At harvesting, root length, plant height, tillers per plant, panicle length, and kernel per panicle were taken from all plants, which were measured and averaged. Entire pots were harvested to determine kernel and biomass yield, as well as the harvest index.

2.6. Statistical Analysis

The data were tested for normal and homogeneity of variances (Bartlett’s test) before analysis. Two-way analysis of variance (ANOVA) was conducted to study the effect of different treatments on soil pH, GHG fluxes over time, and nitrogen dynamics, while one-way ANOVA was applied to analyze cumulative GHGs, soil properties, gene abundance, yield traits, and microbial data. Tukey’s honestly significant difference (HSD) test ($p \leq 0.05$)
was used to separate the significant ANOVA sources by using Statistics 8.1. Further, permutational multivariate analysis of variance (PERMANOVA) was used to assess the impact of different treatments on soil microbial communities. Differences in diversity and composition were determined using the Maaslin2 R package.

3. Results

3.1. Effect of BC, Compost, and BCC on Soil pH and Nitrogen Dynamics

The application of BC, compost, and BCC showed a contrasting impact on soil pH during the study period (Table 1). Initially, there was a non-significant impact ($p \leq 0.05$) of different treatments on soil pH. However, over time, the differences among treatments became more significant ($p \leq 0.05$), and a maximum increase in soil pH was observed with BC application (Table 1). The soil pH value in all treatments reached a plateau after 30 days of incubation, followed by a continuous decrease in soil pH throughout the study period (Table 1). The application of different treatments showed a significant ($p \leq 0.05$) impact on soil $\text{NH}_4^+$-$\text{N}$ and $\text{NO}_3^-$-$\text{N}$ contents during the study period. At the start of the experiment, there was a minor difference in $\text{NH}_4^+$-$\text{N}$ and $\text{NO}_3^-$-$\text{N}$ among treatments. However, this difference increased over time (Table 1). The maximum concentration of $\text{NH}_4^+$-$\text{N}$ (31.20 mg kg$^{-1}$) was observed after 30 days of incubation in control, while the lowest $\text{NH}_4^+$-$\text{N}$ concentration was observed in the BC treatment. The concentration of $\text{NO}_3^-$-$\text{N}$ showed an opposite trend as compared to $\text{NH}_4^+$-$\text{N}$ (Table 1). The maximum concentration of $\text{NO}_3^-$-$\text{N}$ throughout the study period was observed in the BCC treatment, followed by CP and BC, with the lowest $\text{NO}_3^-$-$\text{N}$ throughout the study observed in the control (Table 1).

Table 1. The effects of biochar, compost, and biochar co-compost on soil pH and nitrogen dynamics.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Soil pH</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.381b</td>
</tr>
<tr>
<td>BC</td>
<td>5.393ab</td>
</tr>
<tr>
<td>CP</td>
<td>5.393ab</td>
</tr>
<tr>
<td>BCC</td>
<td>5.400a</td>
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<table>
<thead>
<tr>
<th></th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>$\text{NH}_4^+$-$\text{N}$ (mg kg$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23.283a</td>
</tr>
<tr>
<td>BC</td>
<td>23.233a</td>
</tr>
<tr>
<td>CP</td>
<td>23.267a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>$\text{NO}_3^-$-$\text{N}$ (mg kg$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32.230a</td>
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<tr>
<td>BC</td>
<td>32.230a</td>
</tr>
<tr>
<td>CP</td>
<td>32.430a</td>
</tr>
<tr>
<td>BCC</td>
<td>33.230a</td>
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</tbody>
</table>

BC: biochar, CP: compost, BCC: biochar co-compost. Different letters with mean values ($n = 3$) indicate significant differences ($p \leq 0.05$) with Tukey’s test.

