

# Phytochemical and Physicochemical Characterization of Selected Grass Species and Their Associated Soils from Agro-Ecological Zones of Benue State, Nigeria: Implications for Dual-Function Biopesticide–Biofertilizer Development

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**Abstract:** Grass biomass is a relatively untapped source of bioactive secondary metabolites and lignocellulosic substance that has the potential to be used as feedstock in dual-purpose biopesticide- biofertilizer inputs through pyrolysis (biovinegar and biochar). The aim of the study was to identify phytochemical profile, proximate composition and soil physicochemical properties of the four grass species namely; *Imperata cylindrica*, *Pennisetum purpureum*, *Bambusa vulgaris*, and *Panicum virgatum* that had been harvested in five agro-ecological zones of Benue State, Nigeria, as a preliminary evaluation of their potential as feedstocks for biovinegar and biochar production. Samples of soil and plants were obtained in Makurdi, Otukpo, Katsina-Ala, Obi, and Gboko in the middle of the growing season (June-August 2024). The pH of the soils was moderately low with the values of 5.78 (Otukpo) to 6.31 (Obi), total nitrogen was between 0.09 and 0.10 g kg<sup>-1</sup>; available phosphorus was between 8.90 and 10.20 mg kg<sup>-1</sup>; and exchangeable potassium was between 0.23 and 0. ANOVA One-way ANOVA indicated that there are significant inter-zonal variations in organic carbon (F(4,10) = 28.7, p < 0.001) and available phosphorus (F(4,10) = 6.4, p < 0.05), but total nitrogen was relatively homogeneous across sites (p > 0.05). Phytochemical screening revealed the presence of glycosides and flavonoids in all the four species, the presence of phenolics was confirmed with ferric chloride test and presence of carotenoids was also identified as a separate group of terpenoid pigments. The values of crude protein, crude fibre and ash were 7.2-18.4%, 33.2-42.1% and 6.3-10.3% respectively among species using proximate analysis. The Pearson correlation analysis showed a strong positive relationship between soil organic carbon and total phytochemical yield score (r = 0.82, p < 0.01, n = 20). *Bambusa vulgaris* was the most promising dual-function feedstock species of the species assessed, whereas *Imperata cylindrica* is suggested as an exclusive source of biochar. These results offer a scientific foundation of region-specific feedstock choice in bio-agricultural development of inputs in the Guinea savanna agroecological belt in Nigeria.

**Keywords:** *Phytochemical Screening; Grass Biomass; Bio-Pesticide; Bio-Fertiliser; Biochar; Pyrolyneous Acid; Guinea Savanna Soils; Benue State Nigeria; Secondary Metabolites; Sustainable Agriculture.*

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## I. INTRODUCTION

The economy of Benue State is agrarian with a Guinea savanna agroecological belt that includes alluvial plains and sandy-loam soils that can support various crop systems such as cereals, vegetables, tubers and forages [1]. The smallholder farming industry that contributes more than 80 percent of farm produce is becoming more and more limited by the loss of soil organic matter, dwindling crop production, and rising production cost of synthetic agrochemicals [2,3]. The amount of organic carbon in most of the Guinea savanna soils in the Benue Valley has been found to be lower than the 1.0% that constitutes smallholder soils of the tropics, and this lowers the microbial activity and the capacity of the soils to recycle nutrients [1,4].

The worldwide need to move towards synthetic pesticides and fertilizers to environmentally friendly solutions has triggered a crucial concern that has generated a lot of interest in bio-based agricultural inputs [5]. Two of the most promising innovations in this area are biovinegar (pyroigneous acid, liquid pyrolysis condensate of biomass), and biochar (solid carbon-rich product of slow pyrolysis), which have the potential to form a complementary dual system that can both suppress pests and increase soils [6,7]. Biovinegar acts as a pesticide with its organic acid (acetic, formic, propionic), phenolic, and furan derivatives, whereas biochar enhances soil fertility by increasing the cation exchange capacity, microbial communities, and nutrient retention of sandy soils [8,9]. Grass biomass, producing both fractions, is a very appealing feedstock due to the complementarity of these two products of pyrolysis, which is why it is so appealing to the development of integrated bio-inputs.

The major vegetation in the Guinea savanna is grass species, which is a rich source of biomass in the form of a seasonally renewable resource in Benue State. The comparison of grasses species in West and Central African savanna areas has revealed significant differences in secondary metabolite profiles, proximate composition and lignocellulosic structure of species variants indicating that bioactive compound yields and pyrolysis products can be maximised by selective feedstock choice [10,11]. *Pinisetum purpureum* (Elephant grass) has been extensively researched due to its high biomass productivity, and is reported to contain secondary metabolites, whereas *Bambusa vulgaris* (Bamboo) is well known to have an excellent crude protein and mineral content compared to most herbaceous grasses [12,13]. *Imperata cylindrica* (Spear grass) is an invasive high-fibre species that has been reported to have allelopathic and insecticidal effects and thus is a potential biochar feedstock as well as source of bioactive compounds that suppress pests [14]. *Panicum virgatum* (Switchgrass), which is native to North America, has been also introduced as a high yield lignocellulosic energy crop in West Africa (some parts of it) with high growth rates, stress tolerance, and a known fibre-

rich composition [15]. The accessions assessed in the study were procured in trial plots that had been set up as part of institutional research programmes in Joseph Sarwuan Tarka University, Makurdi which offered a controlled set on which to compare with the three native species.

Polymeric matrix systems that refer to both the natural lignocellulosic matrix of grass biomass itself and biodegradable synthetic polymer encapsulants have been suggested as controlled-release systems of bioactive agrochemicals [16]. Porous biochar, with polymeric coatings, has the potential to tune the release kinetics of pesticidal and nutrient substances into the soil environment, prolonging the efficacy and minimizing the frequency of field application [17].

Though the grass species in Benue State have ecological abundance, and there has been an increasing regional trend in bio-based agricultural inputs, no organized comparative study of phytochemical profiles and soil physicochemical properties across different agro-ecological zones has been done on the candidate species that will be examined in this paper. There is also a lack of understanding of which zones yield grass biomasses with the greatest bioactive compound concentrations or the most appropriate proximate composition to produce biovinegar and biochar using pyrolysis. The objectives of this study were therefore to: (i) describe the soil physicochemical characteristics of five sampling areas in Benue State; (ii) select four native and exotic grass species as sources of important secondary metabolites; (iii) establish their proximate composition; and (iv) establish the relationships between soil organic carbon and grass phytochemical content as a foundation of informed feedstock selection in bio-agricultural input development.

