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# Exploration of Microbial Community Dynamics in Glyphosate Treated Soil Influencing Different Soil Variables

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**Abstract**

Glyphosate is a broad-spectrum systemic organophosphorus herbicide used to control broad-leaved weeds and perennial grasses. The linkage between biodiversity and agroecosystem functioning necessitates elucidation of relationships between biotic and abiotic components, diverse impacts of glyphosate persistence and enzymes mediated catalysis through specified metabolic pathways leading to microbial community dynamics in agroecosystem. Metagenomic based high-throughput sequencing explores glyphosate-induced taxonomic and functional dynamics of microbiome due to longer half-life and ecological toxicity, which necessitate soil quality assessment and prioritize its inclusion in sustainable agro ecosystem. The study was designed to elucidate glyphosate induced shift in nutrients, microbial biomass and enzyme activities influencing microbial community dynamics in agroecosystem. Studies revealed wide variations in available soil nutrients and microbial biomass in different days after glyphosate treatment. FTIR analysis revealed the existence of different chemical moieties with characteristic IR fingerprints ranging from 910 $cm^{-1}$ to 3895 $cm^{-1}$. Microbial profiling of glyphosate-treated soil at different days after treatment showed wide variations in relative distribution of bacterial taxa at different taxonomic level. *Actinomycetota* (33.81-44.76)%, *Pseudomonadota* (34.20-43.16)% and *Acidobacteriota* (4.72-7.35)% were the dominant phyla. Further, the feature selection in glyphosate-treated soil revealed stronger correlation (TN, MBC, MBN, MBP and BSR), which influences microbial community dynamics in glyphosate-treated soil over time. Thus, catabolic profiling offers an effective approach to elucidate functional diversity influencing soil quality.

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**Keywords**
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Glyphosate;
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**Abbreviations**

| | |
|---|---|
| ASC | Agricultural soil (Control)/ without Glyphosate treatment |
| AMPA | Aminomethylphosphonic acid |
| ASC | Agricultural soil (Control)/ without Glyphosate treatment |
| ASG0 | Agricultural soil (Glyphosate treatment: 0 day) |
| ASG7 | Agricultural soil (7 days after glyphosate treatment) |
| ASG14 | Agricultural soil (14 days after glyphosate treatment) |
| ASG21 | Agricultural soil (21 days after glyphosate treatment) |
| ASG28 | Agricultural soil (28 days after glyphosate treatment) |
| BSR | Basal soil respiration |
| EP | Extractable phosphorous |
| FTIR | Fourier Transform Infrared Spectroscopy |
| GLS | Glyphosate |
| MBC | Microbial biomass carbon |
| MBN | Microbial biomass nitrogen |
| MBP | Microbial biomass phosphorous |
| NCBI | National Center for Biotechnology Information |
| OC | Organic Carbon |
| OUT | Operational Taxonomic Unit |
| PCA | Principal Component Analysis |
| PCoA | Principal Coordinates Analysis |
| TN | Total Nitrogen |

**Introduction**

Glyphosate [N-(phosphonomethyl) glycine] is phosphonomethyl derivative of glycine and/or aminophosphonic glycine analogue is a broad-spectrum herbicide that targets broad-leaved weeds and perennial grasses.[1] Its herbicidal action offers competitive inhibition of 5-enol-pyruvyl-shikimate-3-phosphate synthase in shikimate pathway in plants, fungi, bacteria and protozoa.[2,3] This blocks synthesis of aromatic amino acids (tyrosine, tryptophan and phenylalanine), inhibiting protein synthesis, chloroplast degeneration and membrane permeability causing plant death.[4,5] Microbial mediated degradation occurs via two pathways:

(i) utilizing glyphosate as phosphorous source, converting to sarcosine and mineralizing to $CO_2$ and water; (ii) glyphosate as nitrogen source to produce glyoxylate and AMPA.[6] Residual glyphosate and AMPA determine microbial community, which acts as a predictor in determining glyphosate degradation and persistence.[7] The study was designed to show relationships between change in glyphosate induced microbial community structure over time through high-throughput sequencing, emphasizing microbial activities as biomarkers for soil quality and sustainable development rather than degradation kinetics.

Microbial ecologists documented glyphosate induced shift in microbial community structure in agroecosystems using key soil quality biomarkers including soil organic matter,[8,9] microbial biomass pool[10] and enzyme activities.[11] Soil organic matter and clay influence glyphosate sorption, desorption, degradation and bioavailability.[12] Studies on glyphosate treatment revealed changes in microbial community structure and diversity and disruptions of bacterial network.[13] Foliar application impact microbial and functional profiles in nutrient turnover and biogeochemistry explored using metagenomic analysis.[14,15] Repeated herbicide exposure exerts selective pressure for microbial communities to adapt microenvironment or modify genetic profiles by resilience against sub-lethal doses.[16]

Metagenomics based high-throughput sequencing offers comprehensive exploration of glyphosate exposure on the taxonomic and functional dynamics of microbiome. Although glyphosate-based herbicides with varied formulations are extensively used, their long term and formulation dependent on soil microbial communities remains insufficiently understood. Therefore, the present study elucidated glyphosate induced alternations in available nutrients, microbial biomass, enzymatic activities, and their influence on

microbial community dynamics following herbicide application over time. The study also highlighted the role of microbial communities in glyphosate persistence, degradation and resilience leading to the emergence of herbicide-tolerant species and consequent impacts on sustainable agriculture.

## Materials and Methods

### Study Site and Sampling

The field experiments were conducted in the agricultural lands of Godbhaga, Attabira block of Bargarh district, Odisha (SF1). Agricultural lands with no herbicide exposure for at least two years were selected. Randomized block design having 10 m² plots was selected for glyphosate (GLS) treatment and one plot was referred to as 'control' (without glyphosate treatment). Sampling was done in different days after glyphosate treatment (0, 7, 14, 21 and 28). Each site was divided into five blocks and five random samples were collected (0-5 cm depth), pooled into sub-sample and mixed thoroughly to obtain a composite sample.

### Experimental Set up and Glyphosate Treatment

The experiment was conducted in four agricultural plots [Control (without treatment) and three for herbicides treatment]. Glyphosate was sprayed as per the recommended dose *i.e.* Roundup® 360 g/l acid equivalent glyphosate.[17] Soil samples were collected such as without glyphosate treatment (ASC) as well as 0,7,14, 21 and 28 days after glyphosate treatment (ASG0, ASG7, ASG14, ASG21 and ASG28). Effect of glyphosate on organic carbon (OC),[18] total nitrogen (TN),[19] extractable phosphorus (EP),[20] microbial biomass carbon(MBC),[21] microbial biomass nitrogen (MBN),[22] microbial biomass phosphorous (MBP)[23] and basal soil respiration (BSR)[24] was determined.

### FTIR Spectroscopic Analysis

Soil profiles (ASC: without treatment) and different days after glyphosate treatment (ASG0, ASG7, ASG14, ASG21, ASG28) were analyzed by FTIR to determine existence of different functional groups based on absorption.[25] Pellet was prepared with solid powder sample using hydraulic, dried at 60°C for 96 hr and tested in permeation mode compared to KBr pellet (blank) within (400-4000) $cm^{-1}$ spectrum using Bruker (Alpha II model).