3.2. Effect of BC, Compost, and BCC on Fluxes of GHG Emissions

The application of different amendments showed a contrasting impact on the fluxes of GHG emissions (Figure 1). The results indicate that CO$_2$ flux was higher at the initial phase of the study, then showed a downward trend until the 17th day. Afterward, it exhibited an inconsistent trend, increasing again until the 65th day, followed by a continuous decrease until the end of the study. The maximum CO$_2$ emissions were observed in the control, and the lowest CO$_2$ emissions were observed in BCC, which remained consistent with BC application (Figure 1A).
CH$_4$ emissions also showed an inconsistent trend throughout the study. Initially, CH$_4$ emissions were higher at the start of the study and showed a continuous decline until the 17th day. There was an increase on the 20th and 25th days, followed by a continuous decline until the end of the study (Figure 1B). Overall, BC remained the top performer and resulted in the lowest CH$_4$ emissions, comparable to BCC, while the maximum CH$_4$ emissions were observed in control (Figure 1B). The significant differences in CH$_4$ among treatments could be explained by the impact of amendments on soil pH. The increased soil pH from different amendments enhanced methanotrophic activities, which increased CH$_4$ uptake and decreased CH$_4$ emissions.

N$_2$O emissions were significantly high at the start of the study and thereafter showed a decline until the 10th day, then increasing on the 13th and 17th days (Figure 1C). Afterward, N$_2$O emissions showed a decline until the 35th day then an increasing trend until the 45th day, and later continuously decreased until the end of the study. Overall, the lowest N$_2$O emissions were observed with BC application, comparable to BCC, while the maximum N$_2$O emissions were noted in the control (Figure 1C). The application of different treatments also showed a significant impact on cumulative GHG emissions. The lowest cumulative CO$_2$ emissions were recorded with BCC, while the lowest CH$_4$ and N$_2$O emissions were observed with BC, comparable to BCC. The highest fluxes of GHGs were observed in the control (Figure 2).

![Figure 1](Agronomy_2024, 14, 1583_6_of_18.png)

Figure 1. The effects of biochar, compost, and biochar co-compost on CO$_2$ (A), CH$_4$ (B), and N$_2$O (C) emissions. Data are mean values (n = 3) with ±SE.

![Figure 2](Agronomy_2024, 14, 1583_6_of_18.png)

Figure 2. The effects of biochar, compost, and biochar co-compost on cumulative CO$_2$ (A), CH$_4$ (B), and N$_2$O (C) emissions. Data are mean values (n = 3) with ±SE. Different letters with mean values (n = 3) indicate significant differences ($p \leq 0.05$) with Tukey’s test.
3.3. Effect of BC, Compost, and BCC on Soil Nutrients, Gene Abundance, and Microbial Activities

Biochar and BCC showed a remarkable impact on soil properties (Table 2). The maximum pH value was observed with BC (5.82), followed by BCC (5.70) and CP (5.56), with the lowest pH value (5.39) observed in the control (Table 2). The application of BC and BCC also significantly increased the concentration of soil NPK compared to the control. Biochar co-compost increased the soil N, P, and K concentration by 80.59%, 44.27%, and 57.58% compared to the control, while BC increased soil N, P, and K concentration by 59.54%, 31.90%, and 19.69%, respectively (Table 2). The application of different treatments also showed a noteworthy impact on soil organic carbon (SOC) and MBC content. The maximum SOC (19.23 mg kg\(^{-1}\)) was recorded with BC application comparable to BCC (17.31 mg kg\(^{-1}\)), with the lowest SOC (12.49 mg kg\(^{-1}\)) recorded in the control (Table 2).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil pH</th>
<th>Available Phosphorus (mg kg(^{-1}))</th>
<th>Available Potassium (mg kg(^{-1}))</th>
<th>Total Nitrogen (g kg(^{-1}))</th>
<th>Soil Organic Carbon (mg kg(^{-1}))</th>
<th>Soil Microbial Biomass Carbon (mg kg(^{-1}))</th>
<th>Urease Activity (mg NH(_4^+)-N g(^{-1}) day(^{-1}))</th>
<th>Catalase (1 (\mu)mol H(_2)O(_2) g(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.390d</td>
<td>12.830d</td>
<td>59.607d</td>
<td>0.660c</td>
<td>12.49c</td>
<td>297.32c</td>
<td>0.343c</td>
<td>9.900c</td>
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<tr>
<td>BC</td>
<td>5.820a</td>
<td>20.473b</td>
<td>78.632b</td>
<td>0.790b</td>
<td>19.23a</td>
<td>385.000b</td>
<td>0.474b</td>
<td>15.530ab</td>
</tr>
<tr>
<td>CP</td>
<td>5.557c</td>
<td>17.177c</td>
<td>70.600c</td>
<td>0.827b</td>
<td>14.892b</td>
<td>324.394c</td>
<td>0.437b</td>
<td>13.688b</td>
</tr>
<tr>
<td>BCC</td>
<td>5.702b</td>
<td>23.267a</td>
<td>86.000a</td>
<td>1.040a</td>
<td>17.307a</td>
<td>422.667a</td>
<td>0.550a</td>
<td>17.730a</td>
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</tbody>
</table>

