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Alban Nubazung Kpemuonye
Tshwane University of Technology
Titus Yeliku-ang Ngmenzuma
Tshwane University of Technology
Richard Oteng-Frimpong
CSIR-Savanna Agricultural Research Institute
Tiisetso Mpai
University of South Africa
Puffy Soundy
Tshwane University of Technology
Felix Dapare Dakora
dakorafd@gmail.com

Tshwane University of Technology

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Functional 16S rRNA Genomic Analysis of Herbicide-treated Soil Inocula from Legume and Cereal-based Cropping Systems in Smallholder Agroecosystems of Northern Ghana

Alban Nubazung Kpemuonye[1,5], Titus Yeliku-ang Ngmenzuma[1,4*], Richard Oteng-Frimpong[3], Tiisetso Mpai[4,6], Puffy Soundy[1] and Felix Dapare Dakora[2,4*].

[1] Department of Crop Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa. knalbank2012@gmail.com_, zuma.titus@yahoo.com , & soundyP@tut.ac.za
[2] Department of Chemistry, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa. DakoraFd@gmail.com
[3] Crop Improvement Programme, CSIR-Savanna Agricultural Research Institute, Tamale, P.O. Box TL52, Ghana. kotengfrimpong@gmail.com/ ro.frimpong@csir.org.gh
[4] Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Private Bag X6, Florida, 1710, South Africa. tiisetsompai@gmail.com
[5] Department of Science Education, McCoy College of Education, P.O. Box ND12, Ghana. knalbank2012@gmail.com
[6]Agricultural Research Council – Tropical and Subtropical Crops, Hluhluwe, 3960, KZN, South Africa

*Correspondence: DakoraFd@gmail.com; FD Dakora, and zuma.titus@yahoo.com ; TY Ngmenzuma

Abstract

The increasing reliance on herbicides for weed management in smallholder farming systems across sub-Saharan Africa has raised concerns about their long-term effects on soil microbial communities that sustain soil health and crop productivity. This study evaluated the impact of herbicide application on the diversity and population structure of plant growth-promoting microorganisms (PGPM) in agricultural soils collected from 15 farmers' fields in the Upper West Region of Ghana. Soil samples were obtained from herbicide-treated farms and untreated reference sites under legume- and cereal-based cropping systems. Bacterial community composition was characterized using 16S rRNA gene amplicon sequencing, with taxonomic classification performed using the Kraken2 pipeline within the Galaxy platform. Untreated soils consistently exhibited greater microbial taxonomic richness than herbicide-treated soils at both the phylum (29–34 vs. 21–32) and genus (248–495 vs. 182–338) levels. Herbicide application reduced the relative abundance of beneficial phyla, including Firmicutes, Planctomycetes, and Actinobacteria, while enriching stress-tolerant groups such as Acidobacteria and Chloroflexi. At the genus level, important plant growth-promoting taxa, such as *Bradyrhizobium*, *Burkholderia*, *Streptomyces*, *Conexibacter*, and *Planctomyces* declined markedly, whereas xenobiotic-degrading genera including *Rhodoplanes*, *Sphingomonas*, *Geodermatophilus*, and *Nocardioides* increased, indicating microbial adaptation to herbicide-derived compounds. Principal component analysis and agglomerative hierarchical clustering revealed distinct shifts in microbial community composition following herbicide application. Overall, these findings demonstrate that herbicide use alters beneficial soil microbial communities, potentially reducing microbial activities associated with ecosystem functioning such as biological

nitrogen fixation, phosphate solubilization and biocontrol, highlighting the need for environmentally-friendly herbicide management strategies that preserve soil health and agricultural productivity.

Keywords: herbicide, plant growth-promoting microorganisms, soil microbial diversity, 16S rRNA metagenomics, *Bradyrhizobium*, biological nitrogen fixation.

1.0 Introduction

Soil microorganisms constitute the biological engine of terrestrial ecosystems, mediating critical processes such as nutrient cycling, organic matter decomposition, and the maintenance of soil structure [1,2]. Among these microbes, plant growth-promoting microorganisms (PGPMs), including the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Azospirillum*, and many mycorrhizal fungi, have received considerable attention for their roles in biological nitrogen fixation, phosphate solubilisation, siderophore secretion, and the synthesis of phytohormones that directly or indirectly enhance plant growth and productivity [3,4]. The functional role of these microbial communities is therefore not merely of ecological concern but also of agronomic importance, particularly in low-input agricultural systems where smallholder farmers depend heavily on natural soil fertility to sustain crop yields for food security [5].

In Sub-Saharan Africa, herbicide use has increased rapidly over the past two decades, driven by labour shortages, the intensification of cereal and legume cropping systems, and the increasing availability of broad-spectrum formulations such as glyphosate, paraquat, and atrazine [6]. The national agricultural surveys indicate that herbicide adoption among Ghanaian smallholder farmers rose from about 26% in the early 2000s to over 60% by the mid-2010s, with the northern savanna zones, including the Upper West Region, recording some of the steepest increases owing to the predominance of manually intensive, rain-fed farming systems [7-9]. In the Upper West Region, herbicides such as the glyphosate-based products, pendimethalin, and 2,4-dichlorophenoxyacetic acid (2,4-D) are routinely applied to fields used for cultivation of maize, sorghum, millet, and groundnut, often with limited technical guidance on dosage, timing, and environmental safeguards [10,11].

While the agronomic benefits of herbicide application in terms of weed suppression and labour savings are well documented [12], a growing body of evidence suggests that repeated or excessive herbicide use can substantially alter soil microbial community structure and function. Glyphosate, for example, has been shown to inhibit the shikimate pathway not only in target plants but also in sensitive soil bacteria, thereby reducing populations of beneficial taxa such as *Pseudomonas* spp. and certain rhizobial species [13,14]. Atrazine and its metabolites can persist in soil for extended periods and have been associated with shifts in microbial diversity indices, particularly reductions in fungal-to-bacterial ratios and declines in arbuscular mycorrhizal fungi colonisation [15,16]. Similarly, paraquat, though rapidly adsorbed to soil colloids, has demonstrated inhibitory effects on nitrifying bacteria and phosphate-solubilising microorganisms under certain edaphic conditions [17]. Meta-analyses and systematic reviews have confirmed that the magnitude and direction of herbicide effects on soil microbial communities are highly context-dependent, varying with herbicide chemistry, application rate, soil type, climate, and the pre-existing composition of the microbial assemblage [18,19].

