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# Influence of Biochar Fortified with Fungi (*Termitomyces*) on Carbon Stock, Flux, and Yield of Groundnut

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ABSTRACT

The study was conducted to investigate carbon stock, flux, and sequestration potential as well as the response of groundnut (yield) to different fortification approaches. A greenhouse experiment was conducted using four treatment combinations, fortified biochar with *T. albuminosus* (B + T), biochar (B), *T. albuminosus,* and control laid in a Complete Randomized Design (CRD). The result showed increased carbon sequestration, stock, and a subsequent decrease in carbon emission in treatment B + T relative to other treatments. Untreated samples recorded the lowest values with 3.69 g $kg^{-1}$ sequestered carbon 24.97 kg $ha^{-}$ stock 4.92% carbon emission and a net flux of -20.70. The highest groundnut yield was also recorded in the B + T treatment with a value of 1131.10 kg $ha^{-1}$. However, biochar treatment recorded the highest biomass and stover yield 5.62 t $ha^{-1}$ and 3.82 t $ha^{-1}$ respectively. In conclusion, the Fortification of biochar with *T. albuminosus* proved efficient in improving carbon sequestration, increasing carbon stock, and reducing emission as well as other nutrients in soils under cultivation. Also, using B + T as an amendment under optimal growth conditions is recommended for better groundnut production. The study's constraints lie in its execution within controlled greenhouse settings, potentially limiting its applicability to real-world field conditions. Thus, caution should be exercised when extending these findings to field applications, ensuring the validation of the approach's effectiveness.

## INTRODUCTION

The natural equilibrium must be restored and a way must be found to remove atmospheric carbon, to slow down or reverse the effects of climate change (IPCC, 2021). Since $CO_2$ has been identified as the main greenhouse gas contributing to global warming, it is necessary to make significant investments in technology that reduces its effects and emissions as well as enhances C sinks to fulfill an ambitious goal for climate mitigation. (Bovsun et al., 2021; Orlova et al., 2017).

According to Omeke et al. (2023) & Keske et al (2019), soil carbon (C) pools play a significant role in the world's carbon cycle since they contain almost three times as much carbon as the atmosphere and 3.8times as much nitrogen (N) as the combined biotic pool. It can either be a source or a sink of atmospheric carbon, depending on how and where the land is used (IPCC, 2014; Lehmann et al., 2021). Depending on the agricultural management practices and their intensity, mulching, tillage techniques, various soil amendments, and fertilizers have an impact on soil attributes such as soil carbon concentration, organic carbon dynamics, and water retention capacity (FAO, 2021; Groot et al., 2020; Matuštík et al., 2020). The quantity of carbon stored in soil ecosystems is decreased by farm management strategies that promote cultivation and decrease soil disturbance (Aliyu et al., 2023; Huggins et al., 2016). This is mostly because soil productivity is impacted by carbon loss brought on by land intensification and usage conversion from a natural ecosystem to a cultivated agricultural ecosystem (Awoonor et al., 2022; Joseph et al., 2021; Homagain et al., 2015).

The production and application of biochar to fields is one of the most recent technologies tested to increase carbon sequestration in plant biomass and soil, and, in many cases, a soil amendment that is also able to reduce soil greenhouse gas emission (Regmi et al., 2021; Wang et al., 2019; Wu et al., 2019). Termitomyces is a type of fungal genus recognized for its close connection with termite habitats. These fungi commonly establish symbiotic partnerships with termites, aiding in the breakdown of organic materials such as biochar. Biochar is produced by pyrolysis using a large variety of biomass and is characterized by high carbon content, large specific surface area, porous structure, enriched surface functional groups, and mineral components, which improve the overall condition of the soil (Goldstein et al., 2020; Crippa et al., 2021). Biochar is comparatively cheap because of its high production and abundance of raw materials (feedstock). Thus, it is considered to be the best alternative adsorbent for many environmental applications, such as water treatment, soil remediation, and soil improvement. Thus, this work aims to investigate how engineering biochar with an organism will improve carbon sequestration, carbon sink, reduce emissions and serve as an amendment to increase groundnut yield.

## METHODS

### Study Area

This study was conducted at Bayero University Kano (BUK) farm, located within latitude 11.97932$^o$ to 11.98194$^o$N, and longitude 8.41245 $^o$ to 8.42205$^o$E, with altitude varied from 427 to 434 m above the sea level in Sudan Savannah agro-ecological zone. The area is characterized by a mean average temperature of 25±7$^o$C and mean annual rainfall of about 500-760 mm all of which falls between June and October (Nzamouhe, & Omar, 2020).

