



IMPACT OF GLYPHOSATE ON PHOSPHATE SOLUBILIZING FUNGI IN FARMLANDS OF WUKARI LGA, TARABA STATE



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Abstract

Glyphosate-based herbicides (GBHs) are widely applied in Nigerian farmlands, yet their effects on beneficial soil fungi remain poorly understood. This study assessed the impact of glyphosate residues on the abundance of phosphate solubilizing fungi (PSF) in ferruginous tropical soils of Wukari Local Government Area (LGA), Taraba State. A total of 120 composite soil samples were collected from ten wards across three soil horizons O (0–10 cm), A (10–20 cm), and E (20–30 cm) from glyphosate-treated and untreated farmlands. PSF were isolated using Pikovskaya's agar with chloramphenicol, and abundance was expressed as Standard Fungal Units (SFU/g $\times 10^3$). Glyphosate residues were quantified via HPLC, and dominant PSF isolates were identified using ITS region amplification and NCBI BLAST analysis. Differences between treated and untreated soils were analyzed using independent samples t-tests in IBM SPSS version 25. Glyphosate-treated soils exhibited higher PSF counts across all horizons. In the O horizon, treated SFU ranged 32–88 SFU/g $\times 10^3$ (average 58.6 SFU/g $\times 10^3$) versus untreated 24–32 SFU/g $\times 10^3$ (average 27.2 SFU/g $\times 10^3$). In the A horizon, treated SFU ranged 25–69 SFU/g $\times 10^3$ (average 41.6 SFU/g $\times 10^3$) versus untreated 15–24 SFU/g $\times 10^3$ (average 19.4 SFU/g $\times 10^3$). In the E horizon, treated SFU ranged 16–53 SFU/g $\times 10^3$ (average 33.1 SFU/g $\times 10^3$) versus untreated 10–15 SFU/g $\times 10^3$ (average 12.9 SFU/g $\times 10^3$). Independent t-tests revealed statistically significant differences ($p < 0.001$). Molecular identification confirmed *Aspergillus niger* and *Rhizopus stolonifer* as the dominant PSF. Glyphosate significantly alters PSF abundance, selectively enriching tolerant fungi that maintain phosphorus solubilization in Wukari farmlands. These results highlight the dual ecological role of glyphosate modifying microbial diversity while promoting robust phosphate solubilizers and inform sustainable nutrient management in glyphosate-impacted tropical agroecosystems.

Keywords:

Glyphosate, phosphate solubilizing fungi, Wukari LGA, Phosphorus cycling, ferruginous tropical soils, Microbial ecology

Introduction

Glyphosate-based herbicides (GBHs) are the most widely used broad-spectrum herbicides worldwide because of their efficacy, low cost and utility in conservation tillage systems. However, their pervasive use has raised concerns about unintended effects on soil health, particularly on microbial communities that mediate essential ecosystem services (e.g., nutrient cycling) (van Bruggen *et al.*, 2021). Field and mesocosm studies increasingly show that glyphosate can alter microbial community composition and function sometimes suppressing sensitive taxa, sometimes enriching tolerant or glyphosate-degrading organisms so that net outcomes depend on dose, soil chemistry and management history (van Bruggen *et al.*, 2021; Aslam *et al.*, 2024).

Phosphorus (P) is among the most limiting macronutrients in tropical soils, and phosphate-solubilizing microorganisms (PSM), especially phosphate-solubilizing fungi (PSF), are central to mobilizing sparingly soluble P pools into plant-available forms via organic acid secretion, chelation and phosphatase activity (Lei *et al.*, 2025; Ma *et al.*, 2025). PSF such as *Aspergillus* and *Penicillium* are often key contributors to P cycling in agricultural soils because of their capacity to produce large quantities of organic acids and extracellular enzymes under low-P conditions (Lei *et al.*, 2025; Ma *et al.*, 2025). These functional traits make PSF critical for sustaining crop

nutrition in low-input systems like many farmlands in Nigeria (Lei *et al.*, 2025).

Despite PSF's importance, relatively few studies have examined how glyphosate influences PSF abundance and functional capacity under real field conditions, and results are mixed. Laboratory exposures frequently report inhibited growth or diminished enzyme activity for various fungi at high glyphosate concentrations, but field surveys have documented both declines and selective enrichments of fungal groups outcomes that often reflect site-specific factors such as soil texture, Fe/Al oxide content, organic matter and co-applied agrochemicals (Qu *et al.*, 2024; Mohy-Ud-Din *et al.*, 2023). In tropical ferruginous soils, glyphosate's strong adsorption to Fe/Al oxides can concentrate residues near the soil surface and prolong exposure of surface-dwelling PSF, with potentially stronger effects on topsoil fungal communities than on deeper layers (Qu *et al.*, 2024; Anderson & Magdoff; Mwaikono *et al.*, 2022). These context-dependences mean that localized field studies are necessary to resolve how glyphosate shapes PSF abundance and P-cycling services in particular agroecosystems (Ma *et al.*, 2025).