Despite this increasing global evidence, research on the microbiological consequences of herbicide use in West African soils remains limited. The few studies conducted in the region have focused predominantly on the forest and transitional agroecological zones of southern Ghana, Nigeria, and examined general microbial biomass or single functional groups in isolation [20-24]. The Guinea savanna soils of the Upper West Region are characterised by low organic carbon content, sandy-loam textures, seasonal waterlogging, and high temperatures, which present a distinct edaphic and climatic zone where herbicide-microbe interactions may differ fundamentally from those reported in the tropical forest soils [25]. Furthermore, the

spatial heterogeneity of farming practices across the region, where different communities may preferentially use different herbicide types at varying concentrations and frequencies, offers an opportunity to assess their impact on plant growth-promoting microbial (PGPM) communities so far, no study has evaluated the population densities, species composition, and diversity of plant growth-promoting microbial communities across herbicide-treated fields in the Upper West Region.

The aim of this study was to evaluate the effects of herbicide application on the population, diversity and community structure of plant growth-promoting microorganisms in agricultural soils in the Upper West Region, of Ghana. More specifically, it assessed the abundance of key functional groups, characterized microbial diversity using genomic approaches, and examined the relationship between PGPM community composition and herbicide use patterns.

2 MATERIAL AND METHODS

2.1 Description of the study site

Soil samples were collected from paired farmers' fields (herbicide-treated and cultivated sites) planted to maize, soybean, cowpea, millet or groundnut, with ≥3 years or more of herbicide use, and with untreated soil (uncultivated sites), ≥6 years without herbicides in 15 locations (namely, legume-based system: Gbarikong, Lambussie, Saan, Sangu, Baazu, Dapuoh, Konzokalaa and Nanvile or cereal-based system: Tangbonne, Nator, Chaang, Gbierung, Nanga, Takpo and Dakyie). Their respective GPS coordinates within Ghana's Upper West Region (10°.2530" N, 2'.1450" W) are indicated in Table 1. This region is located in the north-western part of Ghana and lies within the Guinea savanna agro-ecological zone, which is characterized by distinct climatic and vegetation patterns [26,27]. The Guinea savanna zone has a unimodal rainfall pattern with an average rainfall of 800–1100 mm/year, and soil texture

classified as sandy-loam with low organic matter content and a pH <6. At the study sites,10 herbicides were used by farmers in their fields (Table 1). Based on trade names, 6 active ingredients were identified, as shown in Table 2.

2.2 Soil sample collection and preparation

Soil samples (in triplicates) were collected randomly from 15 farmers' fields with history of herbicide use together with untreated controls. The samples were obtained from the top 0-20 cm subsurface soil using soil auger sterilised by dipping into 70% alcohol and immediately placed in zip-lock bags. Where possible, the soil samples were placed in cooler boxes with ice packs and later stored at 4°C after sieving (2 mm mesh) [28,29] and transported to South Africa for the genomic analysis. The herbicide-treated and untreated soils (5 g each) from farmers' fields were sent for nutrient analysis. The physicochemical properties of the soil and cropping history of the study sites are presented in supplementary table 1 and 2.

2.3 Total genomic DNA extraction from treated and untreated soils

The soil samples were sent to the Central Analytical Facilities (CAF), DNA Sequencing unit, at the University of Stellenbosch, South Africa for DNA extraction, and sequencing. Total genomic DNA from microbes in herbicide-treated and untreated soils was extracted as described by Jaiswal et al. [30]. The DNA was extracted from 250 mg soil using DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The DNA concentration, quality control, variable region amplification, library preparation, template and sequencing were done using the Ion Torrent S5 method. Metagenomic DNA (mgDNA) concentrations were quantified using the Qubit™ 4 flourometer 1X dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher

Scientific). The NanoDrop® ND-1000 (ThermoFisher Scientific) was employed to determine the purity of the mgDNA samples, using a low TE buffer to blank the instrument (ND-1000-v3.8-users-manual). The Genomic quality score (GQS) was determined on or LabChip GX Touch 24 Nucleic Acid Analyzer (Revvity, Waltham, MA, USA), using the DNA extended range chip and Genomic DNA Reagent Kit (Revvity, Waltham, MA, USA) according to the manufacturer's protocol. The GQS value varies from 0 to 5, with zero indicating the lowest quality DNA and five high-quality, intact gDNA.

An Ion 16S™ Metagenomics Kit (ThermoFisher Scientific) was used to amplify hypervariable regions from polybacterial samples according to the protocol. Target regions were amplified from 2 µl mgDNA across 25 cycles for two primers pools on the SimpliAmp™ Thermal Cycler (ThermoFisher Scientific). The presence of amplified products was verified on the LabChip GX Touch 24 Nucleic Acid Analyzer (Revvity, Waltham, MA, USA), using the X-Mark DNA LabChip and HT DNA NGS 3K Reagent Kit according to the manufacturer's protocol in the NGS 3K Assay Quick Guide. Following verification, PCR products were purified with 1.8x volume AMPure XP reagent (Beckman Coulter, Brea, CA, USA) and eluted in 25 µl Nuclease-Free Water (ThermoFisher Scientific). Purified amplicons were quantified on the Qubit™ 4 Fluorometer using the Qubit Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher Scientific) according to the protocol, with lot number MAN0017455.

2.4 Library Preparation

Library preparation was performed from 50 ng purified PCR product for each sample using the Ion Plus Fragment Library Kit (ThermoFisher Scientific), according to the manufacturer's instructions (Ion™ Plus Fragment Library Preparation User Guide).