The study area and sample collection will be presented in 2.1.2.

## II. MATERIALS AND METHODS

### ➤ Study Area and Sample Collection

In Benue State, North-Central Nigeria, the soil and grass were sampled in five agro-ecological zones Makurdi (Zone A), Otukpo (Zone B), Katsina-Ala (Zone C), Obi (Zone D) and Gboko (Zone E) (Fig. 1). Table S1 (Supplementary Material) has the GPS coordinates, elevation and land-use classification of each sampling zone. Briefly, Makurdi (07°44'N, 08°32'E; ~100 m asl) represents peri-urban agricultural land on the Benue floodplain; Otukpo (07°11'N, 07°42'E; ~152 m asl) is subsistence cropland in the southern Guinea savanna; Katsina-Ala (07°10'N, 09°17'E; ~123 m asl) is mixed farmland-grassland in the Middle Benue Trough; Obi (07°27'N, 09°27'E; ~110 m asl) is Guinea savanna grassland in Nasarawa–Benue border zone; and Gboko (07°20'N, 09°00'E; ~131 m asl) is agricultural grassland in the northern Guinea savanna.

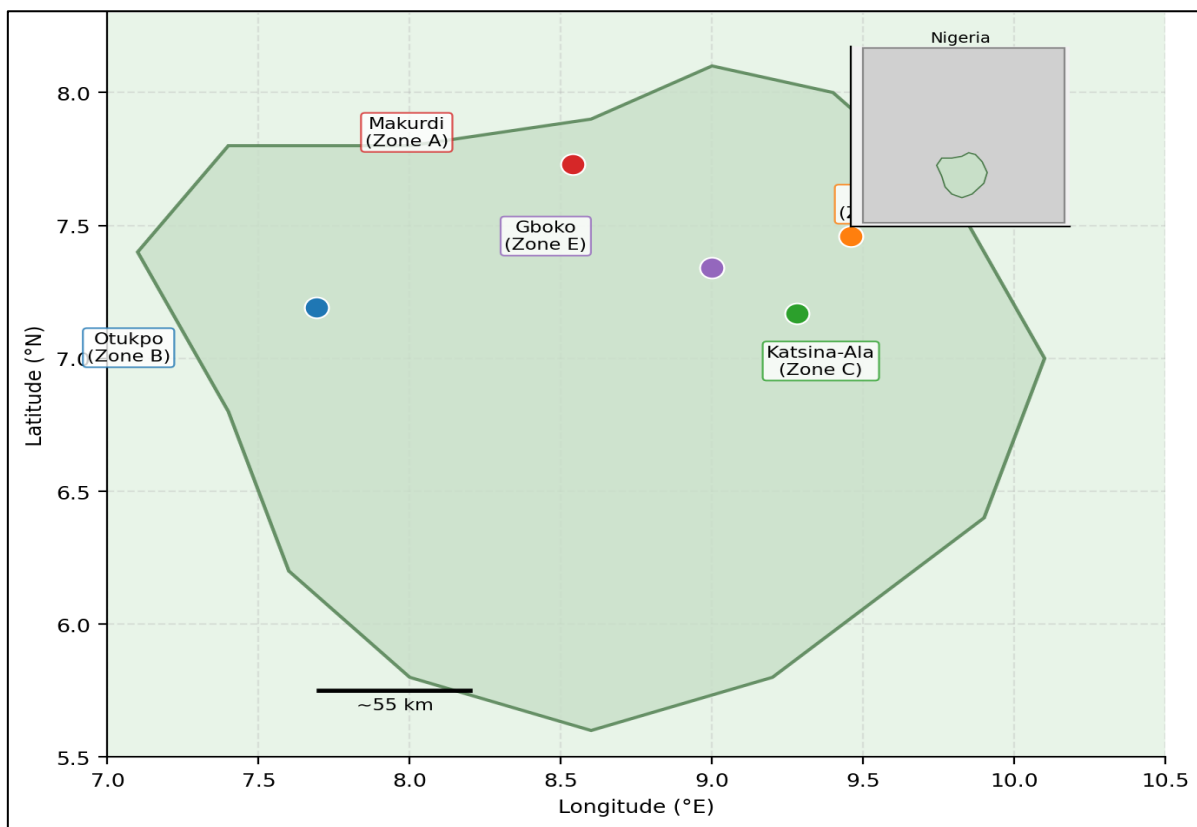


Fig 1 Map of Benue State, Nigeria, Showing the Five Agro-Ecological Sampling Zones (A–E) Used in this Study. Inset Shows the Location of Benue State Within Nigeria.

The targeted grass species included Spear grass (*Imperata cylindrica*), Elephant grass (*Pennisetum purpureum*), Bamboo (*Bambusa vulgaris*), and Switchgrass (*Panicum virgatum*). Sampling was conducted during the peak growing season (June–August 2024) using stratified random sampling. At each zone, three sampling plots of 50 m × 50 m were established, and within each plot, five random soil sub-samples were collected from 0–20 cm depth and composited to yield one representative soil sample per plot, giving n = 3 composite soil samples per zone (N = 15 total soil samples). Soil samples were air-dried at room temperature, ground, and passed through a 2 mm stainless-steel sieve. Grass biomass was harvested from three replicate plots per zone, air-dried at 60°C for 72 hours, combined proportionally, and ground to yield one representative sample per species per zone (n = 3 per species per zone; N = 60 total biomass samples). All laboratory analyses were conducted in triplicate.

➤ *Physicochemical Properties of Soil*

Soil samples were analysed at the College of Agronomy, Joseph Sarwuan Tarka University, Makurdi. The physicochemical properties determined and the respective analytical procedures are described below.

• *Particle Size Distribution*

Soil particle size analysis was performed by the hydrometer method of Bouyoucos [18]. Air-dried soil (50 g) was dispersed with sodium hexametaphosphate (Calgon) solution and hydrometer readings taken at 40 seconds ( $H_1$ ) and three hours ( $H_2$ ) after mixing. Suspension temperature

was recorded simultaneously at 40 seconds ( $T_1$ , °F) and three hours ( $T_2$ , °F) for temperature correction. The particle size fractions were calculated as follows:

$$\text{Sand (\%)} = 100 - [H_1 + 0.2(T_1 - 68) - 2.0] \tag{1}$$

$$\text{Clay (\%)} = H_2 + 0.2(T_2 - 68) - 2.0 \tag{2}$$

$$\text{Silt (\%)} = 100 - (\% \text{Sand} + \% \text{Clay}) \tag{3}$$

Where  $H_1$  = hydrometer reading at 40 seconds ( $\text{g L}^{-1}$ );  $H_2$  = hydrometer reading at three hours ( $\text{g L}^{-1}$ );  $T_1$  = suspension temperature at 40 seconds (°F);  $T_2$  = suspension temperature at three hours (°F); and 0.2 is the hydrometer correction factor for temperature deviation from 68°F [18]. Textural class was assigned using the USDA textural triangle based on the calculated sand, silt, and clay percentages.