### DNA Extraction, Amplicon Sequencing and Bioinformatics Analysis

Total gDNA was extracted by HiPurA DNA purification kit (Hi-Media). About 40ng of extracted DNA was used for PCR amplification using primers such as 5'-AGAGTTTGATGMTGGCTCAG-3' (Forward) and 5'- TTACCGCGGCMGCSGGCAC-3' (Reverse) for 25 cycles. The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed with Illumina adapter sequences. Amplicon purity was determined using Nanodrop and gel electrophoresis. Library was prepared and purified. Amplicon size, integrity and purity were quantified by Qubit dsDNA assay followed by Illumina Miseq with 2x300 PE sequencing. Metagenomic data is de-multiplexed into fasta-format. Raw reads were subjected to quality check using Fastaqc and Multiqc tools, chimeric sequence removed, clustered into OTUs and annotated using NCBI database.

### Sequence Analysis and Data Visualization

The data was rarefied to minimum library size, scaled by total sum scaling (TSS) and visualized with filter data source using rarefaction curve[26] (SF2). Rarefied OTUs tables assessed alpha and beta diversity. Alpha diversity was measured using four parameters (Chao1, Fisher, Shannon and Simpson diversity index). Beta diversity was measured by Bray-Curtis dissimilarity matrix to explore dissimilarities across different soil profiles represented by principal coordinate analysis (PCoA) plot. Pavian tool was used for graphical visualization of microbial taxa.[27] Datasets were imported to Ploty package to visualize differential abundance from phylum-genus level across different soil profiles. Plotly's python graphics library was used for comprehensive analysis revealing bacterial diversity patterns. Further, the stacked bar chart representing relative abundance of bacterial taxa was plotted using Origin 2023 (OriginLab corp., USA).

### Statistical Analysis

Simple correlation analysis between soil variables and enzyme activities in glyphosate treated soil using SPSS (Version 17.0). PCA was performed to discriminate soil profiles in different days after glyphosate treatment using SPSS (Version 17.0).

Redundancy analysis summarized linear relationships between the components of response variables that were redundant with a set of explanatory variables (enzyme activities) among glyphosate treated soil in different days after treatment using XLSTAT-2014 (Version 2.03).

## Results

### Soil Nutrients

The study revealed wide variation in organic C (2.83 - 2.25 mgC/g soil), total N (282.1 - 189.2 mgN/g soil) and extractable P (184.2 - 123.8 mgP/g soil) in glyphosate treated soil with maximum in 0 day and minimum in 28 days after glyphosate treatment (SF3). The analysis of variance showed significant decline in organic C ($r = 0.997$, $p < 0.001$), total N ($r = 0.985$, $p < 0.001$) and extractable P ($r = 0.996$, $p < 0.001$) in different days after glyphosate treatment. The study indicated consistent decline in microbial biomass C (179.13 - 126.35 mgC/g soil), microbial biomass N (21.89 - 13.03 mgN/g soil) and microbial biomass P (12.73 - 7.18 mgP/g soil) in different days after glyphosate treatment, which was found to be significant MB-C ($r = 0.991$, $p < 0.001$), MB-N ($r = 0.988$, $p < 0.001$) and MB-P ($r = 0.998$, $p < 0.001$) (SF4). The OC:TN ratio showed gradual increase (10.03 - 11.89) whereas MBC:OC ratio (6.32 to 5.61), MBN:TN ratio (7.75 to 6.88) and MBP: EP ratio (6.91 to 5.79) showed gradual decline over a period of 28 days after glyphosate treatment (Table 1). Basal soil respiration (BSR) declined significantly after glyphosate application, from 0.435 µg $CO_2$-C/g soil/hr on 0 day to 0.246 µg $CO_2$-C/g soil/hr by 28 days ($r = 0.999$, $p < 0.001$) (SF5).

**Table 1: Integrating quotients such as OC:TN, MBC:OC, MBN:TN and MBP:EP in agricultural soil in different days (ASG0 → ASG28) after glyphosate treatment**

| Integrating quotients | Different days after glyphosate treatment | | | | |
|---|---|---|---|---|---|
| | ASG0 | ASG7 | ASG14 | ASG21 | ASG28 |
| OC:TN | 10.03 | 10.86 | 11.44 | 11.61 | 11.89 |
| MBC:OC (%) | 6.32 | 5.94 | 5.81 | 5.72 | 5.61 |
| MBN:TN (%) | 7.75 | 7.63 | 7.37 | 6.96 | 6.88 |
| MBP:EP (%) | 6.91 | 6.78 | 6.53 | 6.31 | 5.79 |

### FTIR Analysis

FTIR analysis of glyphosate treated agricultural soil in different days (0, 7, 1, 21, 28) after treatment was conducted to identify existence of chemical moieties such as P-O, C-O, carbonyl (C=O), hydroxyl (O-H) and amine (N-H) with characteristic IR fingerprints ranging from 910 $cm^{-1}$ to 3895 $cm^{-1}$ (SF6). Peak appeared at 910 $cm^{-1}$, 999 $cm^{-1}$, 1628 $cm^{-1}$, 3276 $cm^{-1}$ and 3621 $cm^{-1}$ corresponds to the presence of P-O, C-O, carbonyl (C=O), hydroxyl (O-H) and amine (N-H) group respectively within the stretching vibration absorption (ST1).

### Microbiome Across Different Agricultural Soil Profiles

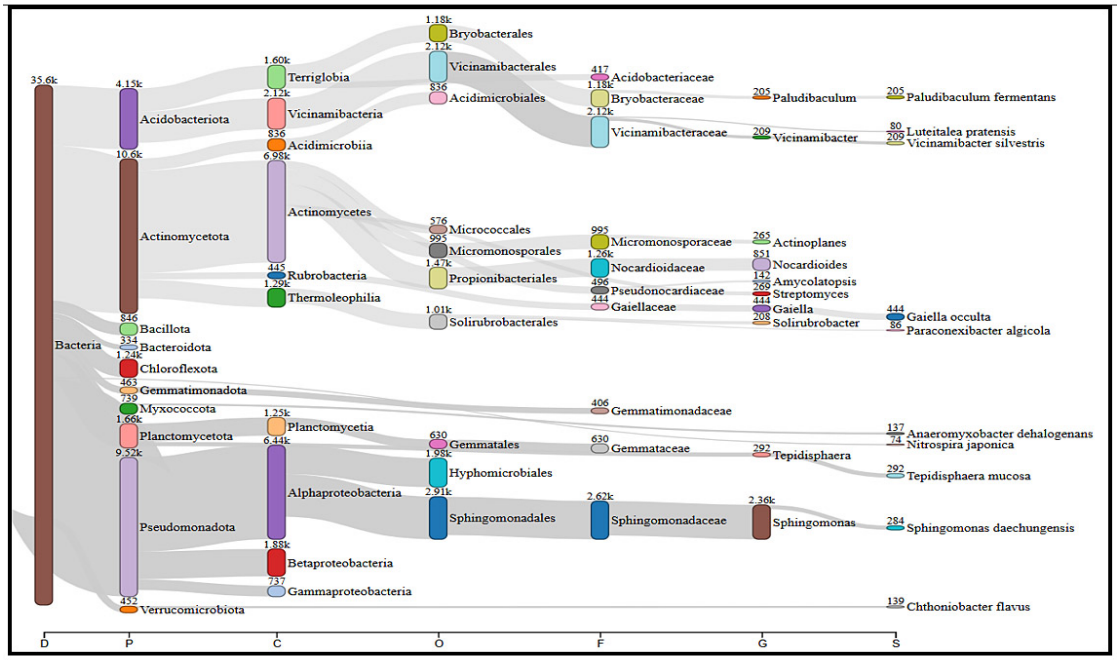
To elucidate the long-term impacts of glyphosate application on bacterial community structure in agricultural soil, the comprehensive microbial profiling of glyphosate treated soil on different days after treatment was performed. The data was presented using Sankey plot that revealed the relative distribution of bacterial taxa from phylum to species level.