Briefly, 79 µl of each purified, PCR product was end-repaired at room temperature for 20 min, using 1 µl end-repair enzyme and 20 µl End-repair buffer in a final volume of 100 µl. The end-repaired products were purified with 1.8x volume AMPure XP reagent (Beckman Coulter). The end-repaired product was ligated to 1 µl IonCode Barcode Adapters (ThermoFisher Scientific). The adapter-ligated, barcoded libraries were purified with 1.4x volume AMPure XP reagent (Beckman Coulter) and quantified using the Ion Universal Library Quantitation Kit; according to the manufacturer's protocol (Ion 16S Metagenomics Kit User Guide). Quantitative PCR amplification was performed using the StepOnePlus™ Real-Time PCR System (ThermoFisher Scientific). Library fragment size distributions were assessed on the LabChip GX Touch 24 Nucleic Acid Analyzer (Revvity, Waltham, MA, USA), using the X-Mark DNA LabChip and HT DNA NGS 3K Reagent Kit, according to the manufacturer's protocol in the NGS 3K Assay Quick Guide. Libraries were diluted to a target concentration of 40 pM. The diluted, barcoded, 16S libraries were combined in equimolar amounts for template preparation using the Ion 510™, Ion 520™, and Ion 530™ Chef Kit (ThermoFisher Scientific). Briefly, 25 µl of the pooled library was loaded on the Ion Chef liquid handler using Reagents, Solutions and Supplies according to the protocol (Ion 510, Ion 520, and Ion 530 Chef Kit User Guide). Enriched, template positive ion sphere particles were loaded onto an Ion 530™ Chip (ThermoFisher Scientific).

2.5 Sequencing and data assembling

Sequencing was performed on the Ion GeneStudio™ S5 Prime System (ThermoFisher Scientific using the Ion S5™ Sequencing Solutions and Sequencing Reagents Kits according to the manufacturer's instructions (Ion 510, Ion 520, and Ion 530 Chef Kit User Guide). Flow space calibration and BaseCaller analyses were performed using default analysis parameters in the Torrent Suite Version 5.18.1 Software. The

taxonomic abundance (%) of microbial communities in each herbicide-treated and untreated soil was assessed by querying read files against dereplicated and removed sets of sequences from the small subunit (SSU) UCLUST. Following the normalization of sequences from each sample, rarefaction analysis was conducted at the genus and phyla levels to evaluate sampling adequacy, employing the alpha rarefaction.py tool from galaxy software.

2.6 16S rRNA metagenomics analysis

The quality and taxonomic classification of the sequence reads were performed on Galaxy online platform (https://galaxy-main.usegalaxy.org). The quality check of the raw reads was assessed using the FastQC (Version 0.74+galaxy1), while Trimmomatic was employed for trimming out the poor-quality raw reads. After the quality check, the paired sequence reads were analysed for taxonomic classification using Kraken2 tool (Version 0.12.1) with default setting. The resulting taxonomic data presented in TSV file format were then saved as excel for further arrangement and visualization. The saved data were presented in the form of appropriate graphs using Microsoft Excel, providing a visual representation of the taxonomic composition of the microbial communities in each soil sample [31]. The accession numbers of the samples generated from the National Center for Biotechnology Information (NCBI) platform are presented in supplementary table 3 and 4.

2.7 Statistical analysis

The data were subjected to multivariate statistical analysis using Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering XLSTAT [32,33] to visualize and elucidate the relationships between the samples and their attributes. The taxonomic abundance of data the sequences were normalized for each sample and

log transformed. The relative abundance of microbial taxa was calculated from the phylum level and the genus level for both herbicide-treated and untreated soils [34,31]. The respective operational taxonomic abundances were organised in a descending order and their numerical numbers determined, as well as best 35 and 10 percentage abundance for the genus and phylum levels respectively determined and used to plot column/bar graphs and pie charts using Microsoft Excel.

3. Results

3.1 Taxonomic abundance of plant growth-promoting microbial phyla in herbicide-treated and untreated soils

The total number of microbial phyla detected in untreated soils from fields with legume-based cropping system was markedly higher, ranging from 29 phyla at Nanvile to 34 phyla at Lambussie and Dapuoh, relative to herbicide-treated soils, which ranged from 21 phyla at Dapuoh to 32 phyla at Saan (Figure 1A), a pattern found across nearly all fields with legume-based cropping system (Figure 1A; Gbarikong to Konzokalaa), with the exception of Saan and Nanvile, which experienced a marginal increase in phylum-level richness with herbicide application (Figure 1A). In contrast, soils collected from cereal-based fields (Tangbonne to Takpo) recorded an increase in phyla with herbicide treatment (Tangbonne, Nator, Gbierung, Nanga and Chaang) (29-30 phyla) compared to untreated soils (27-28 phyla) (Figure 1A). The phyla populations were similar at Dakyie and Takpo irrespective of herbicide application.

Of legume-based fields (Gbarikong to Nanvile), Furmicutes was the most abundant phylum in both the treated and untreated soils, with a ranged from 31.25% at Baazu to 46.83% at Saan in untreated soils (Figures 2.10 and 2.6). After the Fermicutes, the

phyla Proteobacteria, Actinobacteria, Planctomycetes and Acidobacteria were consistently the next most abundant in all fields whether treated or untreated with herbicides. However, at Gbarikong, Proteobacteria was the second most abundant phylum with about 26.13% of sequence reads in untreated soils, and Actinobacteria second with 25.47% reads in treated soils. At Lambussie, Proteobacteria recorded similar abundance levels of 27.21% in untreated and 27.16% in the treated soils. Actinobacteria was the third largest phylum, with a lower abundance in treated (Figure 2.3) relative to untreated soil (Figure 2.4). Soils collected from Saan also harboured Proteobacteria (22.54%), Actinobacteria (20.24%), as second and third largest phyla, followed by Acidobacteria (3.88%), and Planctomycetes (2.05%).

Furthermore, untreated soils from Sangu also revealed higher Firmicutes (38.25%), and Actinobacteria (27.70%) communities compared with herbicide-treated soil (35.84 and 23.48 %, respectively). Bacteroidetes recorded 0.65% in untreated versus 0.40% in treated soils, while Verrucomicrobia was higher in treated relative to untreated soils (Figure 2.7 and 2.8).