• *Soil pH*

Soil pH was measured in a 1:1 soil-to-distilled-water suspension using a calibrated glass electrode pH meter (Mettler Toledo FiveEasy™), following the glass electrode method described by IITA [19]. The suspension was allowed to equilibrate for 30 minutes with occasional stirring before measurement.

• *Organic Carbon and Organic Matter*

Organic carbon was determined by the Walkley–Black chromic acid wet oxidation method [20]. The organic carbon content (%) was calculated as:

$$\%OC = [(V_{blank} - V_{sample}) \times N \times 0.003 \times 100] / (W \times f) \quad (4)$$

Where  $V_{blank}$  = volume (mL) of  $FeSO_4$  used in blank titration;  $V_{sample}$  = volume (mL) of  $FeSO_4$  used in sample titration;  $N$  = normality of the  $FeSO_4$  solution;  $0.003$  = milliequivalent mass of carbon ( $g\ meq^{-1}$ );  $W$  = oven-dry equivalent mass of soil (g); and  $f = 0.77$ , the recovery factor accounting for incomplete oxidation of organic carbon by the dichromate reagent [20,21]. Organic matter (%) was obtained by multiplying organic carbon by the Van Bemmelen factor of 1.724, assuming tropical soil organic matter contains approximately 58% carbon.

- *Total Nitrogen*

Total nitrogen was determined by the macro-Kjeldahl method [22]. Five grams of soil were digested with concentrated  $H_2SO_4$  in the presence of  $K_2SO_4$  and a mercury catalyst at elevated temperature until the digest ran clear. After cooling, the digest was made to volume with distilled water, and ammonia was distilled into 2%  $H_3BO_3$  with 10 N NaOH, then back-titrated with 0.01 N  $H_2SO_4$  to determine ammoniacal nitrogen content.

- *Available Phosphorus*

Available phosphorus was extracted by the Bray-1 method [23]. The soil suspension was centrifuged at 2000 rpm for 15 minutes; the supernatant was mixed with ammonium molybdate solution and stannous chloride ( $SnCl_2$ ) as a reducing agent. Absorbance was measured at 660 nm on a UV-Vis spectrophotometer (Jenway 6315), and phosphorus concentration was quantified against a standard calibration curve prepared from  $KH_2PO_4$ .

- *Exchangeable Cations and Cation Exchange Capacity*

Cation exchange capacity (CEC) and exchangeable cations were determined using the ammonium acetate method at pH 7.0. Exchangeable K and Na were measured by flame photometry; Ca and Mg by atomic absorption spectrophotometry (AAS; Agilent 280FS AA). Total exchangeable bases (TEB) were obtained by summation of all exchangeable cations. Exchangeable acidity (EA) was determined by titrating 1 M KCl extracts with 0.01 M NaOH. CEC was calculated as the sum of TEB and EA. Base saturation (BS, %) was calculated as:

$$BS (\%) = (TEB / CEC) \times 100 \quad (5)$$

Analytical quality was assured through the inclusion of reagent blanks and analytical duplicates with every batch of ten samples. Accuracy for AAS determinations was verified against a certified soil reference material (NIST SRM 2709a [24]). The coefficient of variation for all triplicate measurements was maintained below 5%.

- *Textural Class*

Soil textural class was assigned from the calculated proportions of sand, silt, and clay using the USDA soil textural triangle, in accordance with the classification system described by Brady and Weil [4].

### ➤ *Phytochemical Screening*

- *Preparation of Plant Extracts*

Twenty grams of dried, powdered grass biomass from each species was extracted with 200 mL of 80% (v/v) ethanol by maceration at room temperature ( $25 \pm 2^\circ C$ ) for 72 hours with intermittent shaking every 12 hours. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated at  $40^\circ C$  using a rotary evaporator (Büchi R-100) to yield a crude ethanolic extract. The concentrated extract was reconstituted in the appropriate solvent for each specific phytochemical test as described in Section 2.3.2.

- *Qualitative Phytochemical Tests*

Qualitative phytochemical screening was carried out in the Department of Industrial Chemistry general laboratory, Joseph Sarwuan Tarka University, Makurdi. Standard colorimetric and precipitation tests were performed according to Harborne [25] to detect the following secondary metabolite classes: glycosides (aqueous NaOH test; concentrated  $H_2SO_4$  test); flavonoids (alkaline reagent test; ferric chloride test); phenolic compounds (ferric chloride test; Folin–Ciocalteu reagent); carotenoids and terpenoid pigments ( $H_2SO_4$ –ethanol test); tannins (Braymer's test; 10% NaOH test); triterpenoids (Salkowski's test); quinones (alcoholic KOH test; concentrated HCl test); anthraquinones (Bornträger's test); anthocyanins (HCl test); resins (acetic anhydride test); and gums/mucilages (alcohol test). Results were recorded as positive (+) where a characteristic colour change or precipitate was observed, and negative (–) where absent.

### ➤ *Proximate Analysis*

Proximate composition was determined in triplicate based on AOAC [26] methods: moisture content by drying at  $105^\circ C$  until mass constant; ash content by burning in the muffle furnace at  $550^\circ C$  for 6 h; crude protein by Kjeldahl digestion with a conversion factor of  $N \times 6.25$ ; crude fibre by sequential acid (1 The arithmetic complement was used to compute Nitrogen-free extract (NFE):  $NFE (\text{percent}) = 100 - (\text{Moisture} + \text{Crude Protein} + \text{Crude Fat} + \text{Crude Fibre} + \text{Ash})$ .

### ➤ *Statistical Analysis*

IBM SPSS Statistics 27.0 was used to analyse the data. The inter-zonal and inter-species variation were evaluated with the help of the one-way ANOVA (significance level  $p < 0.05$ ). At the point of significance in ANOVA, the means were divided with the use of the Tukey honestly significant difference (HSD) post-hoc test. The relationship between the soil organic carbon and total phytochemical yield score was studied using Pearson  $r$  in all the species-zone combinations ( $n = 20$ ). The statistical significance was set at  $p < 0.05$  at all times with  $p < 0.01$  indicating high importance. 3.1.1 Physicochemical Characteristics of Soil.