About 35,600 bacterial reads were generated from untreated agricultural soil (ASC), distributed among different phyla. Moreover, the Pseudomonadota (38.8%), Actinomycetota (33.8%), Acidobacteriota (7.3%) and *Planctomycetota* (6.6%) were found to be the four most dominated phyla. *Alphaproteobacteria* (85%) was the most dominated bacterial class within phyla *Pseudomonadota* (Figure 1a). Dominated bacterial order includes *Sphingomonadales* (26.05%), *Propionibacteriales* (9.35%), *Micromonosporales* (4.96%), *Gaiellales* (4.78%) and *Hyphomicrobiales* (4.34%). Besides, *Sphingomonadaceae* was observed as most dominated bacterial family representing about 98% of bacterial taxa within order *Sphingomonadales.* About 37,800 bacterial reads

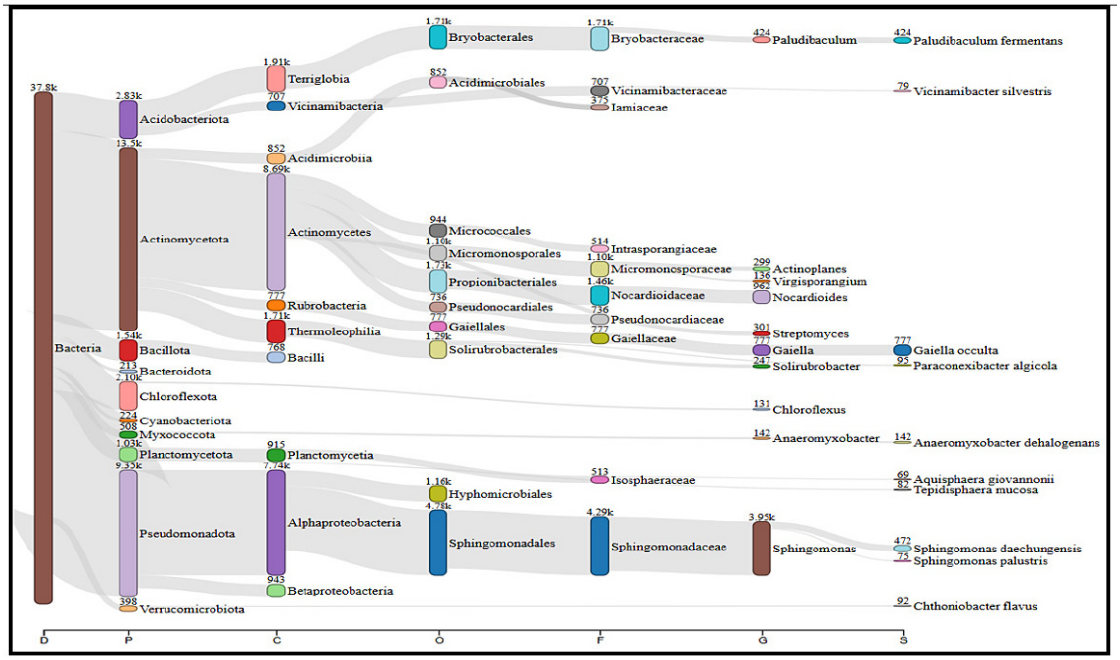
were obtained from glyphosate treated soil (ASG0). The study indicated that *Pseudomonadota* (43.2%), *Actinomycetota* (35.4%), *Acidobacteriota* (5.65%) and *Chloroflexota* (3.8%) were the dominated phyla (Figure 1b). Nevertheless, *Actinomycetes* (64%) followed by *Rubrobacteria* (19%) were observed to be the most abundant bacterial classes within phyla *Actinomycetota.* Additionally, *Sphingomonadales* (35.84%), *Propionibacteriales* (8.66%), *Gaiellales* (6.73%), and *Solirubrobacterales* (4.36%) were the dominated bacterial orders associated with ASG0.

At ASG7, about 39,300 bacterial sequences were generated, which mainly includes *Actinomycetota* (43.91%), *Pseudomonadota* (35.84%), *Acidobact--eriota* (5.2%) and *Chloroflexota* (4.51%) as dominated phyla. *Actinomycetes* (59%), *Rubrobacteria* (20%) and *Thermoleophillia* (17%) were the dominated class within phylum *Actinomycetota*. Major orders include *Sphingomonadales* (29.06%), *Propionibacteriales* (8.23%), *Gaiellales* (8.78%) and *Solirubrobacterales* (7.12%). Additionally, *Sphingomonadaceae* was the most dominated bacterial family representing 98% of order *Sphingomonadales* (Figure 1c). About 38,000 bacterial reads in ASG14 were obtained and classified into different taxa (Figure 1d). *Actinomycetota* (42.73%) *Pseudomonadota* (35.2%) and *Acido bacteriota* (5.14%) were three dominated bacterial phyla. *Actinomycetes* was the most prevalent bacterial class accounted 61% of the bacterial taxa followed by *Rubrobacteria* (20%) within phylum *Actinomycetota.*

A total of 36,000 bacterial reads were generated in 21 days after glyphosate treated soil (ASG21), which were distributed into different bacterial taxa (Figure 1e). The study revealed *Actinomycetota* (37.86%), *Pseudomonadota* (37.12%), *Acidobacteriota* (5.23%) and *Bacillota* (4.55%) as dominated phyla. *Alphaproteobacteria* (34.49%), *Actinomycetes* (23.93%) and *Rubrobacteria* (6.79%) were the dominated bacterial class. *Propionibacteriales* (35%), *Micromonosporales* (26%) and *Streptomycetaceae* (11%) were the dominated orders within class *Actinomycetota*. The bacterial order *Gaiellales* represented 100% bacterial taxa belonging to the class *Rubrobacteria* and 18% of the phylum *Actinomycetota.* About 36,600 bacterial sequences were generated in 28 days after glyphosate treated soil (ASG28). *Actinomycetota* (44.76%) followed by *Pseudomonadota* (35.65%) were the most dominated bacterial phyla in ASG28. The study revealed *Sphingomonadales* (28.32%), *Micromonosporales* (9.55%), *Gaiellales* (8.13%) and *Propionibacteriales* (7.12%) as the dominated orders in ASG28 (Figure 1f).