At Baazu, the untreated soils maintained higher abundance of Proteobacteria (30.70%), Actinobacteria (25.46%), Planctomycetes (5.96%), Acidobacteria (1.90%), Bacteroidetes (0.98%), and Chloroflexi (0.78%), after Firmicutes (34.55%), while the treated soils recorded a high 34.55% Firmicutes, and 27.70% Actinobacteria as well a low 5.96% Planctomycetes populations (Figure 2.9 and 2.10). At Dapuoh, the untreated soils exhibited higher abundances of Firmicutes (38.26%), Proteobacteria (24.19%), Planctomycetes (7.73%), Acidobacteria (4.39 %) and Chloroflexi (0.44%), compared to the treated soils which recorded lower counts of these phyla, though Actinobacteria was higher in treated (28.62%) than untreated soils (20.80%) (Figure 2.11 and 2.12).

The results obtained for untreated soils at Konzokalaa revealed higher abundance of Firmicutes (40.31%), Planctomycetes (7.41%), Acidobacteria (3.51%), Verrucomicrobia (0.76%), and Chloroflexi (0.49%) (Figure 2.14) relative to treated soil which recorded Proteobacteria (30.26%), Actinobacteria (26.07%), and Bacteroidetes (0.95%) (Figure 2.13). Firmicutes was 42.63% abundant in herbicide treated soils from Nanvile compared to 33.79% in the untreated controls. Furthermore, Proteobacteria, Planctomycetes, Verrucomicrobia, Gemmatimonadetes, Chloroflexi, and several minor phyla exhibited greater abundance in untreated soils at Nanvile but lower in population in treated soils (Figure 2.15 and 2.16).

Of the cereal-based cropping system, untreated soil from Tangbonne recorded slightly higher abundance of Firmicutes (38.15%), Actinobacteria (28.24%), and Acidobacteria (3.39%) relative to their herbicide-treated counterparts (36.42%, 22.81%, and 2.15%, respectively). Conversely, treated soil displayed relatively elevated abundance of Proteobacteria (28.15%), Planctomycetes (5.87%), Bacteroidetes (21.06%), Nitrospira, and Chloroflexi (Figure 2.17 and 2.18). At Nator, treated soils recorded marginally higher abundance of Proteobacteria (31.94%), Bacteroidetes (0.88%), Gemmatimonadetes, Nitrospira, and Chloroflexi, in contrast to the untreated soil which exhibited greater abundance of Firmicutes (34.57%), Actinobacteria (29.22%), and Planctomycetes (7.11%) (Figure 2.19 and 2.20).

At Gbierung, the untreated soil displayed 39.20% Firmicutes abundance while the treated soil exhibited high abundance of Proteobacteria, Actinobacteria, Planctomycetes, Acidobacteria, Verrucomicrobia, and Bacteroidetes (Figure 2.21 and 2.22). Treated soil from Nanga also revealed higher abundance of Firmicutes (40.06%) and low level of Thaumarchaeota (0.72%), Gemmmatimonadetes (0.73%), while the

untreated soil recorded high abundance of Proteobacteria, Actinobacteria, Planctomycetes, Verrucomicrobia and Cyanobacteria (Figure 2.23 and 2.24). At Chaang, untreated soils recorded increases in Firmicutes, Planctomycetes, and Acidobacteria, while treated soils showed higher abundance of Proteobacteria, Actinobacteria, Bacteroidetes, Thaumarchaeota, and Chloroflexi (Figure 2.25 and 2.26).

At Dakyie, treated versus untreated soils exhibited marginally higher Firmicutes (39.57% vs. 37.58%) and Proteobacteria (25.01% vs. 21.82%), while untreated soil recorded greater abundance of Actinobacteria, Planctomycetes, Acidobacteria, and Chloroflexi (Figure 2.27 and 2.28). At Takpo, Firmicutes was substantially higher in untreated soils (45.31%) relative to treated soils. The treated soils exhibited higher abundance of Proteobacteria, Actinobacteria, Planctomycetes, and Acidobacteria (Figure 2.29 and 2.30).

3.2 Genus-level microbial abundance in herbicide-treated and untreated soils used

Similar to the phylum-level trends, the total number of genera was higher in untreated soils, especially in the legume-based cropping system (Figure 1B; from Gbarikong to Nanvile) but lower in herbicide-treated soils. The untreated soils from legume fields harboured between 248 and 495 genera, compared to 182 and 338 genera in the treated soils, with the exception of Saan and Nanvile which were the opposite. Conversely, soils from cereal-based fields (Figure 1B; from Tangbonne to Takpo) recorded a higher number of genera in the herbicide-treated fields (290–325) compared to untreated which was relatively lower (212–263 genera).

Figure 1B and Figure 3 indicate microbial abundance at genera level in soils from the study sites. The genera that were dominant in both the treated and untreated soils include *Bacillus*, *Salmonella, Microvirga, Paenibacillus, Conexibacter, Burkholderia, Streptomyces, Priestia, Nocardioides, Geodermatophilus, Skermanella, Rubrobacter, Sphingomonas, Clostridium, Nitrospira, Singulisphaera,* and *Actinomarinicola.* (Figures 3.1–3.30). However, *Bacillus* was the most dominant genus in both herbicide-treated and untreated soils at all 15 field sites (Figure 3) and between treatments, Bacillus was more abundant in untreated soils at five of the eight legume-based fields (Gbarikong, Saan, Sangu, Dapuoh and Konzokalaa) and five of the seven cereal-based cropping system (i.e., Tanbgonne, Nator, Gbierung, Chaang and Takpo) (Figures 3.1-3.30).

But *Paenibacillus*' abundance was generally much higher in treated soils, and in fact highest in 14 out of the 15 fields, with the exception of Takpo where untreated soils recorded 17.85% abundance compared to 3.16% in treated soil. Similarly, *Microvirga* was generally higher in herbicide-treated soils, with 13 out of 15 sites (86.7%) recording greater abundance in treated than untreated controls. In contrast, *Conexibacter* and *Streptomyces* exhibited higher abundance in untreated soils, at 13 and 9 out of the 15 locations, respectively. However, 2 and 6 sites respectively recorded higher *Conexibacter* and *Streptomyces* abundance in treated soils. *Actinomarinicola* also showed higher abundance in untreated soils at 8 of the 15 sites, marginally increased abundance in treated soils at 3 sites, and similar abundance between treatments at the remaining sites (Figures 3.12–3.30).