### Experimental Materials

The experimental materials are Rumen content, Fungi (*Termitomycesspp*), and Groundnut (*Arachis hypogeae*) Samnut 24.

### Collection and Isolation of *Termitomyces albiminosus*

The Fungi (*Termitomyces ssp*) comb was collected from a termite mount positioned around the University orchad, washed with clean water, and sterilized for 3 minutes in 0.5 % sodium hypochlorite (NaOCl) solution. The comb was further rinsed three times in deionized water and blot within folds of sterile filter paper (Whatman No.1). The fungi comb was also transferred onto Potato Destrose Agar (PDAs) media and arranged in a Gallenhamp incubator at a temperature of 27±2$^0$C. The media was monitored daily for mycelia development. The growing fungi were sub-cultured onto fresh PDAs to obtain pure culture. Bitsy features of growing fungi were linked using a manual authored by Muggia et al. (2015). The pure culture of the insulated fungi was preserved in labeled McCartney bottles and stored in the culture herbarium.

Mass production of the fungi was carried out in the Molecular Laboratory to produce the required quantity for the experiment. The fungi comb was analyzed using Near infrared Absorptions (NIRS).

### Biochar Production

The biochar used for the research is large ruminants rumen digesta used as feedstock. Rumen digester was obtained from Kano Abbatoir, air dried for 4 days, moisten, and sterilized to ensure the absence of other organisms. A fraction of the sterilized rumen-content was used to produce biochar using a slow pyrolysis technique at a temperature range between 360 and 380$^o$C. The biochar properties were evaluated according to the International Biochar Initiative (IBI) Standard (2015) before use. The following parameters were established:

1. The pH was measured with a glass electrode pH meter (JENWAY 3520 MODEL) at a 1:2.5 ratio in both water and 1.0M KCl. The electrical conductivity meter (DDS-307 MODEL) was used to measure EC.
2. The organic carbon of the biochar was assessed using the wet oxidation method of Walkley and Black as described by Mustapha et al. (2023).
3. Exchangeable bases (Ca, Mg, K, and Na) were extracted with 1N ammonium acetate (1N $NH_4OAc$) solution buffered at pH 7.0, as described by Mustapha, (2021). The concentration was evaluated using an Atomic Absorption Spectrophotometer (BUCK SCIENTIFIC MODEL, 210 VGP).
4. Available Phosphorus was extracted using a technique described by Mustapha et al. (2021) and measured by spectrophotometry (22PC MODEL at 860nm).

5. Copper, Zn, and Fe were extracted in 0.1 N HCI and measured using atomic absorption spectrophotometry.
6. Nitrogen was determined using 0.5M K2SO, followed by faster colorimetric estimations.
7. The surface areas were calculated using the BET method (Sial et al., 2019).
8. Hydrogen and total C were determined using CHS analyzer 580A.

**Inoculation of the Fungi *Termitomyces***

10 ml $kg^{-1}$ of fungi *Termitomyces* was inoculated into 1 kg of biochar and placed in an incubator for 7 days to allow for complete colonization and initiate the degradation of the biochar by the fungi.

**Treatments and Experimental Design**

Four treatment combinations were used and replicated three times. The first treatment contains bichar alone, *Termitomyces,* Biochar fortified with *Termitomyces*, and control applied at a rate of $10tha^{-1}$ to the designated pots, and laid out in a Complete Randomized Design (CRD) pattern in a greenhouse.

**Pot Preparation and Management**

Soil samples were taken from the University Orchard using a soil auger at depths ranging from 0 to 30 cm. These soil samples were combined to form composite samples, which were air-dried and pulverized using a porcelain pestle and mortar before being put through a 2mm sieve. The Samples used in the Pots were prepared based on the designated treatments. The Pots (35.3cm in size) were arranged in a greenhouse and maintained at field capacity and gravimetric moisture content ($w$ = 20%). The treatment was applied to the pots two weeks before planting, after which two seeds of the test crops were sown per hole.

