

Enhancing Nutrients Availability and Performance of Soybean Using Rumen Content Fortified with *Termitomyces albuminosus* in Soils

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Abstract

The research was conducted to improve nutrient availability using rumen content fortified with *Termitomyces albuminosus* and determine the response of Soybean crops to different rumen content fortification approach. The experiment took place in the greenhouse at the Centre for Dryland Agriculture, located at Bayero University in Kano. Rumen content was subjected to charring, and a portion was inoculated with the fungus *Termitomyces albuminosus*. The experiment included four treatment groups: charred rumen content (B), charred rumen content mixed with *T. albuminosus* (B+T), *T. albuminosus* (T), and a control. The treatments were set up following a Completely Randomized Design (CRD). The results showed that there was no significant difference in Potassium and pH of the raw and charred rumen content. However, the result shows a significant difference in phosphorus content, iron, calcium, magnesium and carbon with a percentage concentration of 13.49%, 16.57%, 6.40%, 8.80% and 3.64% respectively in charred rumen content. The fortification of rumen content decreases the nitrogen content by relatively 12.5%. The effect of the amendments and fortifications tested on Soybean crop shows that application of fortified rumen content (B+T) influences growth and yield of the crop relative to the other treatments. Application of fortified rumen content (B+T) significantly increase soybean's height soybean [increased soybean height] (47.73 cm). The unfortified rumen content positively affect the number of leaves of soybean (49.33). Biomass yield increased with application of fortified rumen content in soybean (2.6 t/ha). It is recommended that charred rumen content should be adopted as a higher soil amendment following the enrichment in nutrient composition after pyrolysis than raw rumen content. For efficient utilization of nutrient in the charred rumen content, fortification with fungi *T. albuminosus* under optimal growth condition is recommended for profitable soybean production.

Keywords: Nutrient availability, Rumen content, Biochar, *Termitomyces*, Soybean

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INTRODUCTION

With the world's population steadily increasing and agricultural resources becoming more limited, global food security has emerged as a major concern. Xie et al. (2011) noted that the increase in agricultural production in the last five decades has largely relied on new crop varieties, pesticides, and mineral fertilizers. Interest around the world has been steadily increasing in using organic materials as natural nutrient sources to support crop production. Organic materials offer valuable benefits for all kinds of crops, from staple grains to fruits and vegetables. Animal waste, wood ash, rice husks, sawdust, mill and brewery by-products, along with other crop residues, are excellent sources of organic fertilizers (Management, 2008).

If not properly managed, abattoir waste such as rumen content can negatively impact the environment, public health, animal well-being, and the economy. Many abattoirs struggle with the safe disposal, treatment, and processing of this waste in ways that meet environmental standards. (Tamenech *et al.*, 2017). Effective disposal, treatment, and processing methods are critical not only for managing public health risks and environmental pollution but also for maximizing the economic benefits of abattoir by-products (Tamenech *et al.*, 2017). By using rumen content (digesta) in agricultural crops, it is possible to reduce the pollution load released into water sources, thus benefiting both the environment and agricultural production (Edvan & Jesus, 2015).

In this regard, utilizing cattle digesta as organic matter in agricultural practices would promote the sustainable use of abattoir waste. Lignin makes up a large portion of rumen digesta, which decomposes slowly and releases fewer nutrients during the short growth period of crops in the field (Management, 2008). Basidiomycetes, the fungi behind white-rot decay, are highly effective at breaking down lignin in wood. Extensive research on these white-rot fungi has shown that three extracellular phenoloxidases lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) are crucial in starting the lignin depolymerization process (Yang & Xu, 2012).

Odontotermes formosanus has its own cellulose-digesting system, but its symbiotic fungus enhances the process by working in synergy with the termite's cellulase enzymes. Yang & Xu (2012) found laccase activity in both the symbiotic fungus *Termitomyces albuminosus* and the fungus combs. This study aims to identify the most effective method for enhancing phosphorus availability in rumen content using *Termitomyces* spp. and to evaluate the response of soybean plants to this fortification. This research aim to identify the best fortification approach for enhancing phosphorus availability in rumen content using *termitomyces spp.* and assess response of soybean plant to fortified rumen content.

MATERIALS AND METHODS

Description of the study area

The study was conducted at the teaching and research farm of Bayero University Kano, located between latitudes 11.97932° and 11.98194°N and longitudes 8.41245° to 8.42205°E, at an elevation of 427 to 434 meters above sea level. The area experiences a tropical wet and dry climate (Aw) based on the Köppen classification. Temperature plays a significant role in the region's climate, with an average of about $25 \pm 7^\circ\text{C}$, staying generally warm to hot year-round.

Experimental Materials

The materials used in the experiment are; rumen content obtained from Kano Abattoir, fungi (*Termitomyces albuminosus*) and three legume species; Groundnut (*Arachis hypogaeae*) Variety Samnut 24, Soy bean (*Glycine max*) Variety TGX 1835-10E and Cowpea (*Vigna unguiculata*) Variety 573-1-1 as test crops.