BC: biochar, CP: compost, BCC: biochar co-compost. Different letters with mean values (n = 3) indicate significant differences (\(p \leq 0.05\)) with Tukey’s test.

Opposite to this, the maximum soil MBC (412.67 mg kg\(^{-1}\)) was noted in BCC treatment followed by BC, and the lowest soil MBC (297.32 mg kg\(^{-1}\)) was observed in the control (Table 2). The application of diverse treatments also induced a significant impact on soil enzymatic activities as compared to control. The maximum urease and catalase activities were observed with BCC that remained comparable with BC and the lowest urease and catalase activities were recorded from control pots (Table 2).

The application of different treatments showed a significant impact on ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) gene abundance (Figure 3A,B). The maximum AOA \((6.38 \times 10^6\) g\(^{-1}\) dry soil) and AOB \((4.37 \times 10^6\) g\(^{-1}\) dry soil) abundance was observed in the control, followed by CP and BCC, and the lowest AOA \((5.15 \times 10^6\) g\(^{-1}\) dry soil) and AOB \((3.60 \times 10^6\) g\(^{-1}\) dry soil) were obtained with application of BC (Figure 3B). Biochar and BCC also showed a significant impact on nitrous oxide reductase (nosZ) and nitrite reductase (nirK) gene abundance (Figure 3C,D). The maximum \(\text{nosZ} \left(5.44 \times 10^7\right)\) g\(^{-1}\) dry soil) and \(\text{nirK}\) genes \((3.73 \times 10^6\) g\(^{-1}\) dry soil) were noted with BC application, followed by BCC and CP, and the lowest AOA and AOB abundance was observed in the control (Figure 3C,D).

Biochar, CP, and BCC applications showed significantly different impacts on bacterial abundance and diversity. The results indicate a significant impact of different treatments on OTUs, with BC application resulting in the maximum OUTs, followed by BCC, and the lowest OUTs were observed with CP application (Figure 4). The application of diverse treatments also showed a remarkable impact on bacterial abundance at the phylum level (Figure 5). Gemmatimonadota, Myxococcota, Acidobacteriota, Actinobacteria, Actinobacteria, Planctomycetota, Bacteroidota, Firmicutes, and Proteobacteria were recognized as the top 10 phyla after the application of different treatments (Figure 5). The application of BCC, CP, and BC significantly increased the richness of Proteobacteria, followed by Firmicutes, Bacteroidota, and Acidobacteriota (Figure 5).
Figure 3. The effects of biochar, compost, and biochar co-compost on AOA (A), AOB (B), nosZ (C), and nirK (D) genes’ abundance. Different letters on bars indicate significant differences ($p \leq 0.05$) with Tukey’s test.

Figure 4. Venn diagram about the effects of biochar, compost, and biochar co-compost on OTUs distribution of bacteria. T1: control, T2: biochar, T3: compost, and T4: biochar co-compost.
Figure 4. Venn diagram about the effects of biochar, compost, and biochar co-compost on OTUs distribution of bacteria. T1: control, T2: biochar, T3: compost, and T4: biochar co-compost.