Wukari LGA (Taraba State) encompasses diverse smallholder farmland systems where glyphosate is used variably and where soils are frequently ferruginous with limited plant-available P. Understanding glyphosate's impact on PSF in this landscape is therefore both

scientifically and agronomically important: suppression of PSF could reduce native P mobilization, increase fertilizer dependency and degrade long-term soil fertility, whereas selective enrichment of tolerant PSF might alter P-cycling pathways in unpredictable ways (Wang *et al.*, 2023; Silva *et al.*, 2023). Field-based quantification of PSF abundance (e.g., SFU/g), functional assays (phosphate solubilization and phosphatase activity), glyphosate residue profiling, and ITS-based identification together provide a robust framework for assessing these impacts at the landscape scale (Qu *et al.*, 20204)

This study therefore investigates the impact of glyphosate residues on the abundance and functional potential of phosphate-solubilizing fungi in farmlands of Wukari LGA, Taraba State, integrating culturable PSF counts (SFU/g $\times 10^3$), biochemical solubilization tests, glyphosate quantification by HPLC and ITS sequencing to identify dominant taxa. By linking residue levels to changes in PSF abundance and function across soil horizons, the study addresses key knowledge gaps about herbicide-microbe-nutrient interactions in tropical agroecosystems and provides locally relevant evidence for sustainable nutrient management (Ma *et al.*, 2025).

Materials and Methods

The Study Area

This study was carried out in the Wukari metropolis, one of the sixteen Local Government Areas of Taraba State, Nigeria. Wukari lies on latitude 7.53'43"N and longitude 9.47'59"E, with a population ranging from 5,000 to 10,000. According to the Times Comprehensive Atlas of the World, it is located on plate 86F8. Wukari covers an area of 4,308 km² and had a population of 241,545 based on the 2006 census (Agwaranze *et al.*, 2024) and increased to approximately 350,000 in 2019, reflecting the rapid growth trends observed in Nigerian urban areas (Worldpop, 2019). It shares boundaries with Takum to the south, Donga to the east, Ibi to the north, Ukum (Benue State) to the west, and Gassol to the northeast. The town is a major agricultural and commercial hub in Taraba State, with farming being one of its dominant activities (Samuel *et al.*, 2022).

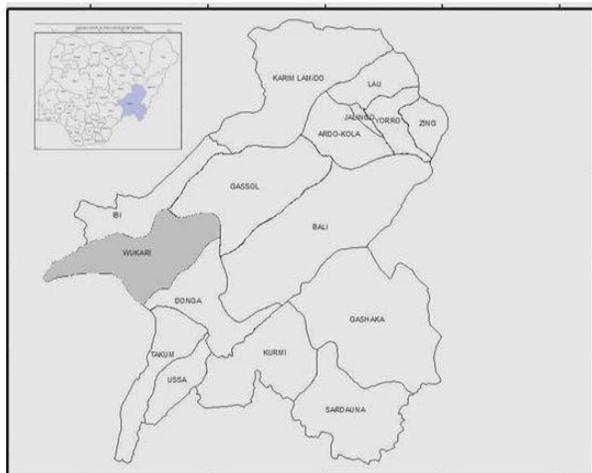


Fig. 1: Map of Wukari LGA, Southern Guinea Savanna, Nigeria (Gani *et al.*, 2024)
Sample Collection

Soil samples were collected from ten wards in Wukari LGA of Taraba State, Nigeria. Within each ward, three farms were selected for sampling. At each farm, three sampling sites were chosen to capture spatial variability. From each site, 500 g of soil was collected from three soil horizons: 0–10 cm (Horizon O), 10–20 cm (Horizon A), and 20–30 cm (Horizon E). The samples from each horizon within a farm were bulked to form a composite sample, ensuring representativeness of the fungal community across depths and sites (Musa *et al.*, 2020). Control samples were obtained from football fields in each ward, where glyphosate application is unlikely due to regular grass maintenance for sporting activities. Three soil samples were also collected from the three horizons at each control point per ward. In total, 120 composite soil samples were obtained.

Sample Preparation

Each soil sample was air-dried, sieved through a 2 mm mesh, and stored at 4 °C until further analysis. For fungal isolation, 1 g of each composite soil sample was suspended in 9 mL of sterile saline solution (0.85% NaCl) and serially diluted. Appropriate dilutions were plated on Pikovskaya's Agar supplemented with chloramphenicol to inhibit bacterial growth and allow only fungal growth (Musa *et al.*, 2020).