Treatments and Experimental Design

The experiment involved four treatments: charred rumen content, T. albuminosus, a combination of charred rumen content and T. albuminosus, and an untreated control. Each treatment was applied to designated pots at a rate of 10 tons per hectare, with T. albuminosus applied at 10 milliliters per hectare. The setup was replicated three times and arranged using a Completely Randomized Design (CRD) inside a greenhouse.

Soil Sample Collection

Soil samples were randomly taken from a depth of 0–20 cm using a soil auger. These samples were combined to form a composite sample, which was then air-dried, ground using a porcelain mortar and pestle, and sieved through a 2 mm mesh to prepare it for analysis.

Collection and Characterization of Rumen Content

Rumen content was obtained from the Kano Abattoir, air-dried for 4 days, and subsequently moistened and sterilized to eliminate any potential organisms. A portion of the sterilized rumen content was charred using the slow pyrolysis method at temperatures ranging from 360–380°C to produce biochar.

Sample of the biochar was grinded and oxides of different elements were analysed using X-ray fluorescence (XFR) Organic Elemental Analyzer. Nutritional composition of the raw and charred rumen content were analysed to determine the best between the two forms of rumen contents.

Collection and Isolation of *T. albuminosus* spp.

Fungal comb samples were collected from a termite mound located near the Institute of Agricultural Research (IAR) in Zaria and transported to the Mycology Laboratory in the Department of Crop Protection at Ahmadu Bello University, Zaria, for the isolation and identification of *Termitomyces albuminosus*. The comb fragments were first rinsed with running water, then disinfected in a 0.5% sodium hypochlorite solution for three minutes. After that, they were rinsed three times with sterile distilled water (SDW) and air-dried on sterile Whatman No. 1 filter paper. Seven sterile pieces of the comb were placed on Potato Dextrose Agar (PDA) plates, labeled, and incubated at $27 \pm 2^\circ\text{C}$ in a Gallenkamp incubator. The plates were observed daily for fungal growth, and actively growing mycelia were transferred to fresh PDA plates to obtain pure cultures. Microscopic identification of the fungus was carried out following the guidelines of Barnett and Hunter (2006). The pure cultures were then preserved in labeled McCartney bottles and stored in the department's culture herbarium.

For mass production, T. albuminosus was grown in the Molecular Laboratory at the Centre for Dryland Agriculture (CDA), Bayero University, Kano, to generate sufficient fungal biomass. Lastly, the comb material was analyzed using Near Infrared Spectroscopy (NIRS).

Inoculation of Fungi *Termitomyces albuminosus*

Direct spray inoculation was employed to inoculate *T. albuminosus* at a rate of 10 ml per kg of charred rumen content. The inoculated mixture was placed in an incubator for seven days to allow the fungus to fully colonize the substrate and begin breaking down the charred rumen content.

Pot Preparation and Management

The pots were prepared according to the specified treatments and placed in a greenhouse, maintaining a field capacity with a gravimetric moisture content of 20%. Two weeks after applying the treatments, two seeds of the test crops were planted in each pot.

Data Collection

Plant Parameters

Data were collected on various growth and yield parameters, including plant height, number of leaves, number of active and inactive nodules, total biomass, and grain yield.

Soil Analysis

Routine analysis was carried out on the composite sample of the soil before treatments application. After the treatments application when the tested crops were harvested, soil samples properly labelled from all pots were analyzed in the laboratory in order to determine the impact of the treatments applied on soil available phosphorus (P), total phosphorus (TP), Nitrogen (N), Calcium (Ca), Carbon (C), Potassium (K), Iron (Fe) and Aluminium (Al). The samples were dried and ashed with a nitric-perchloric-sulfuric acid mixture to determine nitrogen (N) and calcium (Ca). Phosphorus was measured using vanadomolybdate colorimetry, potassium (K) was determined using a flame photometer, and calcium (Ca) and magnesium (Mg) were assessed through EDTA titration (Tel and Hargarty, 1984). Iron (Fe) was extracted with 0.1 N HCl and analyzed using Atomic Absorption Spectrophotometry (BUCK SCIENTIFIC MODEL 210 VGP). Exchangeable acidity (Al^{3+} and H^+) was measured by titration with KCl.

Characterization of Rumen Content

Air-dried and charred rumen content samples were ground for laboratory assessments. The samples were then digested using a mixture of nitric, perchloric, and sulfuric acids to determine nitrogen (N) and calcium (Ca) content. Phosphorus (P) was analyzed via vanadomolybdate colorimetry, potassium (K) was measured using a flame photometer, and calcium (Ca) and magnesium (Mg) levels were determined through EDTA titration (Tel and Hargarty, 1984). Iron

(Fe) was extracted with 0.1 N HCl and quantified by Atomic Absorption Spectrophotometry

(BUCK SCIENTIFIC MODEL 210 VGP). Exchangeable acidity, including aluminum (Al^{3+}) and hydrogen (H^+), was measured using titration with KCl.

Data Analysis

Descriptive statistics and one-way ANOVA were conducted using JMP 15th Edition software, and differences between treatment means were determined using Tukey's HSD test.