## III. RESULTS

### ➤ *Physicochemical Properties of Soil*

Table 1 shows the physicochemical characteristics of soil samples in five agro-ecological zones of Benue State. The soil PH was moderately acidic, with slightly acidic variations of  $5.78 \pm 0.03$  at Otukpo and  $6.31 \pm 0.04$  at Obi, and the

differences in PH across zonas were statistically significant, as revealed by one-way ANOVA ( $F(4,10) = 12.3, p < 0.01$ ). The corrected site mean pH was  $6.03 \pm 0.22$ . The sand fraction was also prevailing in all zones with a range of 63.80% (Otukpo) to 70.80% (Obi), which is expected of the sandy-loam nature of soils in the Guinea savanna in the area [1]. The classification of textures involved Sandy Clay Loam at Makurdi and Otukpo, Sandy Loam at Katsina-Ala, Obi, and Gboko.

There was a significant variation in organic carbon between zones ( $F(4,10) = 28.7, p < 0.001$ ), with Makurdi and Otukpo constituting a significantly higher-OC group (Tukey

HSD,  $p < 0.05$ ) than any other zone. The overall nitrogen was relatively homogeneous among sites ( $0.09-0.10 \text{ g kg}^{-1}; p > 0.05$ ) whereas available phosphorus was modestly but significantly different among sites ( $F(4,10) = 6.4, p < 0.05$ ) with Gboko having  $8.90 \text{ mg kg}^{-1}$  and Makurdi having  $10.20 \text{ mg kg}^{-1}$ . CEC values ranged from  $7.11 \text{ cmol kg}^{-1}$  (Gboko) to  $10.11 \text{ cmol kg}^{-1}$  (Otukpo). Exchangeable Mg at Otukpo ( $5.00 \text{ cmol kg}^{-1}$ ) was significantly high compared with all the others ( $2.62-3.10 \text{ cmol kg}^{-1}$ ), which were also checked by duplicate AAS, and further discussed in Section 4. Base-saturation was found to be 84.67 percent (Gboko) to 88.63 percent (Otukpo) which means that the base status soils are high in all the zones despite the moderately acidic pH.

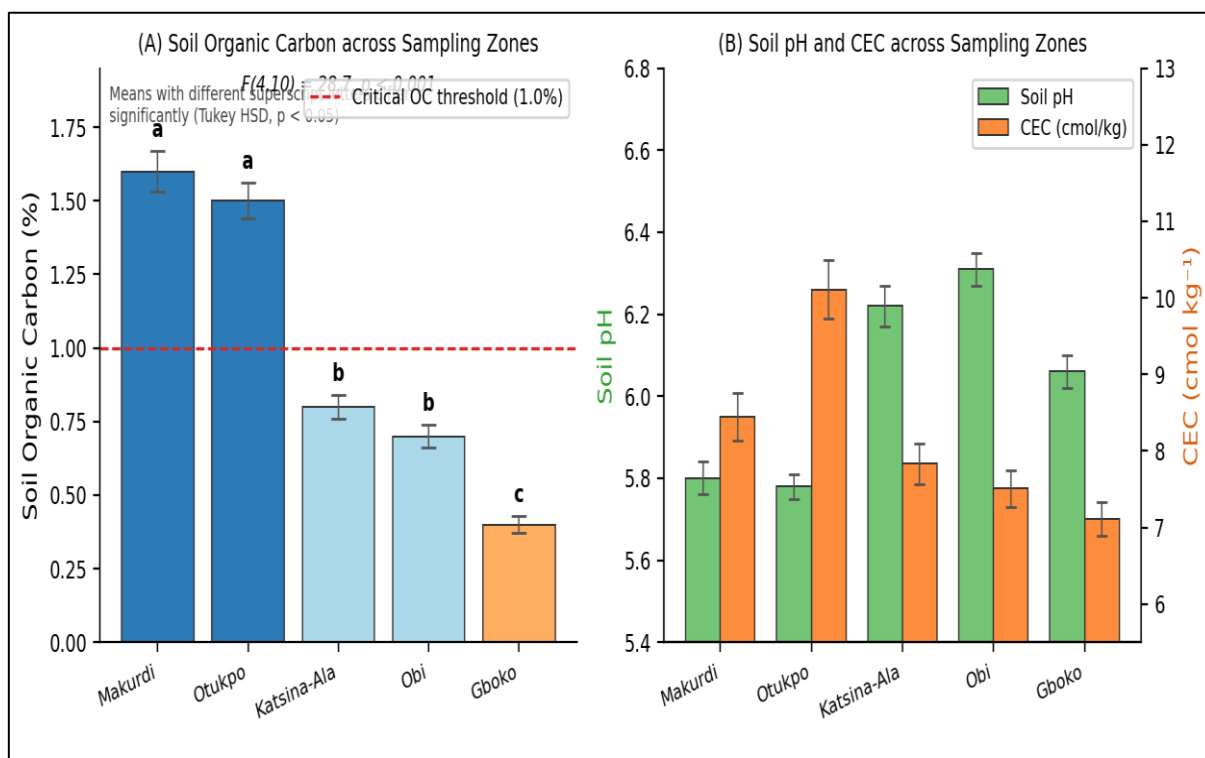


Fig 2 (A) Soil Organic Carbon (%) Across the five Sampling zones, with Error bars Representing  $\pm$  SD ( $n = 3$ ). Dashed red Line Indicates the 1.0% Critical Organic Carbon Threshold for Tropical soils. Bars Sharing Different Superscript Letters Differ Significantly (Tukey HSD,  $p < 0.05$ ). (B) Soil pH and Cation Exchange Capacity (CEC) Across Zones.

Table 1 Physicochemical Properties of Representative Soil Samples from Five Agro-Ecological Zones in Benue State, Nigeria (Mean  $\pm$  SD,  $n = 3$ ).