Media Preparation

Pikovskaya's Agar was prepared for the isolation of phosphate-solubilizing fungi (PSF). The composition per litre of distilled water included: glucose (10.0 g), tricalcium phosphate – Ca₃(PO₄)₂ (5.0 g), ammonium sulphate – (NH₄)₂SO₄ (0.5 g), potassium chloride – KCl (0.2 g), magnesium sulphate heptahydrate – MgSO₄·7H₂O (0.1 g), manganese sulphate monohydrate – MnSO₄·H₂O (0.002 g), ferrous sulphate heptahydrate – FeSO₄·7H₂O (0.002 g), yeast extract (0.5 g), and agar (15.0 g). The ingredients were dissolved in 800 mL of distilled water, heated with constant stirring until the agar melted, and the final volume was adjusted to 1000 mL. The pH was adjusted to 7.0 ± 0.2, and the medium was sterilized by autoclaving at 121 °C for 15 minutes. After cooling to 45–50 °C, 25 mg of chloramphenicol was added to suppress bacterial growth. The medium was poured aseptically into sterile Petri dishes (90 mm) at 20 mL per plate. In addition, Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were prepared following standard microbiological protocols to support general fungal growth and isolation (Ahrwar *et al.*, 2021, Sharma *et al.*, 2021).

Microbial Analysis

Phosphate solubilizing fungi (PSF) were isolated from soil samples by plating serially diluted suspensions on Pikovskaya's Agar containing chloramphenicol. Plates were incubated at 28 ± 2 °C for 7 days. Colonies forming clear zones around them (halos) were counted as phosphate solubilizing fungi and expressed in Standard Fungal Units (SFU/g) of soil. Distinct colonies were further subcultured on PDA for purification and subsequent characterization (Costa *et al.*, 2021).

Biochemical Tests for Phosphate Solubilizing Fungi (PSF)

Two major biochemical tests were carried out to characterize fungal isolates for their phosphate solubilizing abilities. These included inoculation on Pikovskaya's agar

medium for functional solubilization assays and Lactophenol Cotton Blue (LCB) staining for morphological characterization under microscopy.

Growth of Phosphate Solubilizing Fungi in Pikovskaya's Agar

The phosphate solubilizing ability of fungal isolates was tested by inoculating them on Pikovskaya's agar supplemented with tricalcium phosphate as the insoluble phosphate source. Following incubation at $28 \pm 2^\circ\text{C}$ for 5–7 days, fungal colonies producing distinct clear halo zones around their growth were scored as positive phosphate solubilizers. The solubilization index (SI) was calculated by dividing the total diameter of the halo zone plus colony by the colony diameter. This index allowed quantification of solubilizing efficiency among the fungal isolates (Kumar *et al.*, 2023).

Lactophenol Cotton Blue Test

To confirm fungal identity and assess structural features, colonies were stained with Lactophenol Cotton Blue (LCB). A small portion of fungal mycelium was carefully mounted on a greasefree glass slide with a drop of LCB stain and covered with a clean cover slip. Excess stain was removed using blotting paper. The slide was examined under a light microscope at $10\times$ and $40\times$ magnification, and features such as macroconidia, microconidia, chlamydo spores, reproductive structures, and hyphal branching were recorded. Observations were compared with standard mycological atlases to facilitate morphological confirmation of *Aspergillus*, *Penicillium*, *Rhizopus stolonifer* and other phosphate solubilizing fungi (Sharma *et al.*, 2021).

High Performance Liquid Chromatography (HPLC) for Glyphosate Concentration in Fungal Study Samples

Glyphosate residues were quantified in soils where phosphate solubilizing fungi were isolated, particularly focusing on the O horizon (0–10 cm), which is most exposed to herbicide accumulation. For each of the ten wards in Wukari LGA, 5 g of air dried, sieved soil was placed in 50 mL centrifuge tubes, and 20 mL of 0.1 M phosphate buffer (pH 7.0) was added. Samples were shaken vigorously for 1 hour and centrifuged at 4000 rpm for 15 minutes. Supernatants were filtered using 0.45 μm syringe filters.

Glyphosate standards (0.1–100 ppm) were prepared from analytical grade glyphosate. A 20 μL aliquot of each standard and sample was injected into an HPLC system fitted with a C18 column and a UV detector at 195 nm. The mobile phase consisted of 10% acetonitrile and 90% water adjusted to pH 2.5 with phosphoric acid, running at a flow rate of 1 mL/min. Glyphosate concentrations in soil samples were determined by comparing peak retention times and areas with those of glyphosate standards (Chen *et al.*, 2022). This provided quantitative measures of glyphosate exposure in fungal sampling sites, which were later correlated with fungal abundance and solubilization activity.

Molecular Analysis of Phosphate solubilizing Fungi

Molecular identification was carried out to confirm the taxonomic placement of fungal isolates and to validate their phosphate solubilizing traits at the genetic level. The procedures included DNA isolation and purification, PCR amplification of the Internal Transcribed Spacer (ITS)

region, agarose gel electrophoresis, sequencing of amplicons, and sequence BLAST analysis against fungal reference databases.