(a)


Bacteria
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Chloroflexota
Cyanobacteriota
Myxococcota
Planctomycetota
Pseudomonadota
Verrucomicrobiota
Acidobacteriota
Actinomycetota
Terriglobia
Vicinamibacteria
Acidimicrobiia
Actinomycetes
Rubrobacteria
Thermoleophilia
Bacilli
Planctomycetia
Alphaproteobacteria
Betaproteobacteria
Bryobacterales
Acidimicrobiales
Micrococcales
Micromonosporales
Propionibacteriales
Pseudonocardiales
Gaiellales
Solirubrobacterales
Hyphomicrobiales
Sphingomonadales
Bryobacteraceae
Vicinamibacteraceae
Iamiaceae
Intrasporangiaceae
Micromonosporaceae
Nocardioidaceae
Pseudonocardiaceae
Gaiellaceae
Isosphaeraceae
Sphingomonadaceae
Paludibaculum
Actinoplanes
Virgisporangium
Nocardioides
Streptomyces
Gaiella
Solirubrobacter
Chloroflexus
Anaeromyxobacter
Sphingomonas
Paludibaculum fermentans
Vicinamibacter silvestris
Gaiella occulta
Paraconexibacter algicola
Anaeromyxobacter dehalogenans
Aquisphaera giovannonii
Tepidisphaera mucosa
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Sphingomonas palustris
Chthoniobacter flavus
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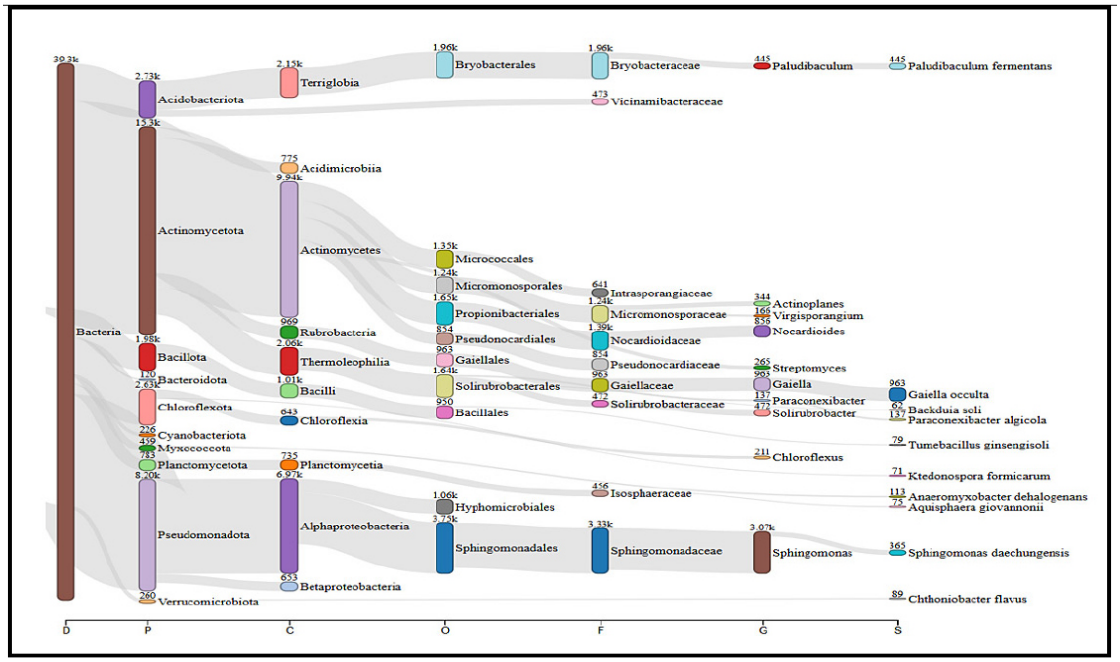
(b)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Actinomycetota
Terriglobia
Acidimicrobiia
Actinomycetes
Rubrobacteria
Thermoleophilia
Bacilli
Chloroflexia
Planctomycetia
Alphaproteobacteria
Betaproteobacteria
Bryobacterales
Micrococcales
Micromonosporales
Propionibacteriales
Pseudonocardiales
Gaiellales
Solirubrobacterales
Bacillales
Hyphomicrobiales
Sphingomonadales
Bryobacteraceae
Vicinamibacteraceae
Intrasporangiaceae
Micromonosporaceae
Nocardioidaceae
Pseudonocardiaceae
Gaiellaceae
Solirubrobacteraceae
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Sphingomonadaceae
Paludibaculum
Actinoplanes
Virgisporangium
Nocardioides
Streptomyces
Gaiella
Paraconexibacter