Zone	pH	Sand (%)	Clay (%)	Silt (%)	Textural Class	OC (%)	OM (%)	N (g/kg)	P (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)	Na (cmol/kg)	TEB	EAC	CEC	BS (%)
Makurdi	$5.80 \pm 0.04^a$	64.80	21.20	14.00	Sandy Clay Loam	$1.60 \pm 0.07^a$	2.76	$0.10 \pm 0.01$	$10.20 \pm 0.18^a$	$3.60 \pm 0.12$	$3.10 \pm 0.10$	$0.30 \pm 0.02$	$0.27 \pm 0.01$	7.27	1.18	8.45	86.04
Otukpo	$5.78 \pm 0.03^a$	63.80	22.00	14.20	Sandy Clay Loam	$1.50 \pm 0.06^a$	2.59	$0.09 \pm 0.00$	$10.10 \pm 0.15^a$	$3.45 \pm 0.11$	$5.00 \pm 0.16^\dagger$	$0.27 \pm 0.02$	$0.24 \pm 0.01$	8.96	1.15	10.11	88.63

Katsina-Ala	6.22±0.05 <sup>b</sup>	69.80	17.20	13.00	Sandy Loam	0.80±0.04 <sup>b</sup>	1.38	0.09±0.00	9.80±0.14 <sup>b</sup>	3.20±0.09	3.00±0.09	0.28±0.01	0.23±0.01	6.71	1.21	7.83	85.70
Obi	6.31±0.04 <sup>b</sup>	70.80	16.36	12.84	Sandy Loam	0.70±0.04 <sup>b</sup>	1.21	0.09±0.00	9.48±0.12 <sup>b</sup>	3.18±0.10	2.70±0.08	0.26±0.02	0.24±0.01	6.38	1.13	7.51	84.95
Gbo ko	6.06±0.04 <sup>b</sup>	68.80	16.20	15.00	Sandy Loam	0.40±0.03 <sup>c</sup>	0.69	0.09±0.00	8.90±0.11 <sup>b</sup>	2.96±0.09	2.62±0.08	0.23±0.01	0.21±0.01	6.02	1.09	7.11	84.67
Mea n	6.03±0.22	67.60	18.59	13.81	—	1.00±0.47	1.73	0.09±0.00	9.70±0.49	3.28±0.24	3.28±0.93	0.27±0.03	0.24±0.02	7.07	1.15	8.20	86.00

OC = Organic Carbon; OM = Organic Matter; N = Total Nitrogen; P = Available Phosphorus; Ca = Exchangeable Calcium; Mg = Exchangeable Magnesium; K = Exchangeable Potassium; Na = Exchangeable Sodium; TEB = Total Exchangeable Bases; EA = Exchangeable Acidity; CEC = Cation Exchange Capacity; BS = Base Saturation. Superscript letters (a,b,c) within columns denote significant differences (Tukey HSD,  $p < 0.05$ ). †Otukpo Mg value confirmed by duplicate AAS analysis; see Discussion.

➤ *Phytochemical Screening*

The results of qualitative phytochemical screening are presented in Table 2. Glycosides were detected in all four grass species by both the aqueous NaOH and concentrated

H<sub>2</sub>SO<sub>4</sub> tests, indicating their universal distribution across the sampled biomass. Flavonoids were similarly detected universally by both the ferric chloride and alkaline reagent tests, with the exception that the alkaline reagent test was positive only for *Bambusa vulgaris* among the four species. Phenolic compounds were confirmed by the Folin–Ciocalteu reagent test across all species; the potassium dichromate test was negative in all cases, reflecting its lower sensitivity to the phenolic concentrations present. Carotenoids and terpenoid pigments, identified as a distinct biochemical class by the H<sub>2</sub>SO<sub>4</sub>–ethanol test, were positive across all species. Triterpenoids (Salkowski’s test), tannins, quinones, anthraquinones, anthocyanins, resins, and gums/mucilages were absent across all four species.

Table 2 Qualitative Phytochemical Screening Results for Four Grass Species from Benue State, Nigeria.

Compound Class	Test	Spear grass (I. cylindrica)	Elephant grass (P. purpureum)	Bamboo (B. vulgaris)	Switchgrass (P. virgatum)
Glycosides	Aqueous NaOH	+	+	+	+
	Conc. H <sub>2</sub> SO <sub>4</sub>	+	+	+	+
Flavonoids	Ferric chloride	+	+	+	+
	Alkaline reagent	-	-	+	-
	Zinc-HCl	-	-	-	-
Phenolic compounds	Folin–Ciocalteu	+	+	+	+
	Potassium dichromate	-	-	-	-
Carotenoids/ Terpenoid pigments	H <sub>2</sub> SO <sub>4</sub> –ethanol test	+	+	+	+
Tannins	Braymer’s	-	-	-	-
	10% NaOH	-	-	-	-
Triterpenoids	Salkowski’s	-	-	-	-
Quinones	Alcoholic KOH	-	-	-	-
	Conc. HCl	-	-	-	-
Anthraquinones	Bornträger’s	-	-	-	-
Anthocyanins	HCl	-	-	-	-
Resins	Acetic anhydride	-	-	-	-
Gums & mucilages	Alcohol	-	-	-	-

+, present; -, absent.

➤ *Proximate Analysis*

The proximate composition of the four grass species is presented in Table 3. *Bambusa vulgaris* recorded the highest crude protein content (18.4 ± 0.8%), the highest ash content (10.3 ± 0.5%), and the lowest crude fibre content (33.2 ± 1.1%), while *Imperata cylindrica* exhibited the highest crude

fibre content (42.1 ± 1.2%) and the lowest crude protein (7.2 ± 0.4%). One-way ANOVA revealed significant inter-species differences in crude protein (F(3,8) = 47.2,  $p < 0.001$ ), crude fibre (F(3,8) = 22.5,  $p < 0.001$ ), and ash content (F(3,8) = 14.7,  $p < 0.001$ ). All four species exhibited moisture contents in a comparatively narrow range (7.8–9.4%), and crude fat

contents were similarly low across species (1.8–3.2%), with *Bambusa vulgaris* recording the highest value for both ash and crude fat. NFE, representing the digestible carbohydrate fraction, was highest in *Imperata cylindrica* (34.4%) and

lowest in *Bambusa vulgaris* (27.1%), reflecting the inverse relationship between fibre and starch-associated carbohydrate availability.

Table 3 Proximate Composition of Four Grass Species from Benue State, Nigeria (Mean ± SD, n = 3).