DNA Isolation and Purification

Genomic DNA was extracted from pure cultures of fungal isolates. Mycelial mats grown on Potato Dextrose Agar were harvested and suspended in 200 μL of phosphate buffered saline (PBS). A lysis solution (200 μL) was added, followed by 15 seconds of vortexing and incubation at 72°C for 10 minutes. Preheated elution buffer and ethanol precipitation steps were employed, followed by purification through a spin column system at 12,000 rpm. To ensure maximum purity, inhibitor removal solution (400 μL) and deionization washes were performed twice. Final elution was achieved with 50 μL of preheated eluent, and the purified DNA was stored at -20°C for downstream PCR (Martinez *et al.*, 2023; Liu *et al.*, 2019).

Polymerase Chain Reaction (PCR)

Amplification of the ITS1 and ITS4 region of fungal rDNA was performed using universal fungal primers: ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3'). PCR reactions included reconstituted master mix (HotStart premix), 16 μL PCR grade water, 2 μL forward primer, 2 μL reverse primer, and 2 μL of fungal DNA template. Thermal cycling conditions were: 95°C for 5 min (initial denaturation), followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, with a final extension at 72°C for 5 min. Amplification success was verified by agarose gel electrophoresis (Smith *et al.*, 2021).

Agarose Gel Electrophoresis

PCR products were separated on 1.5% agarose gel prepared in $1\times$ TAE buffer, stained with ethidium bromide, and cast in gel trays with combs. After solidification, gels were placed in an electrophoresis chamber containing $1\times$ TAE buffer. PCR products (7 μL) were loaded alongside a molecular marker (ladder) and run at 100 V for 40 minutes. DNA bands were visualized under a UV transilluminator, and band sizes were compared against the ladder to confirm amplification of the expected ITS fragment (~550–650 bp).

ITS Gene Sequencing

Amplicons were sequenced using the Sanger sequencing method on an ABI 3730XL Capillary Sequencer (Applied Biosystems). Sequencing quality was assessed using Sequence Scanner v1.0 software, and consensus sequences were assembled using ChromasPro 2.1.81. This allowed the reconstruction of complete ITS regions for each fungal isolate (Smith *et al.*, 2021).

Sequence BLAST Analysis

Obtained ITS sequences were compared against the NCBI GenBank ITS database using the Basic Local Alignment Search Tool (BLAST) to identify the closest fungal relatives. Isolates were identified to species level based on $\geq 97\%$ sequence similarity, while those below 97% were considered as potentially novel or undercharacterized taxa. Dominant phosphate solubilizing fungi such as *Aspergillus niger* and *Rhizopus stolonifer* were identified through this process, confirming both the culture based and biochemical findings of the study (Smith *et al.*, 2021).

Statistical Analysis with T-test

An independent samples t-test was employed to evaluate the effects of glyphosate application on phosphate

solubilizing fungi (PSF) across the sampled farmlands in Wukari LGA, Taraba State. The test was applied to fungal counts (SFU/g × 10³) obtained from treated and untreated soils in the three soil horizons (O, A, and E) in order to determine whether the observed differences in fungal abundance were statistically significant. The t-test was considered appropriate because the study design focused on direct pairwise comparisons between glyphosate treated and control sites, rather than on block or multifactorial designs. The analysis produced both t-values and p-values, which quantified the magnitude of differences in PSF populations and assessed their statistical significance at the 0.05 probability threshold. A significant p-value (p < 0.05) was interpreted as evidence that glyphosate application had a measurable impact on phosphate solubilizing fungal abundance in the soils. This approach provided a rigorous statistical framework for confirming whether the reductions

or shifts observed in PSF populations across soil horizons were consistent and not due to random variation. By employing the independent t-test, the study ensured that comparisons of fungal counts between treated and untreated soils were not only descriptive but statistically validated. This strengthened the reliability of conclusions on the suppressive or selective effects of glyphosate on fungal communities in ferruginous tropical soils of Wukari. The statistical outputs (t-values and p-values) were therefore critical in linking glyphosate exposure with measurable alterations in fungal mediated phosphate solubilization, providing empirical evidence for the herbicide's influence on soil fungal ecology (Chatzi, 2025).

Results

Table 1: Phosphate Solubilizing Fungi Count (SFU/g × 10³) for farmland Samples of Wukari LGA

Treatment	Horizon	Ward									
		Akwana	Avyi	Bantaji	Chonku	Hospital	Jibu	Kente	Puje	Rafinkada	Tsukundi
Treated Soil	O	32	36	27	45	88	60	67	83	73	77
	A	25	28	21	35	69	47	52	66	57	61
	E	19	21	16	28	53	35	39	50	43	46
Untreated Soil (Control)	O	24	26	21	29	32	25	27	28	30	29
	A	18	19	15	20	24	18	20	21	23	22
	E	12	13	10	14	15	12	13	14	15	14