Figure 5. The effect of different treatments on bacterial composition at phylum level. T1: control, T2: biochar, T3: compost, and T4: biochar co-compost.

The results also indicate that different treatments showed a significant impact on soil bacterial communities at the family level (Figure 6). The application of BC and BCC increased the richness of Spirochaetota, Bdellovibrionote of Bacteroidota, Parcubacteria, Proteobacteria, Actinobacteriota, and Actinobacteria (Figure 6).

3.4. Rice Growth and Yield Characteristics

Different treatments showed a variable impact on the root characteristics of rice crops (Table 3). The longer roots (58.27 cm) with the highest fresh (12.23 g) and dry weight (5.44 g) were observed with the application of BCC, followed by BC application. Shorter roots (44.37 cm) with a minimum fresh weight (8.03 g) and dry weight (4.03 g) were noted in the control treatment (Table 3). The maximum plant height (PH: 75 cm) with maximum kernels/panicle (KPP: 75.76) and 1000-grain weight (TGW: 3.14 g) was obtained with BCC, comparable to BC application, and the lowest PH, KPP, and TGW were obtained in the control (Table 3). We also found a significant impact of different treatments on kernel yield (KY), biological yield (BY), and harvest index (HI: Table 3). The application of BCC resulted in the highest KY (29.97 g/pot), BY (62.24 g/pot), and HI (48.16%); however, it remained the same for the BC application. Furthermore, the lowest KY (20.30 g/pot), BY (47.30 g/pot), and HI (42.91%) were recorded in the control treatment (Table 3). Different treatments showed a marked impact on abortive kernel (AK) and sterile kernels (SK), and the maximum percentage of both AK and SK was observed in the control, followed by CP,
and the lowest percentage of AK (7.67%) and SK (10.67%) was observed with the BCC, which were comparable to BC application (Table 3).

Figure 6. The effect of different treatments on bacterial composition at family levels. T1: control, T2: biochar, T3: compost, and T4: biochar co-compost.
Table 3. The effects of biochar, compost, and biochar co-compost on the growth and yield characteristics of rice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RL (cm)</th>
<th>RFW (g)</th>
<th>RDW (g)</th>
<th>PH (cm)</th>
<th>TPP</th>
<th>PL (cm)</th>
<th>KPP</th>
<th>TKW (g)</th>
<th>KY/Pot (g)</th>
<th>BY/Pot (g)</th>
<th>HI (%)</th>
<th>AK (%)</th>
<th>SK (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.367c ± 0.50</td>
<td>8.033c ± 0.07</td>
<td>4.030c ± 0.045</td>
<td>63b ± 0.71</td>
<td>7.00a ± 0.47</td>
<td>10.64d ± 0.067</td>
<td>56.667c ± 0.98</td>
<td>2.13d ± 0.165</td>
<td>20.300b ± 0.47</td>
<td>47.300b ± 0.90</td>
<td>56.667c ± 0.26</td>
<td>42.000a ± 0.67</td>
<td>9.000a ± 0.27</td>
</tr>
<tr>
<td>BC</td>
<td>52.400b ± 0.43</td>
<td>10.400a ± 0.33</td>
<td>4.970b ± 0.063</td>
<td>74a ± 2.06</td>
<td>7.25b ± 0.27</td>
<td>12.34b ± 0.167</td>
<td>70.000ab ± 1.24</td>
<td>2.40b ± 0.222</td>
<td>27.40a ± 0.30</td>
<td>56.073a ± 0.09</td>
<td>46.668ab ± 0.80</td>
<td>5.669b ± 0.28</td>
<td>10.000bc ± 0.47</td>
</tr>
<tr>
<td>CP</td>
<td>47.433bc ± 1.23</td>
<td>9.035b ± 0.25</td>
<td>4.906b ± 0.075</td>
<td>65b ± 0.72</td>
<td>7.32b ± 0.27</td>
<td>11.36c ± 0.066</td>
<td>63.000bc ± 1.25</td>
<td>2.45c ± 0.045</td>
<td>22.23b ± 0.16</td>
<td>50.90b ± 0.29</td>
<td>43.695bc ± 0.69</td>
<td>7.466b ± 0.27</td>
<td>10.075ab ± 0.55</td>
</tr>
<tr>
<td>BCC</td>
<td>58.267a ± 1.23</td>
<td>12.230a ± 0.47</td>
<td>5.44b ± 0.125</td>
<td>76a ± 1.68</td>
<td>8.60b ± 0.54</td>
<td>14.86a ± 0.162</td>
<td>75.668a ± 1.65</td>
<td>3.15a ± 0.017</td>
<td>29.97a ± 0.64</td>
<td>62.24a ± 1.63</td>
<td>49.05a ± 0.18</td>
<td>4.667b ± 0.27</td>
<td>8.35a ± 0.27</td>
</tr>
</tbody>
</table>