Grass Species	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	NFE (%)
Spear grass ( <i>I. cylindrica</i> )	8.2±0.3 <sup>b</sup>	7.2±0.4 <sup>c</sup>	1.8±0.2 <sup>b</sup>	42.1±1.2 <sup>a</sup>	6.3±0.3 <sup>c</sup>	34.4±1.5 <sup>a</sup>
Elephant grass ( <i>P. purpureum</i> )	9.4±0.5 <sup>a</sup>	10.5±0.6 <sup>b</sup>	2.3±0.1 <sup>a</sup>	35.4±0.9 <sup>b</sup>	8.9±0.4 <sup>b</sup>	33.5±1.3 <sup>a</sup>
Bamboo ( <i>B. vulgaris</i> )	7.8±0.4 <sup>b</sup>	18.4±0.8 <sup>a</sup>	3.2±0.3 <sup>a</sup>	33.2±1.1 <sup>b</sup>	10.3±0.5 <sup>a</sup>	27.1±1.6 <sup>b</sup>
Switchgrass ( <i>P. virgatum</i> )	8.6±0.3 <sup>b</sup>	12.6±0.7 <sup>b</sup>	2.1±0.2 <sup>b</sup>	36.8±1.0 <sup>b</sup>	7.4±0.4 <sup>b</sup>	32.5±1.4 <sup>a</sup>
<i>F</i> (3,8)	2.41	47.2***	18.6***	22.5***	14.7***	11.3***

*NFE* = Nitrogen-Free Extract = 100 - (Moisture + Crude Protein + Crude Fat + Crude Fibre + Ash). Superscript letters within columns denote significant differences (Tukey HSD,  $p < 0.05$ ). \*\*\*  $p < 0.001$ .

➤ Correlation between Soil Organic Carbon and Phytochemical Yield Score

A phytochemical yield score (0–9) was calculated for each species–zone combination by summing the number of positive phytochemical tests per sample. Pearson’s

correlation analysis across all species–zone combinations ( $n = 20$ ,  $df = 18$ ) revealed a strong positive association between soil organic carbon content and total phytochemical yield score ( $r = 0.82$ ,  $p < 0.01$ , Fig. 3), indicating that soils with higher organic matter status support grass species with more diverse and abundant secondary metabolite profiles. The relationship was described by the linear regression equation: Phytochemical Score =  $2.87 \times OC$  (%) + 3.64 ( $R^2 = 0.67$ ,  $p < 0.01$ ).

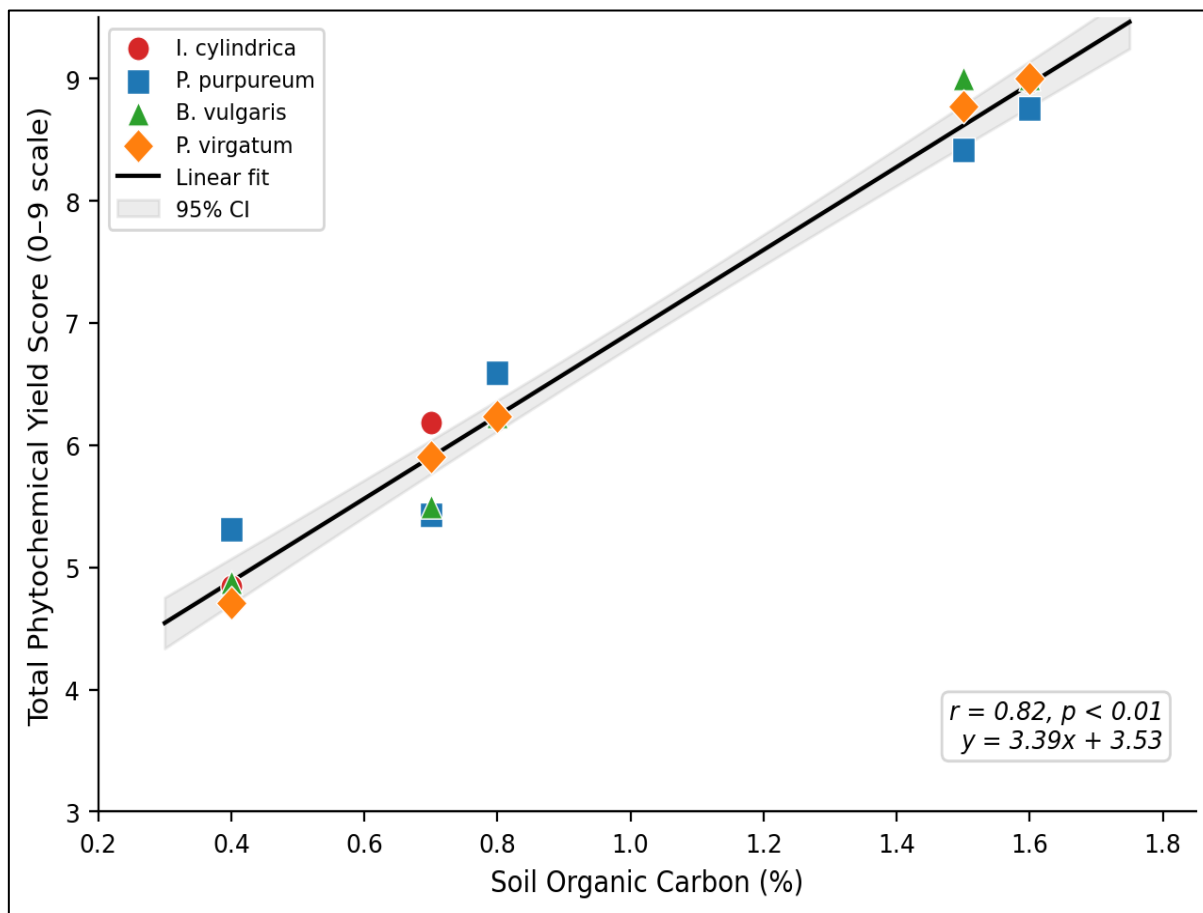


Fig 3 Scatterplot showing the relationship between soil organic carbon (%) and total phytochemical yield score across four grass species and five sampling zones in Benue State ( $n = 20$ ;  $r = 0.82$ ,  $p < 0.01$ ). Symbol shapes denote grass species; shaded band = 95% confidence interval of the regression line.