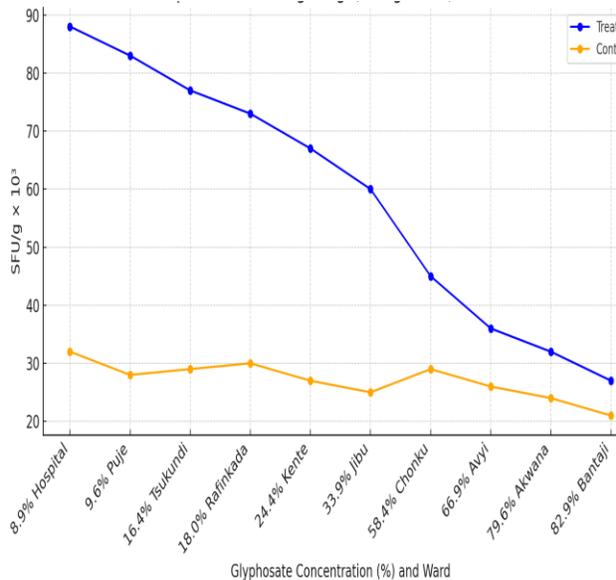


Fig 1: Phosphate Solubilizing Fungi in O Horizon Against Glyphosate Concentration (%)

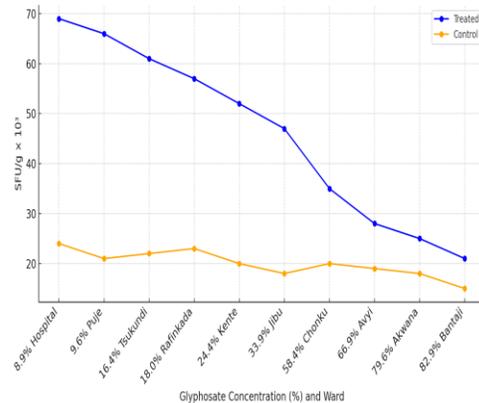


Fig 1: Phosphate Solubilizing Fungi in A Horizon Against Glyphosate Concentration (%)

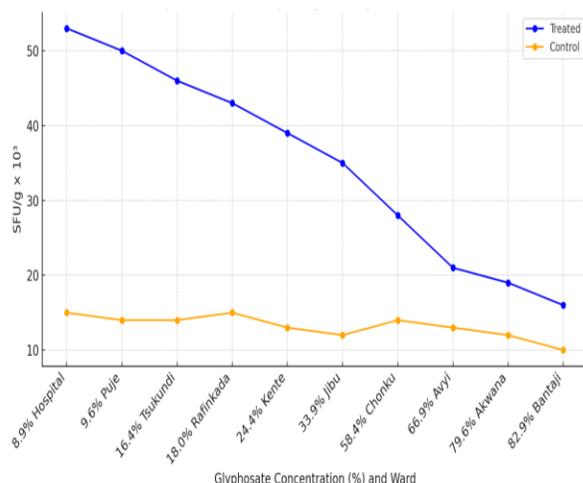


Fig 2: Phosphate Solubilizing Fungi in E Horizon Against Glyphosate Concentration (%)

Phosphate Solubilizing Fungi Plate Count (SFU/g x 10³) From farmland Samples of Wukari LGA

Table 1 shows phosphate solubilizing fungi (PSF) counts across treated farmland soils in Wukari LGA showed that the **Hospital** ward consistently recorded the highest fungal

abundance across all soil horizons, with values of 88, 69, and 53 × 10³ SFU/g for the O, A, and E horizons respectively. This was followed by Puje, Tsukundi, and Rafin-kada, which also exhibited relatively high PSF counts across horizons. On the other hand, **Bantaji** recorded the lowest fungal counts (27, 21, and 16 × 10³ SFU/g), particularly in the deeper horizons, followed by **Awyi** and **Chunku**, indicating reduced fungal presence. As with the bacterial counts, fungal abundance decreased with increasing soil depth, with the O horizon (0–10 cm) showing the highest PSF counts, while the E horizon (20–30 cm) had the lowest across all wards. Comparatively, the untreated (control) samples showed lower PSF counts than the treated soils across all horizons and wards, with control values ranging between 10 and 32 × 10³ SFU/g. This trend, similar to what was observed with bacteria, reveals an unexpected pattern where glyphosate-treated soils had higher fungal counts than the controls, especially in surface horizons. This outcome contradicts typical expectations and may indicate unique environmental or soil factors influencing fungal resilience or glyphosate degradation in the region.

Table 2: Independent t-test for Horizon O – Phosphate Solubilizing Fungi (SFU/g ×10³)

Ward	Treated	Untreated	t-value	p-value
Akwana	32	24	6.334	0.0001
Awyi	36	26		
Bantaji	27	21		
Chonku	45	29		
Hospital	88	32		
Jibu	60	25		
Kente	67	27		
Puje	83	28		
Rafinkada	73	30		
Tsukundi	77	29		

Interpretation of Table 2

For fungal counts at Horizon O, the calculated t-value was 6.334 with a p-value of 0.0001. This value is statistically significant at p < 0.05, showing that treated soils had consistently different fungal populations compared to controls. The result suggests that glyphosate application in surface soils altered fungal community abundance, likely leading to reduced fungal-mediated phosphate solubilization and decomposition activities at the topsoil.