RESULTS AND DISCUSSION

Results

Physical and Chemical Analysis of Experimental Soil

Table 1 shows the physical and chemical properties of the soil used for the experiment. The result shows the particle size distribution of the soil and the textural class which was classified as loam with relative proportion of 42%, 46% and 12% for sand, silt and clay respectively. From the result, soil pH is slightly acidic (pH of 6.10). Magnesium level was 1.92 cmolkg⁻¹. Potassium content of the soil was 1.02 cmolkg⁻¹ rated as average for plant growth. The sodium level of the soil was found to be 0.76 cmolkg⁻¹. Calcium as a soil variable was 6.40 cmolkg⁻¹. The organic carbon of the soil was 3.69 gkg⁻¹. Soil nitrogen was found to be 0.7 g.kg⁻¹ in the soil and the available phosphorus of the soil was 15.95 mg.kg⁻¹, which categorized the soil as highly fertile with respect to available phosphorus. The soil Iron (Fe) was 23.86 mgkg⁻¹ and sulphur level was 0.60%.

Table 1. Physical and chemical analysis of soil

Parameters	Results
% clay	12
% silt	46
% sand	42
Soil Textural Class	Loam
Soil Reaction (Soil pH)	6.10
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
O.M (g kg ⁻¹)	6.36
TN (g kg ⁻¹)	0.73
Available P (mg kg ⁻¹)	15.95
Available Fe (mg kg ⁻¹)	23.86
S (%)	0.60
EA (H^+ + Al^{3+}) (mol kg ⁻¹)	0.11

Role of Fortification Approach on Rumen Nutrient Composition

The effect of fortification on rumen nutritional composition is shown in Table 2. Overall, compositional variables namely phosphorus, iron, calcium and total carbon increased significantly with fortification in comparison to raw rumen content.

However, fortification decreased the total nitrogen content significantly compared to raw rumen content. Variables like pH and potassium did not vary between the raw and charred rumen content. The impact of fortification to phosphorus content, iron, calcium, magnesium, and carbon were relatively higher with 13.49%, 16.57%, 6.40%, 8.80% and 3.64% respectively. Likewise the fortification of rumen content decrease the nitrogen content by relatively 12.5%.

Table 2. Selected properties of raw rumen digest (RRD) and charred rumen digest (CRD)

Parameters	RRD	CRD	P-Value	Relative Change
pH (CaCl ₂)	6.45	6.53	0.1099	
P ₂ O ₅ (%)	4.87 ^b	5.63 ^a	0.0001**	13.49%
Fe (%)	18.70 ^b	21.80 ^a	0.0000**	16.57%
Ca (%)	6.14 ^b	6.56 ^a	0.0446*	6.40%
Mg (%)	1.45	1.59	0.5292	
K ₂ O (%)	0.06	0.04	0.1549	
O.C (%)	18.50 ^b	19.20 ^a	0.0000**	3.64%
TN (%)	4.50 ^a	4.00 ^b	0.0045**	-12.5%

Test significant at 0.05 level of significance are denoted by *, test of significant at 0.01 level of significance are denoted by **. Values without asterisk are not statistically significant. RRD = Raw Rumen Digesta; CRD = Charred Rumen Digesta; RC = Relative change

Plant height of Soybean (*Glycine max*).

Table 5 shows how the different amendments affected soybean plant height. The results indicated that there was no significant effect ($P \leq 0.05$) on plant height at 2 and 6 weeks after planting (WAP). However, the amendments did lead to a significant increase in plant height at 4 and 8 WAP. Amending soybean plants with fortified and unfortified rumen content were statistically the same and produces taller plants than other treatments at 4 WAP, which was 51.97% taller than the treatment used on the shortest plant. At 8 WAP unfortified rumen content gives significantly ($P \leq 0.05$) taller plants (47.7cm) in soybean with relatively 50.23% higher than the control.

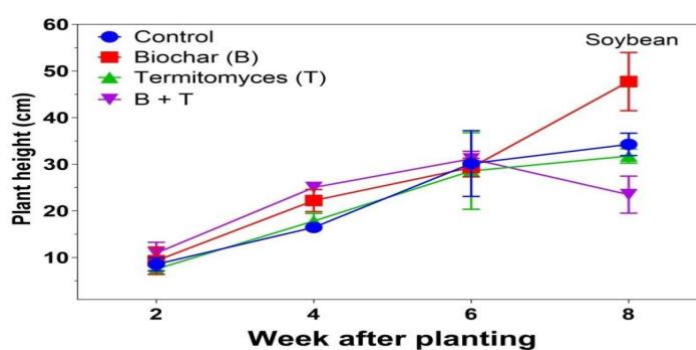


Figure 1. Influence of amendments on plant height of Soybean (*Glycine max*).

Number of leaves of soybean

The result from the experiment table 8 revealed the effect of treatments on number of leaves of soybean. It indicated that except at 2 WAP where no significant difference was observed, application of the treatments had a significant effect on the number of leaves of soybean throughout the study period. At 4, 6 and 8 WAP application of unfortified rumen content proved to be statistically higher than all the treatments and higher than the control with relatively 47.48%, 67.62% and 50.67% respectively.