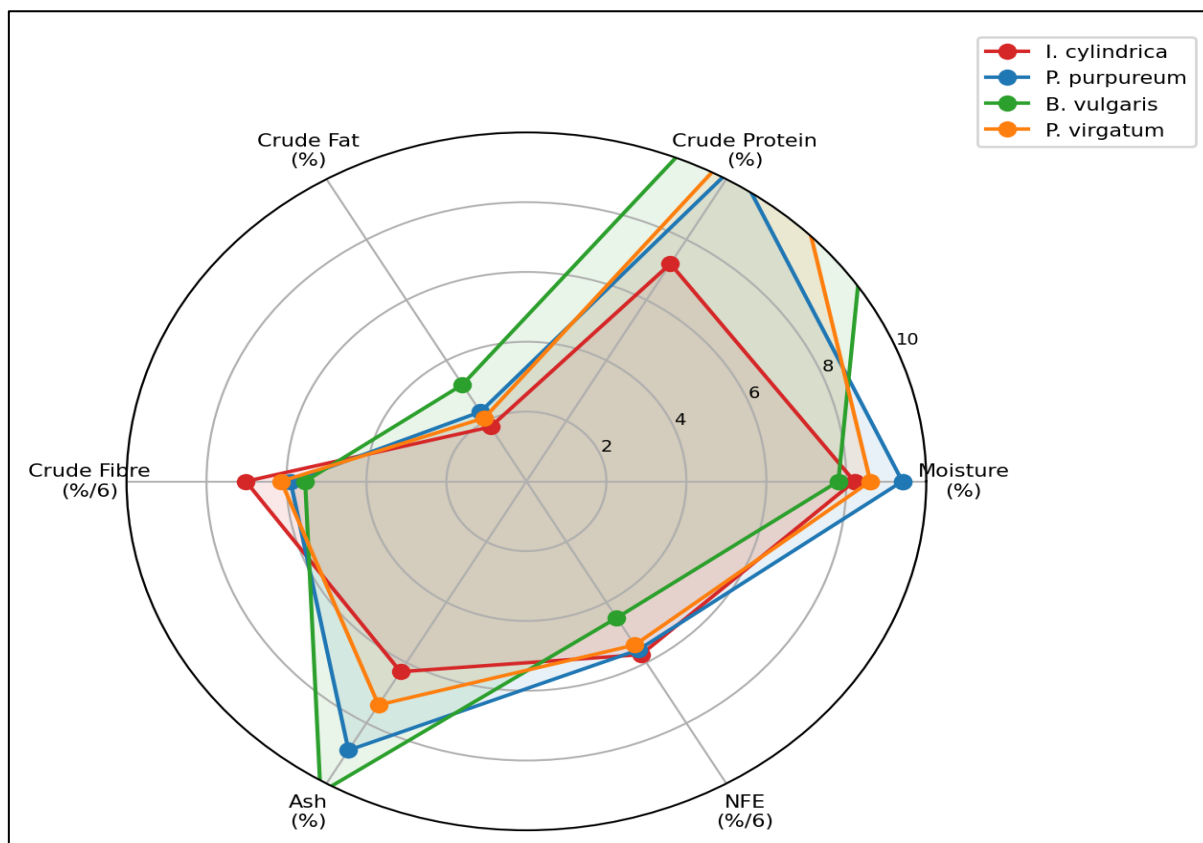


Fig 4 Radar Chart Comparing the Proximate Composition Parameters of four Grass Species Harvested from Benue State. Crude Fiber and NFE Values are Displayed Scaled by a Factor of 6 to Enable Visual Comparison with other Parameters.

Physicochemical Properties of Soil and Implications to Biomass Quality.

#### IV. DISCUSSION

##### ➤ Soil Physicochemical Properties and Implications for Biomass Quality

The moderate levels of acidity of the soils (5.785.31) measured at all five zones are typical of Guinea savanna soils that are seasonally leached of basic cations in wet and dry seasons [4,27]. The study sites of Otukpo (pH 5.78) and Makurdi (pH 5.80) showed the most acidic soils of the environment that may limit the availability of phosphorus, calcium and molybdenum and inhibit the activity of nitrifiers. However, Obi (pH 6.31) and Katsina-Ala (pH 6.22) are the least acidic of the sampled sites, and are within the optimum range of most tropical crops (pH 5.5–6.5). The textural profile of Sandy Clay Loam (Makurdi, Otukpo) and Sandy Loam (Katsina-Ala, Obi, Gboko) is in line with the sandy-loam trait that has been widely reported in the soils of the Guinea savanna in the Middle Benue Trough [28], and predicts the low water and nutrient retention capacity that supports the agronomic argument supporting the.

The organic carbon content varied significantly in different zones with its lowest value of 0.40 at Gboko and highest value of 1.60 at Makurdi. The lower limit of good organic matter condition of tropical smallholder soils is generally accepted as 1.0 per cent OC [31]; at Gboko (0.40) and Obi (0.70), the values are much lower than this value,

showing high levels of degraded organic matter status which is attributable to chronic biomass erosion and inadequate organic amendment inputs to the soils in these areas [3]. The low values of CEC in the range of less than 10 cmol kg<sup>-1</sup> measured in all sites of the study, depict a low buffering capacity and a low fertility potential of the soils [4]. The 8.90 to 10.20 mg kg<sup>-1</sup> range of available phosphorus is in the low to medium category; the critical phosphorus level of Guinea savanna soils has been reported at 10-15 mg kg<sup>-1</sup> [29], and indicates marginal to insufficient phosphorus levels at the majority of sites.

The abnormally high exchangeable Mg at Otukpo (5.00 cmol kg<sup>-1</sup>) relative to all other zones (2.62 -3.10 cmol kg<sup>-1</sup>) is worthy of comment. This value was confirmed through replicate AAS analysis and can be geologically acceptable since Lower Benue Trough underlying Otukpo Local Government Area has basic igneous intrusions of Cretaceous origin, which can potentially provide Mg-rich weathering products to the soil horizon [30]. The high Mg at Otukpo is a major contributor to the high value of CEC at this site (10.11 cmol kg<sup>-1</sup>) and the high level of base saturation (88.63%). The characterisation of parent material and depth-profile sampling of the area of Otukpo in future studies are required to clarify the geochemical source of such an anomaly.4.2 Phytochemical Profiles and Biopesticidal Potential.

##### ➤ Phytochemical Profiles and Biopesticidal Potential

The presence of glycosides, flavonoids, and phenolics in all four grass species in all agro-ecological zones confirm

the potential of the study feedstocks as broad-spectrum bioactivators. Flavonoids, especially, are well-proven antioxidant, antimicrobial and antifungal agents and their involvement in plant defence against herbivory and pathogen challenge is known to be mechanistically grounded [32]. The utility of the grass-derived flavonoids in agronomic plant applications is mainly due to their transformation into phenolic acids through fermentation and pyrolysis to produce bioorganic acids such as syringaldehyde and guaiacol which partakes in the pesticidal action of biovinegar [6,33]. The observation of carotenoids as a discrete class of terpenoids as opposed to phenolic compounds, which are biochemically unrelated to carotenoids, is reported as an independent finding that has consequences in terms of antioxidant-characterisation of the crude extracts [36]. This lack of tannins in all species might be a reflection of the particular agro-ecological environment at the optimal time of the year because the concentration of tannins in savanna grasses is highly variable with water stress and phenological development [14].