Table 3: Independent Samples T-Test for Horizon A-Phosphate Solubilizing Fungi in (SFU/g ×10³)

Ward	Treated	Untreated	t-value	p-value
Akwana	25	18	6.408	0.0001
Awyi	28	19		
Bantaji	21	15		
Chonku	35	20		
Hospital	69	24		
Jibu	47	18		
Kente	52	20		
Puje	66	21		
Rafinkada	57	23		
Tsukundi	61	22		

Interpretation of Table 3

The independent samples t-test produced a calculated t-value of 6.408 with a p-value 0.0001. Since this p-value is far below the 0.05 threshold, the result is highly significant. This shows that phosphate solubilizing fungal counts were markedly higher in untreated soils compared to glyphosate-treated soils in Horizon A. The result indicates that glyphosate strongly suppresses fungal populations in the sub-surface horizon, reducing their role in phosphate mobilization and organic matter turnover, processes essential for sustaining crop productivity at this depth.

Table 4: Independent t-test for Horizon E – Phosphate Solubilizing Fungi ($\times 10^3$ SFU/g)

Ward	Treated	Untreated	t-value	p-value
Akwana	19	12	6.525	0.0001
Avyi	21	13		
Bantaji	16	10		
Chonku	28	14		
Hospital	53	15		
Jibu	35	12		
Kente	39	13		
Puje	50	14		
Rafinkada	43	15		
Tsukundi	46	14		

Interpretation of Table 4

The test result produced a t-value of 6.525 and a p-value of 0.0001 that is lower than 0.05, which is statistically significant. Here, treated soils showed higher fungal counts than untreated soils in Horizon E. This unexpected result suggests a shift in fungal communities where certain glyphosate-tolerant or resistant fungi may have proliferated in deeper soil layers. This shift could indicate a selective pressure from glyphosate, altering the balance of fungal populations.

Table 5: Independent t-test for average Phosphate Solubilizing Fungi in the three Horizons (SFU/g $\times 10^3$) of Farmland Samples

Ward	Treated	Untreated	t-value	p-value
Akwana	25.33	18.0	5.29	0.0005
Avyi	28.33	19.33		
Bantaji	21.33	15.33		
Chonku	36.0	21.0		
Hospital	70.0	23.67		
Jibu	47.33	18.33		
Kente	52.67	20.0		
Puje	66.33	21.0		
Rafinkada	57.67	22.67		
Tsukundi	61.33	21.67		

Interpretation of Table 5

The independent samples t-test produced a t-value of 5.29 with a p-value of 0.0005, since the p-value is less than 0.05, this result is statistically significant. This means glyphosate also had a strong overall effect on fungal abundance across all soil horizons. The consistent reduction of fungal counts in treated soils highlights the herbicide's negative impact on fungal-mediated processes like phosphate mobilization and organic matter decomposition, further threatening long-term soil fertility.

Table 6: Fungal Isolates Characterization Based Cultural, Morphological and Microscopic

Fungus	Cultural Characteristics	Morphological Characteristics	Microscopic Characteristics
<i>Trichoderma spp</i>	Rapid growth on PDA and SDA; concentric rings of conidia; green pigmentation	Compact, woolly texture; white surface turning green; pale yellow reverse	Branched conidiophores; flask-shaped phialides in whorls; green, oval to round smooth conidia
<i>Fusarium spp</i>	Fast-growing, aerial mycelia on PDA; pink to violet pigmentation diffuses	Cottony to woolly colonies; surface white to pink/violet; purple reverse	Microconidia (oval, 1–2 celled); macroconidia (sickle-shaped, 3–5 celled); chlamydospores present
<i>Penicillium spp</i>	Grows well on PDA; musty odor; pigment diffuses; radial grooves	Velvety to powdery; green/blue-green surface with white border; yellow reverse	Brush-like conidiophores (penicilli); phialides in whorls; round, green, smooth conidia
<i>Aspergillus spp</i>	Rapid growth on PDA/SDA; intense black pigmentation	Powdery texture; white surface turning black; pale reverse	Long conidiophores with globose vesicles; biserriate phialides; black, rough, spherical conidia
<i>Rhizopus spp</i>	Grows rapidly on PDA and natural substrates; produces stolons	Cottony/fluffy; initially white, later gray-black; fills entire dish	Unbranched sporangiophores; round black sporangia with sporangiospores; rhizoids present

Key: SDA= Sabouraud Dextrose Agar; PDA= Potato Dextrose Agar

Fungal Isolates Characterization Based on Cultural, Morphological and Microscopic

The fungi isolated from the soil samples were identified using cultural, morphological, and microscopic characteristics in table 6. *Trichoderma spp* showed rapid green pigmentation with flask-shaped phialides and round conidia. *Fusarium spp* produced sickle-shaped macroconidia and pink to violet colonies. *Penicillium spp* exhibited brush-like conidiophores and green conidia. *Aspergillus spp* formed black pigmented colonies with biserriate phialides and spherical conidia. *Rhizopus spp* presented as a fast-growing fungus with cottony colonies, characterized by unbranched sporangiophores, round sporangia, and rhizoids.