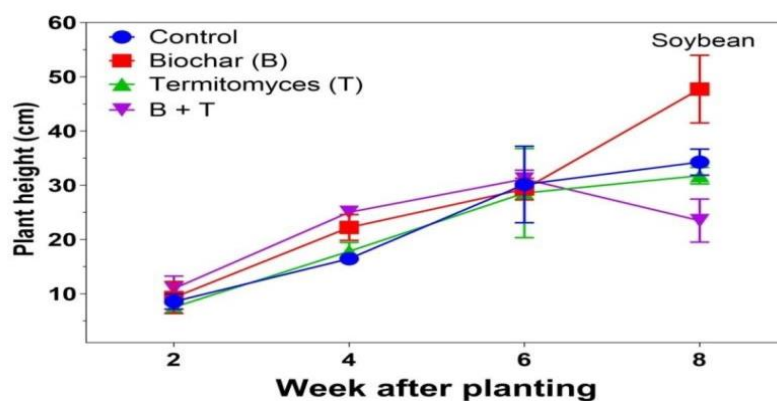


Figure 2. Influence of amendments on number of leaves of Soybean (*Glycine max*).

Root Nodules of Soybean

The result in Table 14, showed the effect of experimental treatments on root nodules of soybean. The result revealed that the treatments have no significant ($P \leq 0.05$) affect on the nodules count, active root nodules and inactive nodules of soybean.

Table 3. Effect of amendments and fortification on root nodules of soybean

Treatments	Noodle Count	Active nodules	Inactive nodules
B + T	7.00 ^b	5.0 ^b	2.66
B	15.00 ^a	11.00 ^a	4.00
T	6.66 ^b	2.00 ^b	4.66
C	11.00 ^{ab}	7.0 ^{ab}	3.66
LS	*	**	NS
SE	1.09	1.32	0.86

B+T = charred rumen content + *T. aluminosus*, B = charred Rumen content, T = *T. aluminosus*, C = Control, LS= level of significant, SE = Standard Error, NS = not significant, ** and * = significant at 0.01 and 0.05 probability level respectively.

Yield characters of Soybean

Table 11 revealed the effect of treatments on biomass, stover and grain yield of soybean. It revealed that, treating soybean with rumen content as amendment significantly increase the yield characters of soybean. The result indicated that application of both fortified and unfortified rumen content gave more biomass yield of soybean with relatively 33.58% than the least treatments. The result further indicated that stover yield is 33.87% relatively higher and significantly ($P \leq 0.05$) higher when treated with both fortified and unfortified rumen content. However, untreated soybean gave the least and was statistically the same as soybean treated with *T. aluminosus*. Grain yield of soybean obtained from the result of the experiment shows that unfortified rumen-content gave significantly (32.75%) higher grain yield than the fortified rumen-content and untreated soybean gave the least yield.

Table 4. Effect of amendments and fortification on yield parameters of soybean.

Treatments	Biomass yield (Mg/ha)	Stover yield (Mg/ha)	Yield per hectare (kg/ha)
B + T	2.65 ^a	1.86 ^a	701.31 ^b
B	2.6 ^a	1.73 ^a	811.46 ^a
T	1.96 ^b	1.33 ^b	558.77 ^c
C	1.76 ^b	1.23 ^b	545.80 ^c
LS	**	**	**
SE	0.11	0.087	17.87

B+T = charred rumen content + *T. aluminosus*, B = charred Rumen content, T = *T. aluminosus*, C = Control, LS= level of significant, SE = Standard Error, NS = not significant, ** & * = significant at 0.01 and 0.05 probability level respectively, Means followed by same letter within a column are statistically the same.

Effect of amendment and fortification of soybean on residual soil properties.

Effect of amendment tested on soybean on residual soil properties is shown in table 17. It expressed that significant ($P \leq 0.05$) difference was observed on nitrogen level of the soil after treatments application. The result further revealed that application of fortified rumen-content was significantly higher than all treatments and gave 32.63% relatively more nitrogen when tested on soybean than control. Also Significant ($P \leq 0.05$) difference was observed on the Potassium level of the soil after treatments, application of fortified rumen-content was found to be statistically (P

≤ 0.05) different in potassium level while all other treatments were statistically ($P \leq 0.05$) the same and gave the least level of residual potassium in soybean soil.

Residual carbon increased by the application of fortified rumen-content and significantly ($P \leq 0.05$) higher by 40.53% more carbon than the least treatment. Application of fortified rumencontent and unfortified rumen-content is statistically ($P \leq 0.05$) the same and significantly ($P \leq 0.05$) higher in the residual total phosphorus than the other treatments and this was relatively 48.8% more than the control. Application of *T. aluminosus* was the least and statistically ($P \leq 0.05$) the same as the untreated soybean. Available phosphorus proves to be significantly ($P \leq 0.05$) higher in soybean treated with fortified rumen-content followed by the unfortified rumen-content while the untreated groundnut was the least in terms of available phosphorus with relatively 57.99% lower. Fortified rumen content increase residual soil calcium of soybean by 22.81% and significantly higher than the control, all other treatments are higher than the untreated soybean.