The genus *Burkholderia* was more abundant in untreated soils, with greater abundance observed at 11 of the 15 sites, but Lambussie, Konzokalaa, Nator and Dakyie recorded marginally higher abundance in herbicide-treated soil (Figures 3.1–

3.30). *Priestia* abundance was higher in untreated soils at 8 out of the 15 fields, with the other 7 locations recording low abundance in treated soils. *Bradyrhizobium*, recorded higher abundance in untreated soils at 8 of the 15 locations. Moreover, at Nanvile, Dapuoh, and Chaang, *Bradyrhizobium* abundance in untreated soils was 0.79%, 1.36% and 0.71% respectively, but absent in treated soils. However, three sites recorded marginally high *Bradyrhizobium* abundance in treated soils (Figures 3.1–30). Similarly, *Planctomyces* was more abundant in untreated soils from 10 of the 15 sites, with only 2 sites the Gbarikong and Sangu sites exhibiting slightly higher abundance in treated soils and Saan, Dapuoh and Tangbonne sites showing similar abundance across treatments. *Rubrobacter* showed elevated abundance in untreated soils at 8 sites, marginal abundance in treated soils from 4 fields, and similar abundance at 3 locations.

In contrast, to the above, *Rhodoplanes* was the dominant genus in 13 out of 15 (Figures 3.1–3.30). Gemmata also increased in 6 out of 15 treated soils especially at Gbierung (Figure 3.21 and 3.22). Similar to *Nocardioides* with higher abundance at 7 of the 15 treated soils and greater in 3 untreated soils.

3.4 Multivariate analysis of microbial community structure in soils

3.4.1 Principal Component Analysis of herbicide-treated soils

The Principal Component Analysis (PCA) biplot for herbicide-treated soils (Figure 4A) showed that the first two principal components (PC F1 and PC F2) collectively explained the 37.26% of the total variance in microbial community composition (PC F1 = 23.74%; PC F2 = 13.52%). The ordination of active observations demonstrated pronounced dispersion across all four quadrants of the biplot, indicating substantial heterogeneity in microbial community structure among herbicide-treated soils.

Samples TUTGHLT37 (Lambussie) and TUTGHTT25 (Takpo) occupied the lower-left quadrant and were strongly associated with oligotrophic and acidophilic taxa, including *Candidatus Solibacter, Candidatus Koribacter, Bradyrhizobium,* and *Acidibrevibacterium*. In contrast, sample TUTGHDT30 (Baazu) was projected into the upper-right quadrant and was strongly correlated with *Conexibacter, Staphylococcus,* and *Tessaracoccus.* Sample TUTGHCT8 (Chaang), positioned at the extreme positive end of PC F1, was closely associated with *Geodermatophilus, Nocardioides, Lichenibacterium* (Sensu lato)*,* and *Microvirga*.

Samples TUTGHNT24 (Nanvile) and TUTGHNT5 (Nator), which occupied intermediate positions in the ordination space, showed affiliation with *Desulfarculus, Rubrobacter, Actinomarinicola,* and *Humisphaera*. The genera *Bacillus, Sphingomonas, Modestobacter,* and *Actinocatenispora* were positioned in the center of the graph. Biplot for untreated soils (Figure 4B) explained a combined 30.84% of total variance across the first two axes (PC F1 = 15.75%; PC F2 = 15.09%). The nearly equal contribution of both principal components suggests that two independent environmental gradients of comparable magnitude govern microbial community assembly in the absence of herbicide treatment. This contrasts with the herbicide-treated biplot, in which PC F1 was disproportionately dominant, implying that herbicide application introduces a single overriding selective pressure that restructures the primary axis of community variation.

Sample TUTGHSU21 (Saan) was an outlier, positioned at the extreme negative end of PC F2 (approximately −8), indicating a highly divergent microbial community. Its isolated position in ordination space, coupled with its association with *Pseudomonas, Nitrospira, Nitrososphaera*, and *Neobacillus*, suggests a community enriched in nitrifying and denitrifying taxa. This profile may reflect localized edaphic conditions,

such as elevated ammonium availability, periodic waterlogging, or distinct soil textural properties favouring ammonia-oxidizing archaea and nitrite-oxidizing bacteria.

Sample TUTGHBU30 (Baazu) occupied an extreme position on the negative PC F1 axis and was associated with *Rubrobacter, Geodermatophilus, Blastococcus*, *Nocardioides*, and *Lichenibacterium*. The enrichment of xerotolerant and radiation-resistant Actinobacteria at this site parallels similar associations observed in the herbicide-treated biplot, suggesting that site-specific edaphic properties, rather than herbicide treatment *per se*, may primarily drive the abundance of these taxa at this location.

A cluster of untreated control samples (TUTGHNU5, TUTGHNU4, TUTGHNU20 from Nator, Tangbonne and Nanga, respectively) was positioned in the upper-right quadrant and associated with *Candidatus Saccharimonas, Frigoribacterium, Globibacterium, Brevibacillus, Klebsiella,* and *Bradyrhizobium*. The co-occurrence of members of the candidate phylum *Saccharibacteria* with nitrogen-fixing *Bradyrhizobium* and plant-associated *Frigoribacterium* suggests a functionally coherent community associated with rhizosphere- or litter-derived carbon inputs in the absence of herbicide stress.

The positive PC F1 axis in untreated soils was further defined *by Singulisphaera, Candidatus Koribacter, Candidatus Solibacter, Acidibrevibacterium*, and *Planctomyces*, taxa could typically be an indication of undisturbed, mature soil ecosystems with stable organic matter cycling. More importantly, the positioning of these oligotrophic markers along the positive PC F1 axis in untreated soils versus the negative PC F1 axis in herbicide-treated soils suggest alteration and reorganization of the dominant ecological actors in response to herbicide treatment.