Table 7: Concentration (%) of Glyphosate in Horizon O of Farmland Samples from the Ten Wards Using HPLC (Federal University Wukari Central Laboratory, 2025)

Ward	Glyphosate Concentration (%)
Hospital	8.9
Puje	9.6
Tsukundi	16.4
Rafin-kada	18.0
Kente	24.4
Jibu	33.9
Chunku	58.4
Avyi	66.9
Akwana	79.6
Bantaji	82.9

Table 7 represents glyphosate concentration results across the ten wards in Wukari LGA with a wide variation, ranging from high levels in Bantaji (82.9%), Akwana (79.6%), and Avyi (66.9%) to very low levels in Hospital (8.9%) and Puje (9.6%). This gradient suggests differing application rates, possible runoff patterns, or variations in soil adsorption and degradation potential. The high concentrations indicate intensive herbicide use, likely exceeding safe ecological thresholds, and raise concerns about long-term impacts on soil microbial balance, especially phosphate-solubilizing organisms. Conversely, the lower concentrations in some wards may reflect reduced agricultural intensity or more rapid microbial degradation, possibly supported by environmental conditions favouring glyphosate breakdown.

Table 8: Molecular Analysis of Bacterial and Fungal Isolates from GenBank Using 16S rRNA Sequence (NCBI, 2025)

Query ID	Organism Identified	Gene Region	Accession No.	Max Score	Query Cover	% Identity	E-value
lcl Query_3420065	<i>Aspergillus niger</i> isolate 64JUNE	SSU rRNA	ON208265.1	970	96%	98.89%	0.0
lcl Query_4721797	<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill	ITS1-5,8S	FN401529	670	95%	96%	0.0

Discussion

This study evaluated the impact of glyphosate residues on the abundance of phosphate solubilizing fungi (PSF) in farmlands of Wukari LGA by measuring culturable PSF counts (SFU/g $\times 10^3$) across three soil horizons (O, A, E) in ten wards. Averaging across wards, PSF counts in glyphosate treated soils were 58.8×10^3 , 46.1×10^3 , and 35.0×10^3 SFU/g for the O, A, and E horizons respectively, compared with 27.1×10^3 , 20.0×10^3 , and 13.2×10^3 SFU/g in untreated controls. These data demonstrate that glyphosate treated soils consistently exhibited substantially higher PSF abundances than untreated soils, a finding that contrasts with many laboratory-based reports of glyphosate toxicity to fungi (Van Bruggen *et al.*, 2021; Santos *et al.*, 2020).

Looking more closely at the ward level fungal data, treated O horizon PSF counts ranged from 27×10^3 SFU/g at Bantaji to 88×10^3 SFU/g at Hospital, while untreated O horizon values ranged only from 21×10^3 to 32×10^3 SFU/g. The paired comparison across wards showed a robust mean increase of 31.7×10^3 SFU/g ($\approx 117\%$ higher) in treated O soils. Statistical analysis confirmed these differences as highly significant (paired $t(9) = 6.334$, $p = 0.0001$).

Similarly, the A horizon revealed treated means of 46.1×10^3 SFU/g versus 20.0×10^3 SFU/g in controls (paired $t(9) = 5.31$, $p = 0.00048$), while the E horizon showed 35.0×10^3 SFU/g in treated versus 13.2×10^3 SFU/g in untreated soils (paired $t(9) = 6.525$, $p = 0.0001$).

These consistent patterns across horizons demonstrate a reproducible enrichment of culturable PSF in glyphosate exposed soils.

The magnitude of enrichment is especially noteworthy given that PSF are generally considered sensitive to agrochemical disturbances. The results suggest that glyphosate may not simply inhibit fungal communities, but instead selectively favour tolerant taxa such as *Aspergillus*, *Rhizopus* and *Penicillium* that can withstand or even metabolize glyphosate and its breakdown products (Sharma *et al.*, 2022; Qu *et al.*, 2024). Glyphosate adsorption to ferruginous soil oxides in Wukari likely concentrate residues in the topsoil, exposing O horizon fungi most directly. This may explain why treated O soils consistently exhibited the greatest fungal counts compared to deeper horizons, where glyphosate exposure is lower but nutrient availability also declines (Anderson & Magdoff, 2023).

Biochemical assays support the observed increases in culturable PSF. Isolates of *Aspergillus niger* and *Rhizopus stolonifer* produced strong solubilization halos on

agar and exhibited robust extracellular enzyme activity, particularly acid phosphatases and organic acid secretion. These traits are consistent with enhanced phosphorus mobilization under glyphosate exposure, suggesting that the fungi not only persist but actively contribute to phosphate cycling under herbicide influence (Lei *et al.*, 2025; Ma *et al.*, 2025). Such functional resilience may provide shortterm benefits to phosphorus availability in farmlands, though long term ecological tradeoffs such as shifts in fungal diversity and potential suppression of glyphosate sensitive taxa require further study (Costa *et al.*, 2021).