Residual Iron and Aluminium level of the soil was significantly ($P \leq 0.05$) higher in soybean treated with *T. aluminosus* followed by the untreated cowpea. Fortified and unfortified rumen content were the least.

Table 5. Influence of amendment tested on soybean on residual soil properties.

Treatments	N	% K%	C %	TP (mg.kg ⁻¹)	AP (mg.kg ⁻¹)	Ca (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Al ³⁺ cmol.kg ⁻¹
B + T	1.05 ^a	1.058 ^a	3.971 ^a	631.97 ^a	38.43 ^a	8.524 ^a	14.54 ^b	0.026 ^b
B	0.62 ^b	1.036 ^b	3.662 ^{ab}	627.82 ^a	34.67 ^{ab}	8.127 ^b	14.13 ^b	0.030 ^b
T	0.56 ^b	1.034 ^b	3.399 ^c	393.94 ^b	16.85 ^{bc}	6.623 ^c	21.79 ^a	0.070 ^a
C	0.65 ^b	1.051 ^a	3.465 ^{bc}	424.24 ^b	12.30 ^c	6.579 ^c	23.32 ^a	0.080 ^a
LS	**	**	**	**	**	**	**	**
SE	0.06	0.029	0.029	31.03	4.44	0.69	0.49	0.005

B+T = charred rumen content + *T. aluminosus*, B = charred Rumen content, T = *T. aluminosus*, C = Control, LS= level of significant, SE = Standard Error, ** & * = significant at 0.01 and 0.05 probability level respectively, Means followed by same letter within a column are statistically same.

DISCUSSION

Initial Soil Analysis

The physical and chemical properties of soils in BUK orchard used in the experiment. The result of the particle size distribution of the soil shows that textural class of the soil is loam with the proportion of 42%, 46% and 12% for sand, silt and clay respectively. From the result of the soil is slightly acidic with pH of 6.10, magnesium level was 1.92 cmol kg⁻¹ which categorized as Average (NSPFS 2005). Potassium content of the orchard soil was 1.02 cmolkg⁻¹ which were rated as average for plant growth according to (Kamaluddeen *et al.*, 2020). The sodium level of the soil was found to be 0.76 cmolkg⁻¹ and considered high as reported by (Kamaluddeen *et al.*, 2020) Calcium of the soil was 6.40 cmolkg⁻¹, which was classified as average for proper soil productively. Organic carbon is an important component of the soil, which serves as a storehouse for plant nutrient. The organic carbon of the orchard soil was 3.69 g kg⁻¹, which classified the soil as very rich in organic carbon. Soil nitrogen content helps soil productivity, deficiency of which affect the crop growth and yield. The experimental soil nitrogen was found to be 0.7 g.kg⁻¹ which is considered average for productive soils. (Kamaluddeen *et al.*, 2020). The available phosphorus of the orchard soil was 15 mg.kg⁻¹, which categorized the soil as high with respect to available phosphorus. The soil was categorised as low in available iron 23.86 mgkg⁻¹ and sulphur level of the soil was categorized as high.