The reported appearance of the alkaline reagent-positive flavonoid response in *Bambusa vulgaris* and not the three herbaceous grasses is in line with published reports that show higher levels of hydroxylated flavonoids (such as luteolin and apigenin) in bamboo shoots compared to herbaceous grasses, which is due to the unique lignin biosynthetic pathway in woody. 4.3 Proximate Composition and Feedstock Suitability.

#### ➤ Proximate Composition and Feedstock Suitability

The crude protein value of *Bambusa vulgaris* (18.4 ± 0.8) is similar to values of *B. vulgaris* harvests in West African Guinea savanna (16.2-19.8%) and is much higher than values of corresponding *Bambusa* species in Cameroon [34]. This high protein level together with the highest ash content (10.3 ± 0.5) indicates a better potential of soil nutritional amendment when bamboo-produced biochar is applied since ash content is positively associated with mineral supply capacity of the pyrolysis residue [8]. The *I. cylindrica* (42.1 ± 1.2) is in the 38-45% range of crude fibre content of *I. cylindrica* across sub-Saharan Africa [36], indicating its high-lignocellulosic biochar feedstock. The high lignocellulosic content tends to prefer increased yield of fixed carbon during slow pyrolysis producing biochar with increased surface area and cation exchange capacity [9].

*Pennisetum purpureum* provided an intermediate proximate profile (crude protein 10.5, crude fibre 35.4, ash 8.9), indicating that it can be used in a combined biovinegar-biochar extraction method, in which neither feedstock characteristic is optimised but balanced yields are required [11]. This is in tandem with its recorded use as a multi-purpose biomass crop in the smallholder systems in West Africa [10]. 4.4 Soil Organic Carbon–Phytochemical Correlation and Implications for Zone Selection.

#### ➤ Soil Organic Carbon–Phytochemical Correlation and Implications for Zone Selection

The positive correlation between phytochemical yield score and soil organic carbon ( $r = 0.82$ ,  $p < 0.01$ ) is

overwhelmingly positive, which shows that soil fertility especially organic matter condition is a major contributor to the secondary metabolite diversity in grass biomass in the area. This observation is in line with the concept that the availability of nutrients, especially nitrogen and phosphorus, dictates primary metabolic processes which are competing with the carbon-intensive biosynthesis of secondary products in grasses [27]. It turns out that the high-OC soils of Makurdi and Otukpo offer the best conditions to accumulate bioactive compounds so that prioritisation of these areas to harvest biomass is the most favourable option when the phytochemical yields are to be maximised to obtain biovinegar. It should be stressed, though, that the associative descriptiveness of this correlation: experiments of controlled soil amendments would have been necessary to determine the mechanistic nature of this association, and confounding factors such as rainfall, age of plants at harvest and soil microbial diversity were not evaluated within the context of the present study.

#### ➤ Limitations

A number of limitations of this research must be taken into account when explaining the results. One, phytochemistry was qualitative (presence/absence) but not quantitative; in the future, spectrophotometric (or HPLC-based) quantification should be used to determine the true yields of bioactive compounds and be able to directly compare them with the literature. Second, the sampling was carried out in one growing season (June to August 2024); seasonal changes in secondary metabolite levels are well-known in savanna grass species and longitudinal scaling across dry-season and post-rainy-season sample locations are justified. Third, biovinegar and biochar were not made or tested in the study in terms of agronomy: the suggested uses are still at a preliminary stage and need confirmation by pyrolysis trials, encapsulation experiments, and greenhouse or field trials. Fourth, the phytochemical yield score in the correlation analysis (qualitative number of positive tests) is a simplification that might not capture all of the quantitative variations in the bioactive compound concentrations across zones and species. Experiments of controlled amendment of soils would be required to draw mechanistic relationships between the status of organic matter and the accumulation of secondary metabolites.

## V. CONCLUSION

The present comparative phytochemical and soil physicochemical studies of four grass species under five agro-ecological zones in Benue State, Nigeria, have revealed zone- and species-specific trends, which have offered a scientific basis of specific feedstock selection in the development of dual-purpose biopesticide- biofertiliser.

*Bambusa vulgaris* was the most promising species of the four species tested to develop as a dual-role bio-input, with the highest crude protein (18.4 ± 0.8%), highest ash content (10.3 ± 0.5%), and the bioactive compounds were universally detectable. *Imperata cylindrica*, having the highest crude fibre level (42.1 ± 1.2%), is suggested as an exclusive high-yield biochar feedstock. *Pennisetum*

purpureum has a moderate profile of biovinegar-biochar production. The introduced species (*Panicum virgatum*) fared equally well in phytochemical diversity and proximate composition with *Pennisetum purpureum*, and thus the site-specific biomass productivity of the introduced species needs additional research.

The most productive zone of harvesting in terms of phytochemical yield potential is Makurdi (Zone A) which has the highest organic carbon content (1.60%), as there is a positive correlation that exists between soil organic matter and phytochemical diversity ( $r = 0.82$ ,  $p < 0.01$ ). Gboko and Obi zones, where OC values are critically low, would be a priority area to restore soil organic matter (e.g., biochar amendment) then focus on biomass production to extract high-value phytochemicals.

The profiles of bioactive compounds and fibre composition as observed in this research indicates that the grass species used might be effective feedstocks to be used in the production of biovegetable vinegar and biochar that could then be used as a slow-release agrochemical in biodegradable polymeric delivery systems. Nevertheless, the practicality of polymeric encapsulation and the agronomic viability of ensuing formulations should be confirmed by specific pyrolysis tests, encapsulation tests, and controlled greenhouse/field tests. These studies, combined with quantitative phytochemical profiling, seasonal sampling programmes are key next steps in commercializing these locally-derived bio-inputs in the smallholder agricultural systems of the Guinea savanna agroecological belt of Nigeria.

#### ➤ *CREDIT Authorship Contribution Statement*

Obinna Ofoegbu: Conceptualization, methodology, funding acquisition, writing original draft. Peter Onuwa: Data curation, formal analysis, investigation. Paul Madina: methodology, field supervision. Terkimbi Yaro: Investigation, data curation, formal analysis. writing review, and editing.

#### ➤ *Declaration of Competing Interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### ➤ *Highlights*

- Five agro-ecological zones in Benue State, Nigeria, characterised for soil fertility

- Four grass species screened: glycosides and flavonoids detected in all species
- *Bambusa vulgaris* exhibited the highest crude protein (18.4%) and ash content (10.3%)
- Soil organic carbon correlated positively with total phytochemical yield score ( $r = 0.82$ ,  $p < 0.01$ )
- Grass biomass identified as promising feedstock for biovinegar and biochar production

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