4. Discussion

The present study aimed to determine the potential of BC and BCC on GHG emissions, soil fertility, and rice productivity. Biochar and BCC significantly increased the soil pH owing to the basic charged groups and alkaline nature of BC, which increases the soil pH [53]. Biochar also decreases the soil aluminum (Al) concentration through its binding on functional groups, increasing base cations, and leading to an increase in the soil pH [54]. Biochar application increased the soil pH initially, which then decreased over time, possibly due to the aging of applied amendments. The results also indicate that NO$_3^-$ increases initially and then decreases, suggesting that nitrification occurred. The nitrification decreased over time, likely resulting in a significant decrease in the soil pH [55].

The results indicated that NH$_4^+$ and NO$_3^-$ were high at the initial period of study, which could be attributed to NH$_4^+$ desorption and the mineralization of organic nitrogen from applied amendments and native soil nitrogen [56]. Biochar and BCC decreased the soil NH$_4^+$ and increased NO$_3^-$ availability (Table 1). Biochar has excellent adsorption properties and absorbs a significant amount of NH$_4^+$ on its surface, thereby leading to decreased NH$_4^+$ availability. Furthermore, compost and BCC increased NO$_3^-$, which could be ascribed to the higher amount of N, increasing soil nitrification [57]. Both BC and BCC increased SOC and MBC owing to effective carbon sequestration [58,59]. The presence of BC modulates the soil microbial composition involved in mineral nutrients, resulting in a substantial increase in soil nutrient availability (Table 2, [60]).

At the start of incubation, CO$_2$ emissions were significantly high and inconsistent, and a decreasing trend was observed over time. The high rate of nitrification provides more N substrates to microbes, thereby increasing CO$_2$ emissions [56]. Therefore, the higher availability of mineral N at the start of the study was a reason behind the increase in CO$_2$ emission. Thereafter, mineral N availability decreased over time, resulting in a significant decrease in CO$_2$ emissions.

BCC significantly decreased CO$_2$ emissions, comparable to BC, while the highest CO$_2$ emissions were observed with compost application. This indicates that compost might contain more degradable carbon compared to BC, which therefore resulted in maximum CO$_2$ emission [61,62]. Biochar is an excellent amendment to improve soil fertility [63,64] and reduce GHG emissions [65]. The significant increase in MBC and lowest CO$_2$ emissions in BCC implies a higher microbial carbon use efficiency. This indicates its excellent potential to increase soil carbon storage through the contribution of microbial necromass [66,67].

Both BC and BCC significantly reduced CH$_4$ emissions (Figure 1), which could be ascribed to BC’s ability to decrease the activity of methanogenic archaea and increase the activity of methanotrophic and proteobacteria [28]. The soil pH is an important factor that significantly affects the activity of methanogens and methanotrophs that control both CH$_4$ uptake and emissions [68]. In the present study, both BC and BCC increased the soil pH, which indicates that the activity of methanotrophs was increased with an increasing soil pH. Furthermore, CH$_4$-monooxygenase is an important enzyme that causes the oxidation of CH$_4$ and is active at a higher pH [69]. This indicates that an increase in the soil pH after BC and BCC application increased the activity of both methanotrophs and the CH$_4$-monooxygenase enzyme, leading to increased CH$_4$ oxidation and uptake, resulting in lower CH$_4$ emissions.