Role of Fortification Approach on Rumen Nutrient Composition

Compared to other organic materials, raw rumen content (RRC) is considered a residue rich in nutrients and organic matter. Rumen content can be used in agriculture as a soil amendment, among other functions. According to Awodun, (2008) rumen content has residual nutrient with slower decomposition rate than most organic manure such as cowdung. Hence the need to determine the best approach for proper utilization of nutrients in rumen content. (Fachini *et al.*, 2021) reported that pyrolysis enriched macro and micronutrients levels of organic material. The results obtained from physical-chemical analysis of rumen content in this study indicate a clear influence of pyrolysis on the properties of rumen content. After the pyrolysis, the pH value of the rumen content was not significantly altered but slightly shifted close to neutrality from 6.43-6.53 for raw rumen content (RRC) and charred rumen content (CRC) respectively. This is due to the low temperature used in the pyrolysis, as report from literatures that pH of materials increased with increasing temperature of the pyrolysis. This conformed with the findings of Faria, (2018) that pH of biochars obtained at low temperature (>300°C) are not greatly altered, while pH increase is obtained at higher pyrolysis temperatures (<300°C). P₂O₅ concentration in CRC was significantly higher with relative increase of 13.49% than in RRC. This increase typically continues up to 700°C, after which phosphorus losses occur due to volatilization. When compared to biochar from various feedstocks and other soil amendments, CRC contains higher levels of phosphorus, making it a promising candidate for use as a phosphorus fertilizer. As the pyrolysis temperature rises, organic phosphorus is converted into inorganic phosphorus, and a greater proportion of non-apatite inorganic phosphorus is transformed into apatite phosphorus, including compounds like Ca₃(PO₄)₂, Ca(H₂PO₄)₂, Mg₃(PO₄)₂, Ca₃Mg₃(PO₄)₄, and Ca₅(PO₄)₃OH. In agreement with (Yuhan *et al.*, 2022) temperature and nature of pyrolysis material determine the Phosphorus enrichment after pyrolysis. Similarly, pyrolysis of dewatered material at 700°C had the highest apatite phosphorus and highest Ca₅(PO₄)₃OH content containing 35.8% of total phosphorus than other pyrolysis products (Yuhan *et al.*, 2022). Relative iron content of the CRC also increases with relatively 16.57% than the RRC because of the higher degree of volatilization. Reactions during pyrolysis can affect the iron content of rumen content, as high temperature can cause decomposition of organic compounds, and these compounds chemically bound to iron in the material may result in change in its chemical form. The specific effect of iron content depend on the composition and conditions of the material being treated. Katrin *et al.* (2019) found that the relative iron content of the material increases with elevated pyrolysis temperatures, due to a higher degree of devolatilization, resulting in 3.8 wt% Fe for FeHTC-800. After pyrolysis, the Mg and (Ca) were found to be higher in CRC. The calcium (Ca) content was higher in CRC compared to RRC with relative increase of 6.4%. Magnesium does not undergo any chemical transformations during pyrolysis of rumen contents; however, if magnesium compounds or alloys are present in the material that is undergoing pyrolysis, indirect effects on magnesium content may occur due to the high temperatures of pyrolysis which can cause physical separation or change in the chemical state of the magnesium compound with possible liberation of magnesium or its transformation into different chemical forms. As demonstrated by Yuhan *et al.*, 2022, Ca and Mg increased with increasing temperature from 300°C to 700°C. The increased amount of mineral relative to organic material is mostly due to the loss of organic materials at higher

temperatures (Figueiredo *et al.*, 2017) and the relative enrichment in minerals in the resulting biochar (Li *et al.*, 2018). The metal elements Ca, Mg, Al, and Fe may react with phosphorus, forming phosphates like $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}(\text{H}_2\text{PO}_4)_2$, AlPO_4 , $\text{Mg}_3(\text{PO}_4)_2$, $\text{Ca}_3\text{Mg}_3(\text{PO}_4)_4$, and FePO_4 , immobilizing phosphorus in biochar. The presence of metals (Ca, Mg, Fe, and Al) in organic materials promotes the formation of minerals during pyrolysis, which affects the morphology of phosphorus in biochar (Yuhan *et al.*, 2022). RRC was low in potassium content and did not respond significantly to pyrolysis. Consequently, the charred rumen content (CRC) was also found to be poor potassium. This is one of the limitation when using CRC as amendment. Hence the need for alternatives to provide potassium sources. Enriching organic material with potassium fertilizers and/or co-pyrolysis of organic material with other materials that are rich in Potassium are necessary in organic material that are low in potassium (Najafi *et al.*, 2020), this is to supplement potassium as an essential component of many organic compounds, such as potassium salts, which are commonly found in organic matter.

Result of this study further revealed that pyrolysis enhances the organic carbon of rumen content by 3.65%, this occur as a result of carbonization of rumen content. Furthermore, Xi *et al.* (2020) found that as the pyrolysis temperature of biochar increases and its application amount grows, the improvement in soil organic carbon content becomes more pronounced. The pyrolysis cause the breakdown of complex organic compounds in the rumen content into a simpler ones, with the release of volatile organic compounds as gases. The raw rumen content become carbon rich residue that is stable and can be used as a soil conditioner or for carbon sequestration.

This result reported the significant decrease in total nitrogen after pyrolysis of rumen content. Charred rumen content (CRC) decrease in nitrogen by the relatively 12.5% less than the raw rumen content (RRC), resulting from high mobility of nitrogen in idle state, *let along* under pyrolysis where higher temperature is introduced. Several factors contribute to nitrogen loss during pyrolysis. Elevated temperatures can cause nitrogen to be lost as nitrogen-containing gases, such as ammonia (NH_3) and nitrogen oxides, which are released during the breakdown of nitrogen-rich organic compounds. Nitrogen losses during pyrolysis are often substantial, ranging from 19% to 77% of the initial nitrogen content (Liu *et al.*, 2015). Additionally, some studies have reported that applying biochar at high rates can lead to nitrogen immobilization and reduced nitrogen availability, due to its high carbon-to-nitrogen (C/N) ratio, potentially resulting in lower crop yields (Lehmann *et al.*, 2002; Li *et al.*, 2018).