The statistical and biochemical evidence demonstrates that glyphosate application in Wukari farmlands is associated with a significant enrichment of phosphate solubilizing fungi, particularly in the O horizon. This enrichment likely reflects selective pressures that allow tolerant fungi to dominate under glyphosate exposure, with implications for both nutrient cycling and longterm soil microbial ecology.

Conclusion

This study provided a comprehensive assessment of the impact of glyphosate on phosphate solubilizing fungi (PSF) across three soil horizons (0–10 cm, 10–20 cm, and 20–30 cm) in farmlands of Wukari LGA, Taraba State. Using fungal plate counts (SFU/g $\times 10^3$), HPLC quantification of glyphosate residues, and molecular sequencing, it was revealed that glyphosate treated soils had consistently higher PSF counts than untreated controls, with statistically significant differences across all horizons ($p < 0.001$). The O horizon supported the highest PSF populations, reflecting both enhanced organic inputs and stronger glyphosate exposure, while the A and E horizons showed progressively lower counts.

Molecular identification confirmed *Aspergillus niger* and *Rhizopus stolonifer* as the dominant phosphate solubilizing fungi in the study area. This species, known for its tolerance to chemical stress and ability to solubilize insoluble phosphate through organic acid secretion, likely contributed to the enrichment observed in treated soils. These findings highlight that glyphosate, while potentially disruptive to sensitive fungi, can select for robust PSF populations capable of maintaining phosphorus solubilization in agricultural systems.

Glyphosate application in Wukari farmlands does not reduce culturable PSF abundance but instead enhances their presence, particularly in surface soils. While this may temporarily support phosphorus availability, the ecological consequences of longterm fungal community shifts such as

reduced diversity or dominance of tolerant taxa warrant ongoing monitoring. The study underscores the need for balanced herbicide management practices that sustain both crop productivity and microbial-mediated soil fertility in tropical agroecosystems.

Recommendations

Based on the findings of this study, several recommendations are proposed for sustainable soil and microbial management in Wukari LGA and similar agroecological zones:

1. Farmers are advised to reduce both the frequency and concentration of glyphosate application. Overuse not only suppresses weed populations but also alters the community structure of phosphate solubilizing fungi (PSF), which are essential for maintaining soil fertility and crop productivity.
2. Weed management should be diversified by integrating practices such as crop rotation, cover cropping, mulching, and selective mechanical weeding. These approaches help control weeds while minimizing adverse impacts on fungal populations.
3. Application of PSF-based bioinoculants and compost should be encouraged to replenish and stabilize fungal communities, thereby enhancing phosphate availability in glyphosate-affected soils.
4. Agricultural extension workers and researchers should promote periodic monitoring of fungal populations to guide farmers in sustainable land use, helping to detect shifts in PSF abundance or diversity caused by herbicide application.
5. Local agricultural policies should incorporate clear monitoring mechanisms, guidelines on glyphosate use, and education programs on its ecological implications. Particular emphasis should be placed on the protection of beneficial fungi that sustain soil fertility in ferruginous tropical soils.
6. Awareness and sensitization programs should be organized to educate farmers on the ecological role of PSF, their contribution to nutrient cycling, and the long-term risks of glyphosate misuse. Improved farmer knowledge can foster sustainable soil stewardship and better decision-making on agrochemical inputs.

Contribution to Knowledge

This research contributes significant new insights to the field of agricultural microbiology, soil ecology, and herbicide impact studies, especially in the context of ferruginous tropical soils. The major contributions include:

1. Glyphosate application was found to significantly alter the abundance of phosphate solubilizing fungi (PSF), with treated soils consistently showing higher culturable PSF counts compared to untreated soils, highlighting the selective enrichment of tolerant fungal taxa.
2. PSF were more abundant in farmland soils than in long-term fallowed soils, emphasizing the role of continuous cropping, organic inputs, and root exudates in supporting and stimulating fungal communities involved in phosphate solubilization.
3. Molecular identification confirmed *Aspergillus niger* and *Rhizopus stolonifer* as the dominant phosphate solubilizing fungi in Wukari soils under glyphosate exposure. This demonstrates that resilient fungal taxa persist and remain functionally important despite herbicide stress.

4. By integrating fungal plate counts, glyphosate residue analysis using HPLC, and molecular sequencing, this study offers a novel, multilayered approach to understanding herbicide–fungus–soil interactions in Nigeria’s ferruginous soils, an area where data are sparse.

5. The findings present evidence that while glyphosate may not always reduce fungal abundance, it could narrow fungal diversity, potentially leading to ecological homogenization and reduced functional redundancy in soil ecosystems.

6. The confirmation of *Aspergillus niger* and *Rhizopus stolonifer* as robust and efficient phosphate solubilizers in glyphosate-impacted soils underscores their potential application as a biofertilizer candidate, opening pathways for further research into PSF-based biofertilizer formulations for sustainable agriculture in Africa.

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