Apart from this, BC also contains oxygenated functional groups and has a higher surface area, which allows CH$_4$ adoption on the BC surface and leads to a reduction in CH$_4$ emissions [29]. The BC and BCC used in the current study had a higher C:N ratio, which also contributed to a reduction in methane emissions. Moreover, BC also improves soil aeration and bulk density, enhancing the soil’s sink capacity and reducing CH$_4$ emissions [70,71]. The application of BC also promotes the methanotrophic CH$_4$ intake and subsequent CH$_4$ oxidation by methanotrophic organisms at the root surface, therefore, leading to a reduction in CH$_4$ emissions [72,73].

The soil pH is an important factor that fundamentally affects N$_2$O emissions from soils [53]. The application of BC and BCC significantly increased the soil pH and reduced
the \( \text{N}_2\text{O} \) emissions, consistent with earlier studies indicating that BC application reduces \( \text{N}_2\text{O} \) emissions by increasing the soil pH [74–76]. Biochar application significantly decreases \( \text{N}_2\text{O} \) emissions due to its liming effect, which reduces the \( \text{N}_2\text{O}/\text{N}_2 \) ratio and results in lower \( \text{N}_2\text{O} \) emissions [29,77]. Thus, the reduction in \( \text{N}_2\text{O} \) emissions following BC and BCC application could be attributed to the liming effect of BC, which might favor \( \text{N}_2 \) formation [78]. Nitrous-oxide reductase (\( \text{N}_2\text{OR} \)) is an important enzyme involved in the conversion of \( \text{N}_2\text{O} \) to \( \text{N}_2 \) during nitrification. It is documented that a lower soil pH can decrease the assembly and function of the \( \text{N}_2\text{O} \) reductase (\( \text{N}_2\text{OR} \)) enzyme, which reduces \( \text{N}_2\text{O} \) to \( \text{N}_2 \) in denitrification, leading to lower \( \text{N}_2\text{O} \) emissions [79]. The addition of BC and BCC enhanced the soil pH, which might increase the functioning of \( \text{N}_2\text{OR} \), leading to a marked reduction in \( \text{N}_2\text{O} \) emission (Figure 1). The positive impact of BC and BCC on reducing \( \text{N}_2\text{O} \) production can also be ascribed to their capacity to inhibit nitrification (Table 1) and decrease \( \text{N}_2\text{O} \) losses from denitrification.

The bacterial-encoded \textit{nosZ} gene is involved in the synthesis of \( \text{N}_2\text{OR} \) enzymes. In the present study, BC and BCC increased \textit{nosZ} abundance and subsequent \( \text{N}_2\text{OR} \) activity, resulting in a marked \( \text{N}_2\text{O} \) production [80]. The increase in \textit{nosZ} activity was linked with an increase in the soil pH following different amendments. The increase in the soil pH increases the abundance of \textit{nosZ} encoding the \( \text{N}_2\text{O} \) reductase, which increases the ratio of denitrification product (\( \text{N}_2/\text{N}_2\text{O} \)), thereby reducing the \( \text{N}_2\text{O} \) emission [81,82].

The maximum \( \text{N}_2\text{O} \) emission was observed at the start of the study, followed by a continuously decreasing trend. This could be attributed to the transformation of mineral nitrogen and an increase in N availability. A significantly higher AOA and AOB abundance was seen under the control. The addition of BC and BCC decreased the abundance of both AOA and AOB, with AOA showing more abundance than AOB. This indicates that the nitrification effect in this study was dominated by AOA rather than AOB. These results coincide with the findings of Ye and Zhang [83], who found that AOA can outnumber AOB. Likewise, Shi et al. [84] and Wu et al. [85] also reported that an increase in the soil pH after BC application decreased AOB abundance. The application of fertilizers can also decrease AOA and AOB abundance by changing the concentration of soil available phosphorus [86]. The increase in nutrient availability after BC and BCC might have allowed the plants and microbes to increase access to limited nutrients, thus maintaining a dynamic balance of stoichiometry. Biochar and BCC mediated increases in P availability increased N absorption by plants, reducing the availability of N to AOA and AOB microbes, thereby inhibiting their growth and abundance. Therefore, a reduction in AOA and AOB abundance could also be another important reason for BC and BCC-mediated decrease in \( \text{N}_2\text{O} \) emissions.