Influence of Amendments on Growth of Soybean

The amendment of legumes with charred rumen content (fortified and unfortified charred rumen content) proves significant increase in the height of groundnut, cowpea and soybean with higher relative increase (Figure 1). Fortified rumen (B+T) content gave the tallest plants of soybean while *T. albuminosus* and the untreated plant (control) gave the shortest plants. The performance of fortified rumen content is attributed to the ability of *T. albuminosus* to decompose complex organic compounds in exchange for carbon and consequently making most nutrient available in the soil and utilized by the legume crops. Biochar application has been shown to enhance nitrogen uptake from the soil (Sadaf *et al.*, 2017), with higher nitrogen concentrations leading to an increase in

8PSII and qP, while decreasing NPQ (Liu *et al.*, 2013). his effect may be attributed to biochar's ability to enhance leaf chlorophyll content, which supports the synthesis of enzymes and electron carriers crucial for photosynthetic carbon assimilation, thereby improving the photosynthetic efficiency of leaves (Sadaf *et al.*, 2017). Biochar enhances soil physical and chemical properties such as cation exchange capacity (CEC), bulk density, water retention, permeability, and biological activity to promote plant growth with increased crop productivity (Behera *et al.*, 2015). Biochar is also resistant to decomposition and can remain in the soil for long periods of time, providing extended agricultural benefits (Steiner *et al.*, 2007). To further enhance biochar, organisms such as *T. albuminosus* are often added because this fungus naturally accelerates the degradation and decomposition of organic matter including biochar at a faster rate than it would degrade on its own (Otani, 2017) as described above. This is in line with the observation that symbiotic fungi can degrade lignin, thus rendering cellulose more readily decomposable than native cellulose (Ohkuma 2003)

In support of this hypothesis, Van Zwieten *et al.* (2010) showed that biochar enhances plant growth through supplying nutrients that stimulate plants to grow; the elevated cowpea height could be attributed to the improved soil fertility as a result of higher levels of biochar in the soil. This is further supported by Tariku *et al.* (2017), who reported that the application of biochar increased garden pea growth, including plant height and leaf number, similar to our results where fortified rumen content most significantly enhanced leaf numbers in groundnut and soybean, while unfortified rumen content resulted in elevated leaf numbers in cowpea.

Multiple studies have shown that applying biochar can enhance plant photosynthesis, chlorophyll concentration, and transpiration rates (Agegnehu *et al.*, 2015). Sarma *et al.* (2017) reported that biochar notably improved the photosynthetic rate of plants, indicating that its application can boost both soil nutrient availability and the physiological performance of legumes.

Influence of Amendments on Nodulation of Soybean

Biochar application enhances legume modulation, as evidenced by this study that showed application of fortified rumen content increased more root nodules of soybean. Results also demonstrated significant differences between treatments and control but no difference between the two forms of applied rumen content. The increase in nodule number may be due to the synergistic effect of biochar with fortification by *T. albuminosus*, which probably improved nutrient levels either through altering plant metabolism (thus modifying root exudate composition) or improving nutrient solubility and availability (Kumar *et al.*, 2022). Rumen biochar could also promote nodulation by retaining soluble nutrients in the soil that are essential for root development and nodule initiation (Asuming-Brempong *et al.*, 2023). Moreover, the added rumen biochar

(especially when enriched with microorganisms) could potentially act as a source of micronutrients such as molybdenum that is required for rhizobia-mediated nodule formation; similar results were reported from other studies where biochar application increased nodulation in legumes (white clover (Rillig *et al.*, 2010), red clover (Mia *et al.*, 2014), but we also observed here that the amendments did not have a significant impact on soybean nodule count, which is different from an observation of Tagoe *et al.* (2008) who found increased nodulation in biochar-amended soils. The quality of the biochar, application rates and type of legumes as well as soil conditions can influence its effect on nodulation. Some studies have shown positive effects, while other have no significant impact or even negative effects on nodulation.

Influence of Amendments on Yield and Yield Components of Soybean

Legume (soybean) yield components response to application of rumen content indicated that application of fortified rumen content gave the highest biomass yield in soybean. Rumen biocharbased inoculant carriers with *Termitomyces* have been shown to significantly increase biomass and soybean yield, reducing fertilizer demand and promoting crop production sustainability. However, there is no conclusive evidence that *T. albuminosus* enhances the symbiotic relationship between legumes and rhizobia (biofertilizers), suggesting that further research is needed. Ghazi and Karnwal (2017) investigated the use of rice straw-derived biochar as a carrier for rhizobia and found that it enhanced bacterial colonization and survival. Similarly, biochar-based inoculants were reported to improve root and shoot biomass, nodulation, and nutrient uptake (Tripti *et al.*, 2015). In the present study, the application of rumen content improved soybean grain yield, while fortified rumen content notably enhanced groundnut and cowpea yields compared to the control. In contrast, unfortified rumen content specifically increased soybean grain yield. These findings align with those of Yamato *et al.* (2006), who reported a 50% increase in legume yields when 10 t ha⁻¹ of biochar was applied along with fertilizer in poor soils. Likewise, Agegnehu *et al.* (2015) found that applying 10 t ha⁻¹ of biochar increased peanut pod yield by 23% relative to the use of inorganic fertilizer. Furthermore, Tan *et al.* (2018) showed that the application of rice husk and cottonseed husk biochars at 50 t ha⁻¹ increased peanut yields by 16.8% and 14.4%, respectively, compared with treatments without biochar; application of biochar also enhanced yields of legumes, wheat, maize, and rice by around 30%, 11%, 8%, and 7%, respectively (Liu *et al.*, 2013), primarily due to the improvement in soil fertility, water retention, reduction in soil acidity, stimulation of microbial activity, and decrease in nitrogen leaching. Still, it must be noted that biochar can sometimes have a negative effect on legume yields; this depends on the impact of biochar application on both soils and plants.