3.4.2 Agglomerative Hierarchical Clustering

Agglomerative hierarchical clustering (AHC) of herbicide-treated soil samples (Figure 5A) resolved three distinct clusters at an empirical truncation threshold of approximately 70 dissimilarity units. Cluster C1 comprised seven samples (TUTGHNT24, TUTGHSK31, TUTGHGT36, TUTGHCT8, TUTGHNT4, TUTGHNT5, TUTGHDT28) for sites: Nanvile, Konzokalaa, Gbarikong, Chaang, Tangbonne and Nator, respectively, which merged at relatively low dissimilarity values (<30). This cluster corresponds to the samples positioned predominantly along the positive PC F1 axis in the PCA biplot.

Cluster C3 consisted of three samples (TUTGHST21, TUTGHDT30, TUTGHST35) for sites Saan, Baazu, and Sangu, respectively, merging at a moderate dissimilarity of approximately 35. Cluster C2 comprised five samples (TUTGHTT25, TUTGHLT37, TUTGHDT17, TUTGHGT12, TUTGHNT20 from Takpo, Lambussie, Dakyie Gbierung and Nanga, respectively with internal dissimilarity values generally below 45.

The dendrogram for untreated soils (Figure 5B) similarly identified three clusters, although the branching architecture differed substantially from the treated soils. Cluster C2 comprised five samples (TUTGHSU21, TUTGHDU28, TUTGHLU37, TUTGHCU8, TUTGHKU31 from Saan, Dapuoh, Lambussie, Chaang and Konzokalaa, respectively, with a maximum within-cluster dissimilarity of approximately 48. Notably, sample TUTGHSU21 from Saan, the extreme outlier identified in the PCA, merged with the remainder of C2 at the highest within-cluster dissimilarity, corroborating its distinct community composition, while maintaining sufficient shared taxa to justify cluster membership. Cluster C1 was the smallest group, containing three samples (TUTGHNU20, TUTGHTU4, TUTGHNU5 from Nanga, Tongbonne and Nator,

respectively), corresponding to the Nanga, Tangbonne, and Nator locations, respectively. Cluster C3 was the largest group, comprising seven samples that displayed low internal dissimilarity and aligned with intermediate PCA positions. The comparatively lower maximum inter-cluster dissimilarity in untreated soils (approximately 142) relative to herbicide-treated soils (approximately 175) suggests that, in the absence of herbicide effect, microbial communities exhibit somewhat greater overall compositional convergence, although significant site-to-site heterogeneity may exist.

3.5 Comparative Synthesis

Collectively, the multivariate analyses reveal several key findings regarding the impact of herbicide treatment on soil microbial community structure. First, herbicide treatment was associated with increased community divergence among sampling sites. The higher proportion of variance captured by PC F1 in herbicide-treated soils (23.74%) compared with untreated soils (15.75%), combined with the greater maximum inter-cluster dissimilarity observed in the AHC analysis (175 vs. 142), suggests that herbicide application increased beta-diversity among sites. This finding is consistent with the "stress-divergence" hypothesis, which indicates that environmental disturbance drives communities toward alternative stable states contingent upon local ecological and edaphic conditions [35].

Second, herbicide treatment appeared to reorganize the dominant ecological gradients structuring microbial communities. The shift in ordination-space placement of oligotrophic markers (*Candidatus Solibacter, Candidatus Koribacter*) from positive PC F1 loadings in untreated soils to negative PC F1 loadings in treated soils indicates that herbicide application can restructure the primary axis of community variation. This

reorganization likely reflects the introduction of xenobiotic degradation capacity as a new axis of functional selection overlaying pre-existing environmental gradients.

Third, despite the overarching effects of herbicide treatment, both treated and untreated soils exhibited three-cluster solutions with substantial inter-cluster dissimilarity, indicating that intrinsic edaphic properties; including soil type, pH, organic matter content, and moisture regime, remain important determinants of community assembly irrespective of herbicide management status. The persistence of site-specific microbial signatures across treatments underscores the role of local environmental filtering in shaping soil microbial communities.

Fourth, the analyses identified candidate indicator taxa for herbicide response monitoring. The enrichment of copiotrophic *Actinobacteria*, particularly *Geodermatophilus* and *Nocardioides*, and the concomitant depletion of *Acidobacteria* in certain herbicide-treated clusters, contrasted with the maintenance of functionally diverse communities comprising nitrifiers and nitrogen-fixers in untreated soils, provides a suite of candidate biomarkers for assessing herbicide impacts on soil ecosystem functioning.

These findings underscore the importance of integrating ordination and hierarchical clustering approaches to disentangle the multifactorial drivers of microbial community variation in herbicide-impacted agricultural soils. Furthermore, they highlight the need for long-term monitoring programmes to determine whether herbicide-induced community divergence represents a transient change or a persistent ecological shift with lasting consequences for soil fertility and plant growth promotion.

4 Discussion

4.1 Herbicide-induced shifts in plant growth-promoting microbial phyla and genera

The results of this study demonstrate that herbicide application exerts measurable and ecologically significant effects on soil microbial community structure, with pronounced implications for plant growth-promoting (PGP) functionality in agricultural soils. At the phylum level, the untreated soil consistently harboured greater taxonomic richness, ranging from 29 to 39 phyla compared with 21 to 32 phyla in herbicide-treated soil across the sampling sites (Figure 1A). This reduction in phylum-level diversity under herbicide pressure is consistent with findings by Zaller *et al.* [36], which showed that herbicide residues in soil can significantly diminish microbial taxonomic richness, and that pesticide exposure can also narrow the breadth of functional guilds present in agricultural soils [37]. Similarly, at the genus level, specific PGP taxa were affected by herbicide treatment and revealed patterns that have direct implications for the management of biological nitrogen fixation and plant-microbe symbioses in tropical cropping systems. The untreated soils from legume-based cropping systems harboured between 248 and 495 genera compared to 182 to 338 genera in the herbicide-treated fields (Table 1 and Figure 1B), representing reductions in genus-level that are consistent with reports by Beaumelle *et al.* [38] and Köninger *et al.* [39], who documented herbicide-driven declines in soil microbial diversity across multiple European agricultural sites. The more modest differential observed in cereal-cultivated soils (290–325 genera in treated soil vs. 212–263 genera in untreated soil) (Figure 1B) reinforces the hypothesis that cropping system and rhizosphere ecology mediate microbial susceptibility to herbicide disturbance. The observation that legume-cultivated soils exhibited greater number of phyla and genera in untreated and treated soils compared to cereal-based systems suggests that cropping history and the

associated rhizosphere microbes can modulate the susceptibility of soil communities to herbicide effect [40].