**Analytical Procedures**

The following parameters were determined in the soil samples. The Bouycous Hydrometer method was used to determine the particle size distribution (Mustapha *et al.,* 2021). The USDA textural triangle was used to define the textural classifications. Soil bulk density was measured using the core method described by Nabayi et al. (2019). Soil pH was measured with a glass electrode pH meter (JENWAY 3520 MODEL) in a 1:2.5 ratio in both water and 1.0M KCl. The electrical conductivity (EC) meter (DDS-307 MODEL) was used to measure it. The wet oxidation method of Walkley and Black as described by Mustapha et al. (2023) was used to determine organic carbon (OC). Exchangeable bases (Ca, Mg, K, and Na) in the soil were extracted with 1N ammonium acetate (1N $NH_4OAc$) solution, buffered at pH 7.0 as described by Mustapha, (2021). Concentration was determined with an Atomic Absorption Spectrophotometer (Buck Scientific Model, 210 VGP). Available Phosphorus was extracted using a technique described by Mustapha et al. (2021) and was read using a spectrophotomer (22PC MODEL at 860nm wavelength). Micronutrients Copper, Zn, and Fe were extracted in 0.1 N HCI and determined by atomic absorption spectrophotometry. Lead (Pb) was extracted using the method described by Lenntech, et al. (2013) and was read using atomic absorption spectrophotometer.

**Soil Organic Carbon Stock (SOCs) Determination**

The soil organic carbon was calculated using the soil's bulk density, organic carbon (OC), and depth $SOCs = \sum DbiCiDi$

Where:

SOCs denotes the soil organic carbon stock (kg C $ha^{-1}$)

$D$bi denotes the bulk density (g $cm^{-3}$) of layer i.

$C$i represents the fraction of organic carbon (g C $g^{-1}$) in layer i

$D$i is its thickness (depth in cm).

**Net Carbon Flux**

Net C flux was calculated as the difference between carbon emission ($CO_2$) from a procedure outlined by (Omeka, *et al.,* 2023). Where the flux is calculated carbon emission and soil organic carbon stocks (SOCs). Carbon emission was measured in-situ in a procedure using alkaline absorption method outlined by (Olaniyan *et al.,* 2020). Reading was taken at 5-day intervals.

Net Flux = C emission – C sequestration

**Plant Parameters**

Yield was determined from the biomass and grain yield after harvest.

**Data Analysis**

Descriptive statistics and one-way analysis of variance (ANOVA) were carried out using JMP® 15 edition. Means were separated using Tukey HSD.

## RESULTS AND DISCUSSION

### Physical and Chemical Properties of the Experimental Soil

The physical and chemical characteristics of the soils used in the experiment are shown in Table 1. According to Table 1's relative proportion of soil particles, the soil is classified as a loam, and its pH is 6.10, making it somewhat acidic. According to Dawaki, Haruna, and Samdi (2018), the exchangeable bases Ca, Mg, K, and Na in the soil were low to moderate, and it is likely that major cations will be low in irrigated areas, especially those that succumb to pressure from year-round farming. In addition, the soil's organic carbon content was low (table 1). The amount of nitrogen in the soil was discovered to be 0.7g.kg-1, which was also considered poor by (Imadojemu et al., 2017). The low amount of total nitrogen found in this study may be due to the mobility of nitrogen in soils, which causes losses through multiple mechanisms like ammonia volatilization, particularly in conditions of high temperature, which are typical of the region's climate (Popkin, 2021). Similar processes like denitrification, chemical and microbiological fixation, leaching, and runoff may cause low levels of total nitrogen in the soil (Musa et al., 2017). According to Fekadu et al. (2018), the soil's accessible phosphorus level was 2.5 mg kg-1, which translates to a low amount of phosphorus that can be attributed to its propensity to be fixed by various ions. Sulfur (S), copper (Cu), lead (Pb), and iron (Fe) were all at very low levels.

### Biochar

The result from the analysis (Table 2) shows that the biochar is safe for usage and environmentally friendly. This is because it contains a minute concentration of the metals classified as dangerous based on the IBI standard (Bovsun *et al.,* 2021). The biochar is also classified as in the first class as it contains more than 60% C from dry mass (Table 2).

Table 1. Physical and Chemical Parameters of the Soil

| Parameters | Results |
|---|---|
| % clay | 12 |
| % silt | 46 |
| % sand | 42 |
| Soil Texture | Loam |
| BD (g $cm^{-3}$) | 1.28 |
| pH | 6.10 |
| EC ($dSm^{-1}$) | 0.02 |
| Mg ($cmol.kg^{-1}$) | 1.92 |
| K ($cmol.kg^{-1}$) | 1.02 |
| Na ($cmol.kg^{-1}$) | 0.76 |
| Ca ($cmol.kg^{-1}$) | 6.40 |
| O.C ($g.kg^{-1}$) | 3.69 |
| TN ($g.kg^{-1}$) | 0.73 |
| Available P ($mg.kg^{-1}$) | 15.95 |
| Fe ($mg.kg^{-1}$) | 23.86 |
| Cu ($mgkg^{-1}$) | 0.92 |
| Pb ($mgkg^{-1}$) | 0.04 |
| S (%) | 0.60 |