Solirubrobacter
Chloroflexus
Sphingomonas
Paludibaculum fermentans
Gaiella occulta
Baekduia soli
Paraconexibacter algicola
Tumebacillus ginsengisoli
Ktedonospora formicarum
Anaeromyxobacter dehalogenans
Aquisphaera giovannonii
Sphingomonas daechungensis
Chthoniobacter flavus
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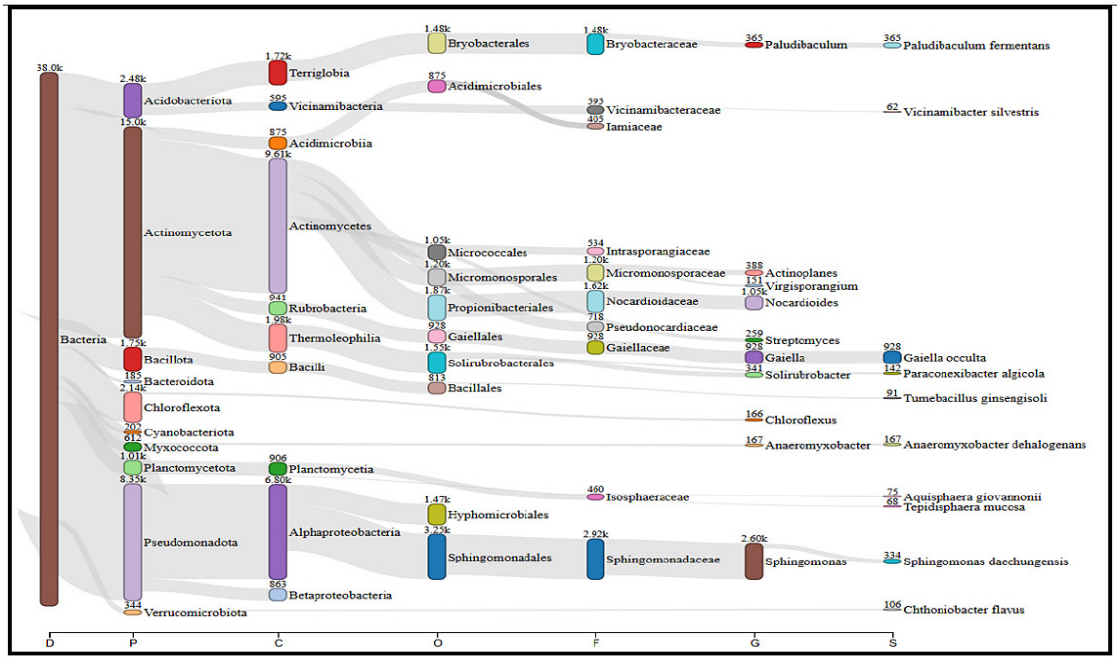
(c)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Planctomycetia
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Aquisphaera giovannonii
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Sphingomonas daechungensis
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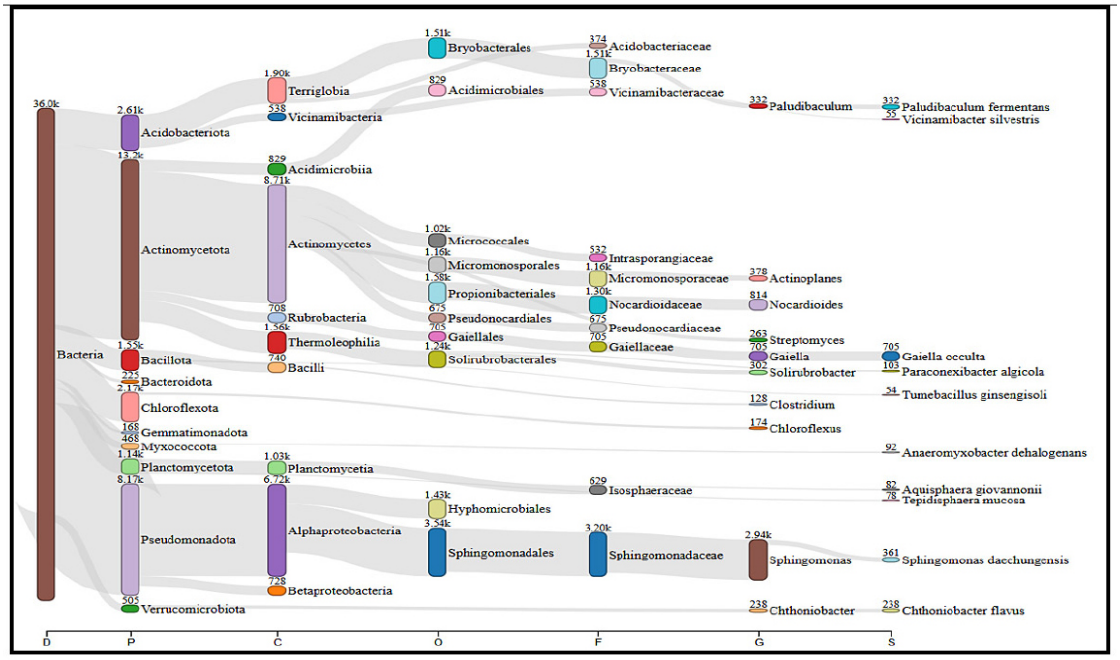
(d)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Planctomycetota
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Terriglobia
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Vicinamibacteria
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Acidimicrobiia
8.71k
Actinomycetes
708
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Propionibacteriales
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Hyphomicrobiales
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Sphingomonadales
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Vicinamibacteraceae
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Intrasporangiaceae
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Gaiellaceae
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Streptomyces
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Chloroflexus
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Tumebacillus ginsengisoli
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Anaeromyxobacter dehalogenans
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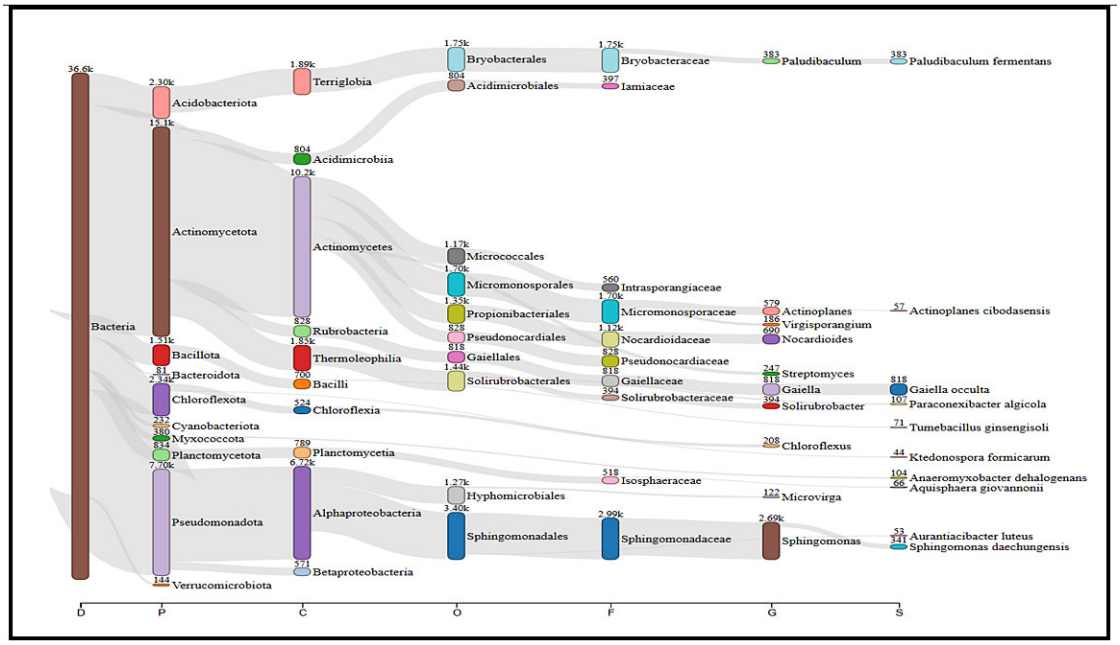
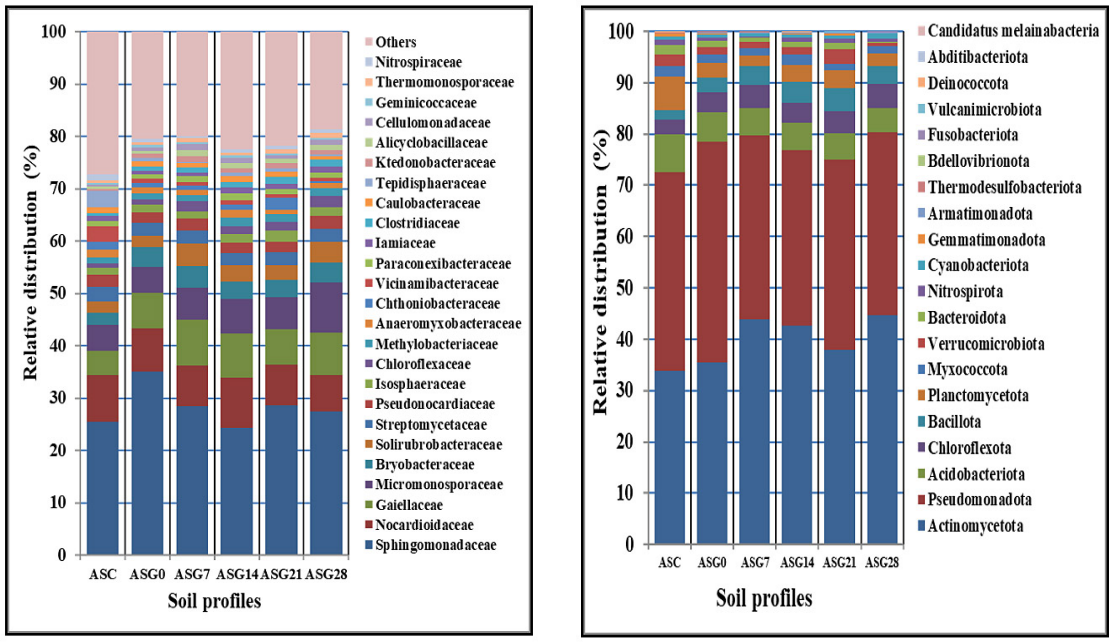