Proteobacteria, *Actinobacteria*, and *Firmicutes* which are widely recognised as keystone contributors to PGP functions, including biological nitrogen fixation, phosphate solubilisation, siderophore production, and phytohormone biosynthesis [41-44] were differentially affected by herbicide treatment in a field-dependent manner. In a majority of the legume-cultivated sites, untreated soil exhibited higher relative abundance of Firmicutes, a phylum encompassing genera such as *Bacillus*, *Paenibacillus*, and *Clostridium* that are central to soil nutrient cycling and biocontrol [45,46]. For instance, at Saan, the Firmicutes constituted approximately 46.83% of the community in the untreated soil compared to 34.79% in the treated soil (Figure 2.5 and 2.6), representing a substantial decline that may compromise the capacity of the soil microbiome to support plant nutrition and defence. Similarly, *Bacillus*, the most abundant genus across all sampling sites and treatments, also exhibited resilience to herbicide application, but was nonetheless more abundant in untreated soils at majority of the study sites. *Bacillus* species are among the most extensively studied PGP bacteria, with well-documented ability to solubilise phosphate, produce IAA and gibberellins, form biofilm, and induce systemic resistance in plants [47]. The reduction of *Bacillus* abundance under herbicide treatment, probably decreased the magnitude of PGP services delivered to crop plants, particularly during early seedling establishment when microbial inoculant effects are most critical [3]. This argument agrees with the report by Rose *et al.* [18], which found that glyphosate-based herbicides significantly reduced Firmicutes abundance in rhizosphere soils while Druille *et al.* [48] observed the negative effects of glyphosate on beneficial soil microbial communities in grassland ecosystems. The genus-level reductions in

abundance are consistent with herbicide application reducing the PGP activity of *Bacillus* isolates in chickpea rhizosphere soils Ahemad and Khan [49]. Tsotetsi *et al.* [50] and Ezaka *et al.* [51] also reported a dose-dependent inhibition of *Bacillus* PGP traits by many commonly used herbicides.

The behaviour of *Bradyrhizobium* under herbicide treatment is perhaps the most agronomically consequential findings of this study. *Bradyrhizobium* is the main microsymbiont responsible for nodulation and nitrogen fixation in soybean and several tropical legumes, and its absence in herbicide-treated soils has implications for legume productivity and soil N economy [52]. *Bradyrhizobium* abundance was higher in untreated soils at 8 out of 15 sites and was not among the 35 most abundant genera found in the herbicide-treated soils at Nanvile, Dapuoh, and Chaang, strongly which suggests that herbicide application probably exerted a lethal effect on this nitrogen-fixing genus. Machado *et al.* [53] and Martins *et al.* [54] similarly found significant reductions in *Bradyrhizobium japonicum* populations following imazaquin and flumetsulam application in soybean fields. Furthermore, Angelini *et al.* [55], also showed that glyphosate and imazethapyr could reduce *Bradyrhizobium* cell viability and nodulation competitiveness both *in vitro* and under field conditions. The consequences of a diminishing *Bradyrhizobium* populations with herbicide use in smallholder farming systems of Sub-Saharan Africa, would impact on food security, especially where nitrogen fertiliser inputs are zero or insufficient and BNF is the primary source of soil nitrogen fertility [56,57].

The substantial reduction in *Burkholderia* population in herbicide-treated soils at 11 of the 15 sites represents another significant finding with implications for PGP functionality. Some members of the genus *Burkholderia* are N_2-fixers [58], including recently reclassified *Paraburkholderia* and *Caballeronia* and therefore recognised as

versatile PGP agents capable of phosphate solubilisation, production of antimicrobial compounds, and facilitation of mycorrhizal colonisation [59,60]. Some *Burkholderia* species specifically engage in symbiotic nitrogen fixation with tropical legumes belonging to the Mimosoid clade [61]. The observed sensitivity of *Burkholderia* to herbicide treatment in this study is consistent with the report by Machado *et al.* [53], which showed that herbicide application reduced populations of non-rhizobial nitrogen-fixing bacteria in legume rhizospheres, and could partly explain the reduced nodulation frequently detected in herbicide-treated legumes [62].

Conexibacter and *Streptomyces*, are members of the phylum Actinobacteria, that were respectively abundant in 13 and 9 untreated soils. Functionally, *Streptomyces* is widely recognised for its production of antibiotics, siderophores, and hydrolytic enzymes that contribute to plant disease suppression and nutrient acquisition [63,64]. Therefore, its decline under herbicide application in this study could affect its biocontrol functionality, potentially increasing plant susceptibility to soil-borne pathogens [65]. *Conexibacter* has been associated with stable organic matter turnover and is considered an indicator of soil ecosystem maturity [66]. However, its consistent reduction in herbicide-treated soils alter soil organic matter turnover and nutrient cycling processes.

The genus *Clostridium* is important in anaerobic nitrogen fixation and organic matter fermentation in waterlogged environments [67]. Its sensitivity to herbicide exposure in this study has been reported by Shehata *et al.* [68], who showed that glyphosate has a bactericidal effect on *Clostridium*. The reduced *Clostridium* population in herbicide-treated soils in this study is concerning because free-living nitrogen fixation by *Clostridium* and related diazotrophs represents an important source of nitrogen input in tropical agriculture [69].

The genus *Planctomyces*, belongs to the phylum Planctomycetes and was substantially more abundant in untreated soils. Because its members degrade sulfated polysaccharides and heteropolymeric organic compounds [70], their decrease in herbicide treated could impair the mineralisation of complex organic residues, thereby reducing the availability of plant-accessible nutrients. Similarly, the reduced abundance of *Rubrobacter*, an extremotolerant Actinobacterium with roles in organic matter stabilisation and soil aggregate formation [71], at many of herbicide-treated fields suggests that herbicide application may adversely affect soil structural integrity and carbon sequestration.