Furthermore, the percentage concentration of O and H is low signifying the strength of the carbon rings (Park et al., 2014; Malyan et al., 2021) This also translates to lower H/C and O/C ratios indicating the quality of the material, which is directly proportional to its decomposition rate (Bovsun et al., 2020; Azzi et al., 2019). The pH of the biochar is slightly acidic (6.53) with an electrical conductivity of 0.03 $dSm^{-1}$. The biochar has a surface area of 78.920 $m^2g^{-1}$ and all the other nutrients are within range translating the quality of the biochar.

Table 2. Biochar Properties

| Parameters | Concentration |
|---|---|
| Surface Area ($m^2g^{-1}$) | 78.920 |
| pH | 6.53 |
| EC ($dSm^{-1}$) | 0.030 |
| Mg (%) | 0.159 |
| O (%) | 12.21 |
| H (%) | 4.92 |
| K (%) | 0.004 |
| Na (%) | 0.076 |
| Ca ($cmol.kg^{-1}$) | 0.656 |
| C (%) | 78.20 |
| TN (%) | 0.040 |
| Available P (%) | 0.056 |
| Fe (%) | 1.89 |
| Zn (%) | 0.152 |
| Cu (%) | 0.072 |
| Pb (%) | 0.001 |
| S (%) | 0.92 |
| H/C (%) | 0.062 |
| O/C (%) | 0.15 |

**Effect of Biochar Treatment on Carbon Sequestration, Storage, Carbon Emission, and Carbon Flux**

The effects of biochar treatment on soil carbon sequestration, carbon stock, emission, and flux are shown in Table 3 for the studied soils. The results of the treatments show that B + T and B sequestered the maximum amounts of organic carbon, at 6.71 and 5.62 g kg$^{-1}$, respectively. Following this, organic carbon concentrations of 4.65 g kg$^{-1}$ were found after T treatment. Under C, the lowest carbon concentration values were found (Table 3). Additionally, the results in Fig 1 demonstrate that the maximum carbon stock values (27.99 and 26.90 kg ha$^{-1}$) were achieved from B + T and B, respectively. With values of 24.97 and 25.93 g kg$^{-1}$, control (C) and test (T) had the lowest carbon stocks, respectively. In contrast, the control has the most carbon that is being grown (Table 3), followed by the Termitomyces treatment with 4.27% of carbon lost. The least amount of carbon was lost in the B + T and B treatments (Table 3). Table 3 also displayed how biochar treatment affected net carbon flux. The net flow values were consistently negative, with the largest net flux being found in soils with Termitomyces (T) treatment followed by untreated soils (C) treatments. The soils treated with biochar (B) had the lowest net flux, which was measured at -25.35, followed by soils treated with fortified biochar (B + T) at -23.69 (Table 3). According to Odunze et al. (2017) and Mukhina, (2017), intensive crop production causes nutrient mining and lowers carbon sequestration (stock) and net carbon flux. High negative net carbon emissions and fluxes, however, also imply that there may be room to boost soil organic carbon to enhance soil fertility and productivity.

Table 3 Effect of Biochar Treatment on ca Carbon Sequestration, Storage, and Emission

| Treatment | OC (g kg$^{-1}$) | $CO_2$ (%) | Net Flux |
|---|---|---|---|
| B + T | 6.71$^{a}$ | 2.65$^{c}$ | -25.34$^{a}$ |
| B | 5.62$^{ab}$ | 3.21$^{b}$ | -23.69$^{b}$ |
| T | 4.65$^{bc}$ | 4.27$^{a}$ | -21.01$^{c}$ |
| C | 3.69$^{c}$ | 4.92$^{a}$ | -20.70$^{c}$ |
| SE | 0.21 | 0.67 | 2.2 |

B+T = Biochar content + *Termitomyces*, B = Biochar, T = *Termitomyces*, C = Control, SE = Standard Error. Values followed by the same letter are not statistically different at $p < 0.001$.