(f)

**Fig. 1: Bacterial community structure and taxonomic hierarchy from phylum to species level (D: domain, P: phylum, C: class, O: order, F: family, G: genus, S: species) in untreated and glyphosate treated soil over days revealed through Sankey plot.**

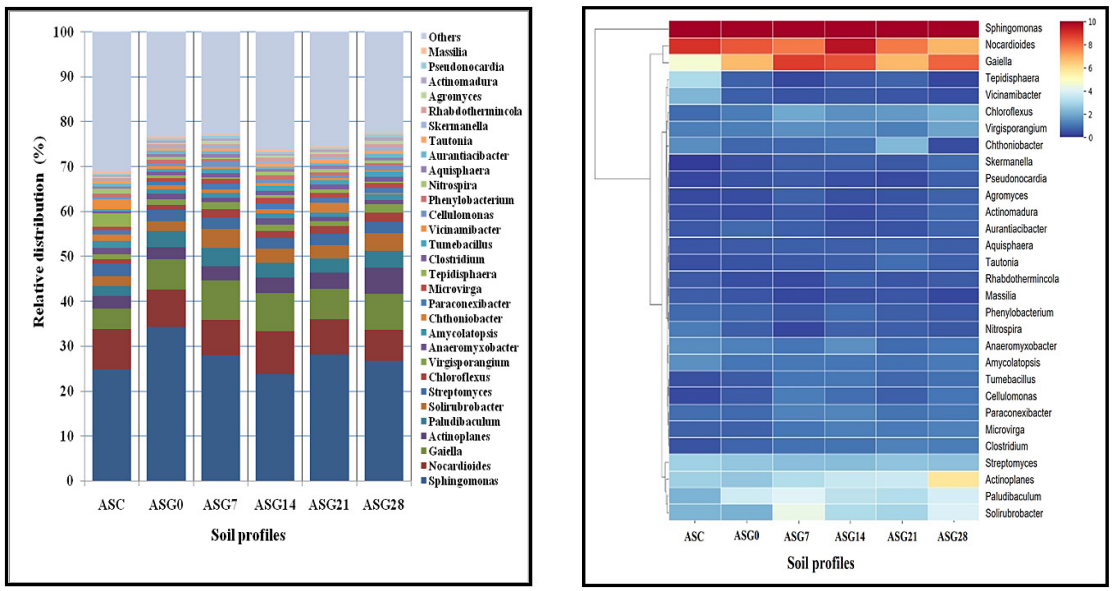
**Bacterial Community Dynamics at Taxonomic Levels Across Soil Profiles**

Relative abundance of top 20 bacterial phyla showed wide variations among untreated and glyphosate treated agricultural soil profiles (Figure 2a). *Actinomycetota* (33.81- 44.76)%, *Pseudomonadota* (34.20-43.16)%, *Acidobacteriota* (4.72-7.35)%, *Chloroflexota* (2.86-4.67)% and *Bacillota* (1.94-4.55)% were dominated phyla. Distribution of *Actinomycetota* was minimum in ASC, which showed increasing trend in glyphosate treated soil. Relative distribution of *Pseudomonadota* was highest in ASG0, which gradually declined over time. The study indicated dominance of *Sphingomonadaceae* (24.33-34.99)%, *Nocardioidaceae* (6.86-9.60)%, *Gaiellaceae* (4.67-8.79)% across soil profiles (Figure 2b). Relative distribution of *Sphingomonadaceae* was highest in ASG0 that declined over time. Bacterial genera *Sphingomonas* (23.72-34.26)%, *Nocardioides* (6.85-9.57)%, *Gaiella* (4.67-8.73)% and *Actinoplanes* (2.59-5.75)% were the dominated taxa (Figure 3a). Wide variations in certain bacterial taxa in *glyphosate* treated soil can be attributed to their abilities either to degrade glyphosate (Figure 3b). Bacterial species such as *Sphingomonas sp.* (20.05-29.39)%, *Nocardioides sp.* (6.17-8.58)%, Gaiella occulta (4.67-8.13)%, *Sphingomonas daechungensis (*2.98-4.09)% and *Paludibaculum fermentans* (2.15-4.09)% were the dominated bacterial taxa across untreated and glyphosate treated soil profiles (Figure 4a). *Gaiella occulta* exhibited significant variation with minimum in untreated soil ASC (4.67%), which increases in glyphosate treated soil profiles with relative abundance in ASG7 (8.78%) (Figure 4b).



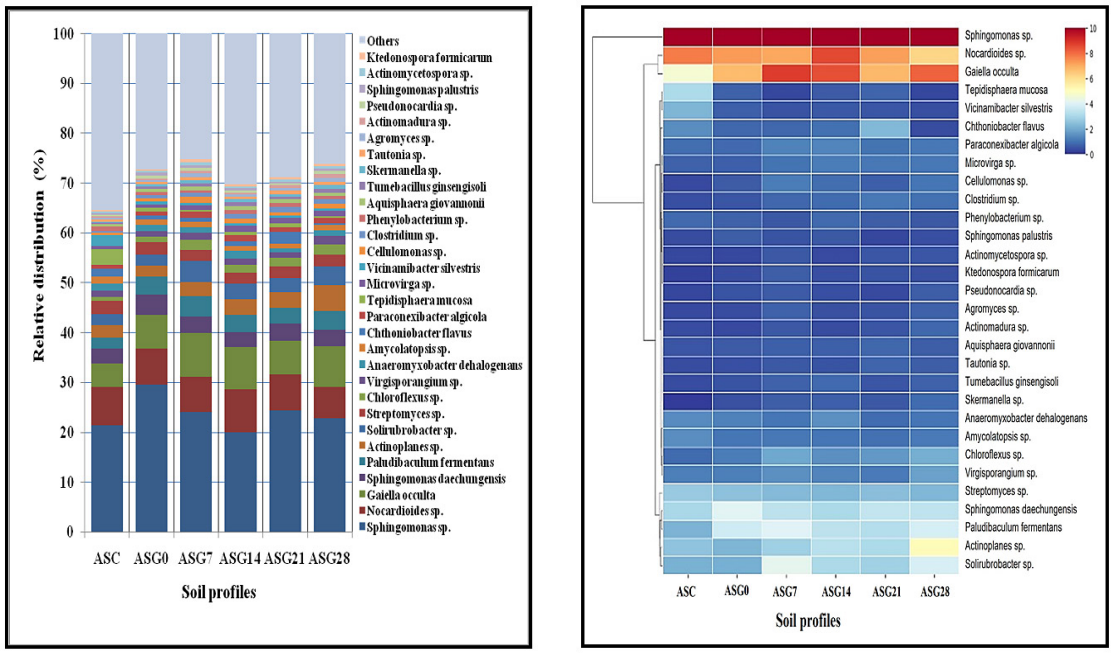

(a) (b)

**Fig. 2: Bacterial community dynamics in agricultural soil without treatment (ASC) and different days after glyphosate treatment (ASG0 → ASG28). Relative abundance (%) of (a) top 20 phyla and (b) top 25 families represented as stacked column bar.**




(a) (b)

**Fig. 3: Bacterial community dynamics across untreated (ASC) and glyphosate treated agricultural soil profiles (ASG0 → ASG28). (a) Relative abundance of bacterial genera represented by stacked bar column; (b) Taxonomic cluster heatmap displayed relative abundance of top 30 genera plotted based on their absolute z-score. Color codes represented with red (higher abundance) and blue (lower abundance) for each genus.**