Biochar and BCC showed a marked improvement in yield and biomass production (Table 3). The higher availability of N and P explained the difference in biomass and yield after the application of different treatments. These findings are in line with earlier studies indicating that BC and BCC can increase crop growth and yield by improving soil nutrient availability [87–89]. Our findings also indicate that nutrients present in compost were retained during composting, and the addition of BC further improved nutrient retention, leading to improved plant performance [37]. Furthermore, BCC also increased the P and K availability, water retention, and soil carbon contents [90,91], which could also reason behind improved rice growth and productivity in the current study. Biochar co-compost reduced sterile and abortive kernels, which could be attributed to improved nutrient availability, SOC, and microbial activities.

The relative abundance and diversity of soil bacteria are important indicators of soil functioning [92]. The abundance of \textit{Proteobacteria}, \textit{Firmicutes}, \textit{Bacteroidota}, and \textit{Acidobacteriota} was significantly increased following BC and BCC application. This is consistent with the findings of Xia et al. [93], who reported that BC application can increase bacterial abundance and diversity. Further, Lei et al. [94] also found a significant relationship between \textit{Firmicutes} and \textit{Proteobacteria} and variations in \( \text{N}_2\text{O} \) and \( \text{NH}_3 \) emissions. The results indicate that all the treatments increased the abundance of \textit{Actinobacteria}, which inhibit the proliferation of methanogens, thereby reducing the \( \text{CH}_4 \) emissions [95]. The results also indicated that
biochar treatments decreased \( \text{NH}_4^+ \)-N over time, which reduced the availability of N substrates for nitrification and denitrification processes, thus resulting in reduced \( \text{N}_2\text{O} \) emissions. All the treatments increased the abundance of \textit{Proteobacteria}, which are involved in carbon decomposition, and led to an increase in soil organic carbon and a decrease in \( \text{CO}_2 \) and \( \text{N}_2\text{O} \) emissions [96]. Biochar and BCC facilitate changes in the soil bacterial community, therefore leading to abated GHG fluxes and improved rice yield. Moreover, the BC and BCC might also create new ecological niches and provide more nutrients (Tables 1 and 2) for microbial growth, thereby resulting in improved bacterial abundance and diversity, which in turn affects GHG emissions [97,98].

5. Conclusions

The application of all the organic amendments significantly increased rice growth and productivity. However, biochar co-compost and biochar provided better benefits compared to compost alone. BC and BCC increased rice growth and productivity by increasing the nutrient (NPK) availability, soil carbon, soil enzyme activity, genes abundance, and diversity of soil bacteria. Furthermore, BCC significantly reduced \( \text{CO}_2 \) emissions, while biochar significantly reduced \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) emissions, with similar results observed for BCC. The reduction in GHGs following BC and BCC was linked with an improved soil pH, modulated gene abundance, nitrogen dynamics, and bacterial abundance. These findings suggest that BCC offers an excellent opportunity to reduce GHG emissions while improving rice productivity. The present short-term experiment was conducted under controlled conditions, which is a major limitation of this study. Therefore, field studies must be conducted under a wide range of soil and climate conditions to determine the impact of BCC on soil fertility, rice productivity, and microbial activities. Besides this, the present study was conducted in acidic soil, highlighting the need for more studies in neutral and alkaline soils before fully assessing the impact of BC and BCC on soil fertility, rice productivity, and microbial activities.

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Data Availability Statement: Data are contained within the article, and further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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