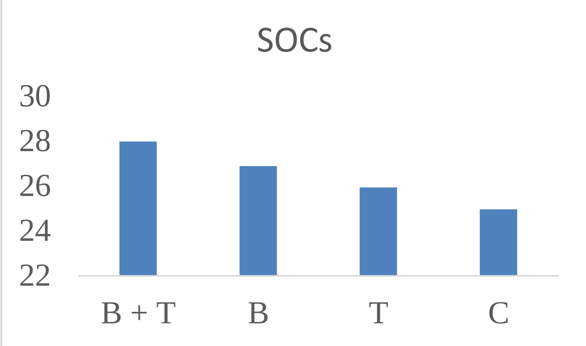


Fig 1. Soil carbon Stocks

Since the B + T application had the largest increase in carbon content in comparison to other treatments, it has the greatest potential to improve soil carbon. This could be a result of the T. albuminosus' capacity to synthesize and break down nutrients from the soil, including carbon. This is consistent with research by Olaniyan et al. (2020), who found that adding biochar to the soil boosts its nutritional content. Additionally, the biochar's vast surface area and microporous structure alter the community structure of fungi by adsorbing nutrients, which in turn gives the fungi room to grow and increase their relative abundance as well as the amount of carbon stored and sequestered (Liu et al., 2018).

**Influence of Biochar Amendments on Yield of Groundnut**

The yield and yield characteristics of groundnuts after treatment application were determined (Table 4), and the results revealed that the treatments had a substantial impact on the biomass production, stover yield, and grain yield of groundnuts. The outcome indicated that a treatment using solely biochar produced a higher biomass yield of groundnuts than the control (P 0.05). The relative biomass yield was 44.83% higher than the control. The second-best yield came from the reinforced biochar, behind the one biochar treatment alone (Table 4). The control had the lowest yield (3.31) while Termitomyces administration produced a statistically marginally better outcome (P 0.05) than the control. Groundnuts treated with biochar alone produced the most stover, followed by groundnuts treated with fortified biochar, and groundnuts treated with Termitomyces produced the least. However, the groundnut having reinforced biochar had a grain yield that was 37.69% higher than the control.

Table 4. Influence of amendments on yield parameters of Groundnut.

| Treatments | Yield Parameters | | |
|---|---|---|---|
| | Biomass yield (t $ha^{-1}$) | Stover yield (t $ha^{-1}$) | Yield per hectare (kg $ha^{-1}$) |
| B + T | 4.63[b] | 3.43[b] | 1130.10[a] |
| B | 5.62[a] | 3.82[a] | 1083.94[b] |
| T | 3.33[c] | 2.38[d] | 941.45[c] |
| C | 3.10[c] | 2.82[c] | 922.72[c] |
| LS | ** | ** | ** |
| SE | 0.14 | 0.06 | 8.48 |

Key: B+T = Biochar content + *Termitomyces*, B = Biochar, T = *Termitomyces*, C = Control, SE = Standard Error. Values followed by the same letter are not statistically different at $p < 0.001$.

Groundnut biomass and yield are considerably increased by rumen biochar-based inoculant carriers with *termimotyces*, which also lowers the need for fertilizer and supports the sustainability of crop production. Further research is advised because there is now insufficient proof that *termitomyces* increased the symbiotic performance of groundnut with rhizobia. According to Egamberdieva et al. (2017), the biochar-based inoculant boosted root and shoot biomass, nodulation, and nutrient uptake. In this study, the use of reinforced biochar greatly increased the grain production of groundnuts. The findings were in line with those of Opachat et al. (2018) who found that applying 10t $ha^{-1}$ of biochar along with fertilizer to barren soil enhanced legume output by 50%. In a similar vein, Aggenehu et al. (2015) found that applying 10t $ha^{-1}$ biochar considerably boosts pod output by 23% when compared to inorganic fertilizer. This may be linked to biochar's capacity to boost microbial activity, decrease nitrogen leaching, reduce soil acidity, improve soil fertility, and improve water retention. The impact of biochar on legume output, however, may not always be favorable as a result of a variety of circumstances, and it is crucial to highlight. While groundnut is important the effect of biochar and T. *albuminosus* fortification on other crops was not investigated. Thus, the broader agricultural implications of these treatments should be explored. Furthermore, the study was conducted in a controlled greenhouse setting, potentially limiting its applicability to real-world field conditions. Thus, caution should be exercised when extending these findings to field applications, ensuring the validation of the approach's effectiveness.

## CONCLUSION

To sum up, treatment B + T led to a rise in carbon sequestration and stock, alongside a consequent decrease in carbon emissions. This enhancement contributed to an improved carbon content in the soil, thereby enriching its nutrient composition. Additionally, by reducing $CO_2$ emissions, this approach mitigated environmental risks like global warming. Furthermore, the groundnut yield saw an increase, positioning this fortification method as a viable alternative to inorganic fertilizers. It's crucial to implement proper management practices to prevent actions that could deplete carbon stocks and degrade soil quality.

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