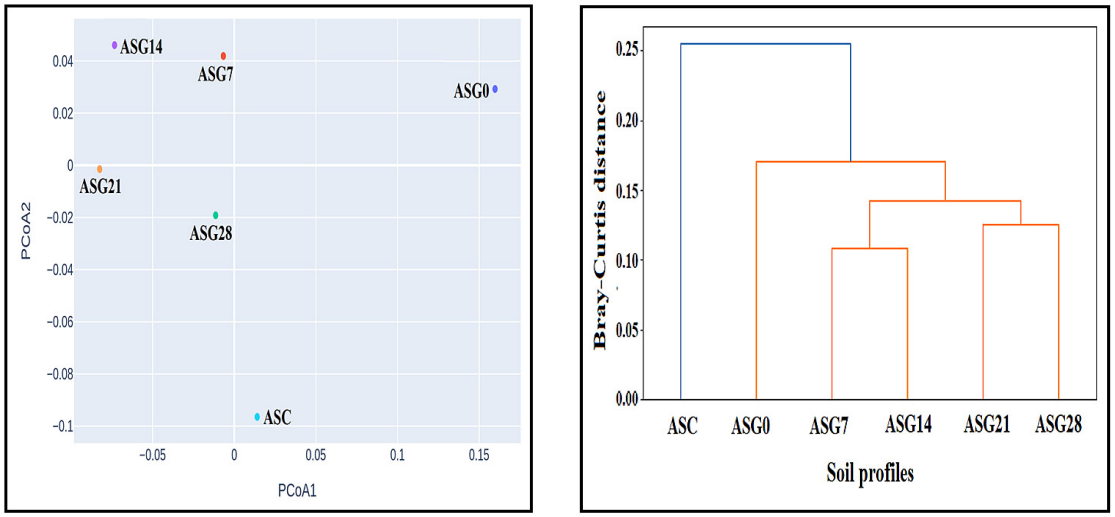

(a) (b)

**Fig. 4: Bacterial community dynamics in agricultural soil without treatment (ASC) as well as different days after glyphosate treatment (ASG0 → ASG28) revealed the (a) Relative abundance of bacterial communities at species level was represented by stacked bar column; (b) Taxonomic cluster heatmap displayed relative abundance of top 30 species plotted based on their absolute z-score. Color codes represented with red (higher abundance) and blue (lower abundance) for each species.**

**Diversity Indices Based on Relative Distribution**

Chao1 index varied from 177 (ASG0) to 193 (ASC) across different soil profiles. Higher Chao1 index value by ASC indicated rare bacterial communities compared to glyphosate treated soil profiles. Similarly, fisher value varied from 30.17 (ASG0) to 34.81 (ASC) across different glyphosate treated soil. Higher fisher value in ASC indicated greater species richness, while lower Fisher's value in ASG0 indicated lower species evenness. Moreover, Shannon's diversity index correlates both species richness and evenness, which varied from 3.28 (ASG0) to 3.67 (ASC). Greater value of Shannon's diversity index exhibited by ASC revealed dominance of certain bacterial species compared to treated soil profiles. Further, Simpson diversity index varied from 0.85 (ASG0) to 0.91 (ASC).

Principal coordinates analysis (PCoA) based on Bray-curtis index value indicated the variance between ASC and different days after glyphosate treatment soil profiles. Study suggested that bacterial community across different soil profiles were well segregated (Figure 5a). Further, dendrogram was constructed using Ward clustering algorithm, which revealed closer relatedness between ASG7 and ASG14, ASG21 and ASG28 (Figure 5b). However, ASC and ASG0 were found to be distantly related with distinct bacterial communities.

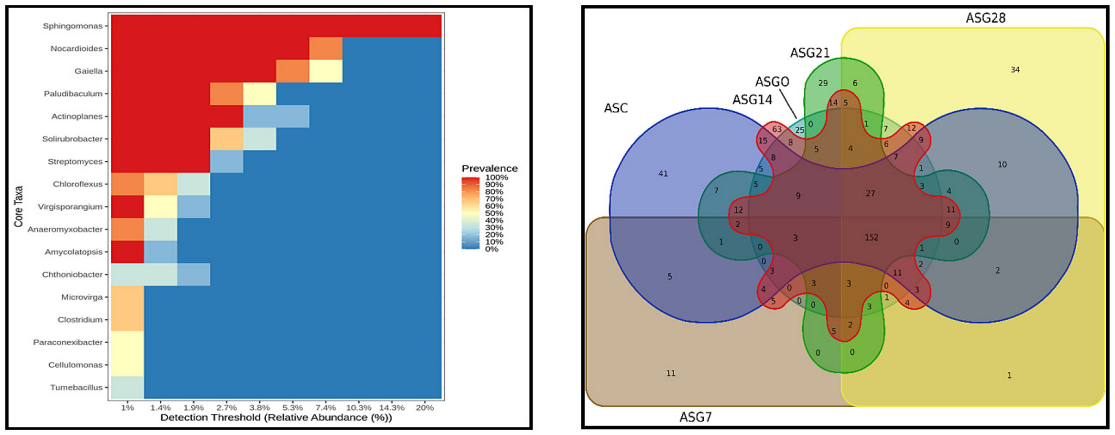



(a) (b)

**Fig. 5: (a) Principal coordinates analysis (PCoA) plot; (b) Dendrogram constructed using Ward clustering algorithm based on Bray-Curtis dissimilarity matrix between bacterial communities across soil profiles such as ASC (untreated soil) and different days after glyphosate treated agricultural soil (ASG0 → ASG28).**

## Core Microbiome Analysis

The core microbiome analysis revealed the presence of *Sphingomonas, Nocardioides, Gaiella, Paludibaculum, Actinoplanes, Solirubrobacter and Streptomyces* across untreated and glyphosate treated soil profiles (Figure 6a). The study clearly indicated that untreated ASC and different days after glyphosate treated soil profiles (ASG0 → ASG28) shared large number of bacterial taxa. However, bacterial taxa unique to individual soil profiles were represented by heterogeneous bacterial communities, depicted by Venn diagram. The result depicted the existence of 41 unique bacterial taxa associated with ASC (without glyphosate tretaement). Moreover, the unique bacterial taxa associated with different days after glyphosate treated agricultural soil such as ASG0, ASG7, ASG14, ASG21 and ASG28 were found to be 25, 11, 63, 29 and 34 respectively (Figure 6b).




(a) (b)

**Fig. 6: (a) Core microbiome identified for bacterial communities with prevalence threshold of 25% and relative abundance threshold of 1%; (b) Venn diagram revealed bacterial community dynamics across different soil profiles such as ASC (untreated soil) and different days after glyphosate treatment agricultural soil (ASG0 → ASG28).**

**Features Selection of Importance using Heatmap**

The features selection technique involves soil variables in dataset, which reveal most significant impact on the predictive power of a model, represented by heatmap (Figure 7). Features selection in glyphosate treated soil display patterns and correlations in heatmap in which vibrant colors signify stronger correlation (TN, MBC, MBN, MBP and BSR) with standalone value 1, while lighter colors represent weaker interactions among soil variables.