Influence of Amendment Tested on Soybean on Residual Soil Properties

Biochar is known to improve long-term soil fertility by raising the CEC and holding nutrients such as calcium, magnesium, and potassium (leading to greater nutrient levels over time). The impact of biochar depends on its properties and the type of soil it is applied into. These two forms of nitrogen are important for plant growth and microbial activity (Sun *et al.*, 2019), and once incorporated into the soil, their movement, distribution, and leaching depend on a variety of physical and chemical characteristics of the biochar itself and its interactions with the soil matrix as well as application rate and type of soil (Li *et al.*, 2020). When fortified rumen content was tested with soybean, residual soil nitrogen increased by 40.95% compared to controls, indicating that fortified rumen content significantly improved soil nitrogen content. The high pore structure and large surface area of biochar enhance its ability to adsorb nitrogen and reduce leaching, thereby increasing the N content in the soil (Abujabhah *et al.*, 2018). It is also possible that the increase in nitrogen content resulting from *T. albuminosus* fortification may be related to other factors such as texture of the soil, initial microbial biomass and nutrient levels, and biochar type.

Wardle *et al.* (2008) reported that addition of biochar stimulated microbial growth in forest soils in northern Sweden; our results are similar: higher application rates of biochar increased microbial biomass, which may improve carbon availability in the soil and promote microbial growth. Durenkamp *et al.* (2010) observed that biochar could reduce soil microbial biomass, which aligns with findings by Tian *et al.* (2023), who reported that while biochar application increased soil total nitrogen and ammonium nitrogen, it reduced nitrate nitrogen content by 2.6–12.5%. This contrasts with Wang *et al.* (2020), who found that increased biochar application rates significantly raised soil nitrate and ammonium nitrogen contents.

In this study, fortified biochar also enhanced soil carbon, phosphorus, and potassium levels relative to other treatments, with these increases being statistically significant. This effect may stem from the nutrient content of the biochar itself (Table 1) and the ability of *T. albuminosus* to degrade and synthesize nutrients from the soil or rumen content. Gao *et al.* (2021) found that biochar applications increased potassium content in the soil and influenced the fungal community structure, as biochar adsorbed nutrients and provided space for fungi to grow, boosting their relative abundance. Wu *et al.* (2022) noted an increase in the soil total carbon, nitrogen as well as potassium with increase in biochar, thus, improving the nitrogen and potassium availability within the rhizosphere. Biochar's rich composition allows it to provide various exogenous nutrients for plant use (Sohi *et al.*, 2010).

The large surface area of biochar and its microporous structure enhances its nutrient adsorption capacity (Peng *et al.*, 2019), reducing nutrient loss and helping retain soil moisture and nutrients, while limiting nutrient migration and transformation (Liu *et al.*, 2015).

In this experiment, aluminum and iron content in soils treated with legumes was found to be significantly higher in plants treated with *T. albuminosus* and untreated plants, while plants treated with fortified and unfortified rumen content showed the least amount. This result supports the idea that biochar increases soil CEC and raises soil pH, leading to the precipitation of exchangeable and soluble aluminum and iron as insoluble hydroxides, which reduces their concentrations in the soil solution. Shetty *et al.* (2021) observed a similar reduction in soluble and exchangeable aluminum forms when biochar (wood biochar at 20 t ha⁻¹) was added, playing a key role in aluminum detoxification. However, Novak *et al.* (2018) reported

negligible effects of biochar in reducing aluminum toxicity in acidic mine soils enriched with aluminum. The effectiveness of biochar in ameliorating aluminum toxicity depends on the calcium carbonate equivalent of the biochar.

CONCLUSION

Sustainable agriculture aims to maintain or enhance soil fertility without depleting natural resources. Recycling nutrients, helps conserve valuable resources like nitrogen, phosphorus, and potassium, which is essential for agricultural production. These nutrients are often mined or produced through energy intensive processes, so recycling and utilizing organic materials reduces the need for resources extraction. Organic materials such as rumen content, crop residues, compost manure among others, can enhance soil structure, moisture retention and microbial activities. By recycling the nutrient in these materials locally, farmers can reduce their carbon footprint and energy consumption, contributing to climate change. This promote the idea that waste from one process can become a valuable input for another, reducing waste generation and negative environmental consequences. In this study, recycling rumen content have proved to have positive impact in legume growth and yield, and increased soil health by positively influencing the availability of essential nutrients of the soil.

Compliance with Ethical Standards

Peer Review

This article has been reviewed by independent experts in the field using a rigorous double-blind peer review process.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed equally to the study design, data collection, analysis, and manuscript preparation.

Ethics Committee Approval

Ethical approval was not required for this study.

Consent to Participate / Publish

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Data Availability

Not applicable.

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