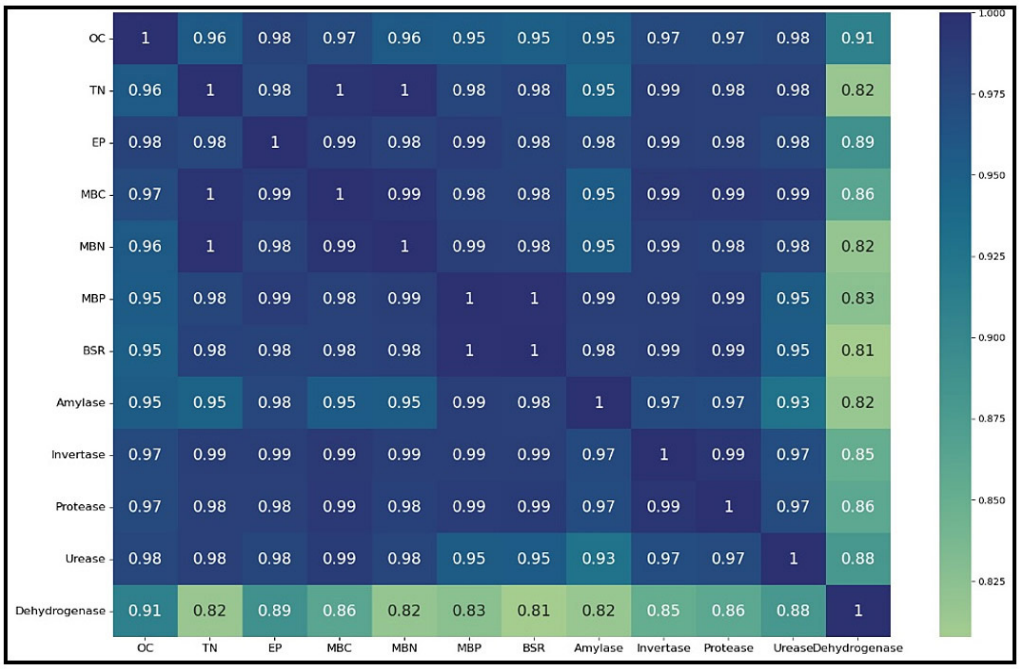



**Fig. 7: Features selection (TN, MBC, MBN, MBP and BSR) based on correlation among soil variables in different days after glyphosate treatment (ASG0 → ASG28).**

**Discussion**

The organic C play crucial role in modulating its mobility, soil persistence and adsorption.[28,29] Studies substantiated variability in organic C level in glyphosate treated soil based on organic matter inputs[30,31] and decline in microbial biomass.[32,33] Studies reported that glyphosate application induced gradual decline in microbial biomass and activity by altering C mineralization,[8,34] N assimilation[35,36] and P mobilization.[5,37,38] Glyphosate treatment in agricultural soil with low OC:TN ratio (10.03) suggested that surplus organic N beyond microbial demand is readily mineralized by heterotrophs.[39,40] The variation in OC:TN ratio is due to the glyphosate mediated alterations in nutrient cycling by inhibiting shikimate pathway,[41,42] degradation influencing microbial community structure,[3] persistence and mobility in agroecosystem. Its negative effects were assessed using different integrating quotients. Decline in MBC: OC ratio (6.32 to 5.61) over a period of 28 days after treatment indicated shift in microbial community structure influencing their potency for C mineralization due to decline in glyphosate persistence. Similar decline trends in MBN:TN ratio (7.75 to 6.88) and MBP: EP ratio (6.91 to 5.79) substantiated the role of microbial biomass in N mineralization and P immobilization in glyphosate treated soil that act as critical limiting nutrients.[43]

Minimal BSR exhibited in 28 days after treatment indicated lower microbial turnover due to substrate scarcity, glyphosate induced toxicity[44] and binding of glyphosate residues reduces labile pool of organic matter.[45] The study indicated that relatively

higher microbial metabolic quotient was observed in 0 day (28.284 x $10_{-4}$ g $CO_2$-C/g microbial C/hr) and minimum in 28 days (19.469 x $10_{-4}$ g $CO_2$-C/g microbial C/hr) after glyphosate application (SF4). Significant reduction in microbial metabolic quotient was observed over different days after application ($r = 0.944$, $p < 0.001$). Microbial metabolic quotient reflects efficacy of microbial communities involved in substrate/energy utilization, serving as potential soil quality biomarker.[46] Higher $qCO_2$ supports microbial community with 'r- strategy' ecotype, while lower $qCO_2$ associated with complex detritus and favors 'k- strategy' ecotype.[47] FTIR analysis revealed that intensities of several characteristic absorption peaks decreased in glyphosate treated soil over time. Similar finding was reported in the glyphosate treated agricultural soil.[25] Gradual decline in absorption peaks in glyphosate treated soil was due to glyphosate degradation over time[48] with lower concentration in ASG28 compared to ASG0.[49]

*Metagenomic* analysis revealed that *Actinomycetota, Pseudomonadota, Acidobacteria and Chloroflexota* were found as prominent bacterial phyla across untreated and glyphosate treated agricultural soil. *Abundance of Pseudomonadota, Actinomycetota, Acidobacteria* and *Chloroflexota* in untreated and glyphosate treated agricultural soil were reported.[7,38,50-52] Glyphosate is reported to trigger the shift in microbial community structure after repeated application.[8,53] Studies reported that microbial community dynamics in agricultural soil is influenced by glyphosate treatment combined with seasonal variations, cropping patterns and soil management practices.[7,52] The dominance of phyla *Pseudomonadota (Proteobacteria) and Actinomycetota (Actinobacteria)* in glyphosate treated soil was reported.[54-56] Bacterial communities were reported to degrade glyphosate after application in agricultural soil and utilize as the source of nitrogen, phosphorus and carbon.[6,7] Moreover, glyphosate has been reported to influence sensitive bacterial taxa by reducing competition, allowing the proliferation of resistant bacterial taxa.[38,56] Several studies have been substantiated the subtle changes in bacterial community after glyphosate application in agricultural soil over time.[7,53] Diversity indices were used to estimate richness and abundance of bacterial communities, which was found to be higher in ASC compared to treated soil profiles.[50,57-59] Principal coordinates analysis (PCoA) based on Bray-curtis index value revealed closer relatedness between glyphosate treated soil with distinct bacterial communities have been substantiated.[7,56] Core microbiome analysis reported that glyphosate promotes the shift in bacterial community structure in agricultural soil.[56,60] Repeated glyphosate exposure creates favorable conditions for microbial growth by acting as selective pressure leading to microbial adaptation across the glyphosate treated agricultural soil.[5,61] Besides, the features selection highlights redundant and/or irrelevant features improving model efficiency by enabling dimensionality reduction without compromising accuracy.[62]

## Conclusion

The present study demonstrated that glyphosate application can induce temporal variations in microbial community composition and soil functional attributes, as reflected by alternations in microbial biomass pool size, basal soil respiration and enzyme activities. However, the magnitude of microbial responses may vary with glyphosate dose, soil properties, environ mental conditions, and exposure duration. Bacterial community structure and biochemical indicators can therefore serve as sensitive biomarkers for monitoring glyphosate-induced changes in soil quality over time. However, longer-term studies incorporating diverse microbial groups and field conditions are needed to clarify the persistence, recovery, and broader ecological consequences of these effects.

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## Conflict of Interest

The authors do not have any conflict of interest.

## Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Data Availability**

All the metagenomic data generated during this study are available in the NCBI database (BioProject PRJNA1395345).

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials.

**Authors Contribution**

- **Debajani Shyamal:** Sampling, conducting experiments.
- **Ankita Agrawal:** Data compilation, analysis, writing.
- **Amisha Mohanty:** Methodology, data collection.
- **Aparupa Naik:** Statistical analysis.
- **Amiya Kumar Patel:** Conceptualization, final approval of manuscript.

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