The co-occurrence of Acidobacteria and Chloroflexi populations in many herbicide-treated soils in this study further corroborates the hypothesis that herbicide application can selectively favour oligotrophic, stress-tolerant taxa at the expense of copiotrophic PGP-associated phyla [72]. Acidobacteria are widely regarded as bio-indicators of nutrient-poor, acidic, disturbed soil conditions [73], and their increased abundance in the treated soil could reflect a competitive release following the suppression of faster-growing PGP taxa. Similarly, the greater abundance of Chloroflexi in the treated soil at several study sites is consistent with the hardiness of members of this phylum to persist under environmentally-stressful conditions, including xenobiotic contamination [74]. These phylum-level shifts could have implications for soil ecosystem functioning, as the displacement of metabolically versatile PGP phyla by stress-tolerant but functionally limited taxa can reduce the soil's capacity to support plant growth through nutrient mobilisation, pathogen suppression, and hormonal signalling [75].

The abundance of *Rhodoplanes* in herbicide-treated soils in 13 out of 15 sites is consistent with the metabolic versatility of this genus, which includes the capacity for

phototrophic and chemoheterotrophic growth in contaminated and anthropogenically impacted soils [76,77]. That the genus *Sphingomonas*, occurred abundantly in 10 out 15 herbicide-treated field soils in this study as it is known to degrade a wide range of xenobiotics, including herbicides, polycyclic aromatic hydrocarbons, and chlorinated compounds [78]. The high abundance of *Sphingomonas* in herbicide-treated field soil probably reflects its ability to catabolise herbicide and use their degradation products and carbon as energy sources.

Similarly, both *Geodermatophilus* and *Nocardioides*, recorded an increase in abundance in the herbicide-treated soils at 9 and 7 out of the 15 study sites respectively, and are associated with desiccation tolerance, UV resistance, and xenobiotic metabolism [79,80]. Their proliferation under herbicide stress is likely to reflects a competitive advantage in chemically disturbed environments. The increase of *Gemmata* in herbicide-treated soils especially at Gbierung, where it reached 10–20% abundance, reflects patterns found in contaminated soils [81] and may be attributed its ability to uptake and process of macromolecular substrates [82]

The substantial abundance of *Salmonella* DNA in the herbicide-treated soils from 11 out of the 15 study sites is consistent with findings by Kurenbach *et al.* [83], who showed that exposure to herbicides including glyphosate and 2,4-D altered the sensitivity of *Salmonella enterica*. The herbicide-mediated proliferation of *Salmonella* in agricultural soils could pose risks to food safety, particularly for crops such as cowpea that are frequently consumed with minimal processing.

The site-specific exceptions observed in this study, such as the marginal increase in phylum-level sequence abundance in treated soil at Saan and Nanvile, and the elevated Firmicutes population in the treated soil at Nanvile (42.63% vs. 33.79% in the untreated soil) and Baazu (34.55% treated vs. 31.25% in untreated) (Figures 2.15,

2.16, 2.9 and 2.10) underscore the complexity of herbicide-microbiome interactions. These deviations probably reflect the mediating influence of local edaphic factors, including soil pH, organic matter, soil texture, and moisture regime, which have been shown to strongly control microbial community responses to chemical stress [84-86]. The variable response of Actinobacteria across sites is rather interesting. While this phylum declined in the untreated soils at several legume-cultivated sites, it exhibited elevated abundance in the herbicide-treated soil at sites Saan and Gbierung (Figures 2.5, 2.6, 2.21 and 2.22). Actinobacteria consists of both PGP-associated genera (e.g., *Streptomyces*, *Frankia*) and genera capable of xenobiotic degradation (e.g., *Nocardioides*, *Rhodococcus*), and the selective enrichment of the latter functional group under herbicide pressure could explain the apparent increase in phylum-level abundance despite a net loss of PGP functionality [87’88].

Planctomycetes, a phylum increasingly recognised for its contributions to carbon and nitrogen cycling in soils [89,82], was generally more abundant in the untreated soil across legume-cultivated sites, with the exception of sites Lambussie and Saan (Figures 2.3, 2.4 and 2.5, 2.6). The sensitivity of *Planctomycetes* to herbicide application is concerning from a soil health perspective, as members of this phylum participate in anaerobic ammonium oxidation (anammox) and contribute to the degradation of complex organic polymers [90-92]. The decline of *Planctomycetes* in treated soil, coupled with the reduction of *Firmicutes* and the variable response of *Proteobacteria*, suggests that herbicide application could disrupt multiple complementary pathways of nutrient cycling simultaneously, potentially creating functional bottlenecks in soil biogeochemical processes [93].

Among cereal-cultivated fields, the differences between treated and untreated soils in phylum-level richness at the same site was smaller than that observed in legume fields

(Table 1 and Figure 1A). This pattern could be attributed to the absence of a legume-specific rhizosphere effect, which is known to selectively enrich nitrogen-fixing and PGP-associated taxa [94], implying that the baseline community in cereal soils may be less sensitive to herbicide-induced disruption of symbiotic and associative nitrogen-fixing associations. Nevertheless, the consistent enrichment of Proteobacteria and Bacteroidete*s* in treated cereal soils at several sites, including Tangbonne and Nator, suggests that herbicide degradation intermediates probably serve as carbon sources for specific copiotrophic lineages within these phyla, a phenomenon documented for soils receiving repeated herbicide applications [95].

4.2 Multivariate patterns and ecological implications

The multivariate analyses provided an integrated perspective on the ecological consequences of herbicide application on soil microbial community structure. The PCA of herbicide-treated soil showed that the primary axis of community variation was aligned with a gradient from oligotrophic, acidophilic communities (rich in *Candidatus solibacter*, *Candidatus koribacter*, and *Bradyrhizobium*) to copiotrophic, Actinobacteria-dominated assemblages (rich in *Geodermatophilus*, *Nocardioides*, and *Microvirga*). This gradient is consistent with the oligotroph–copiotroph ecological framework proposed by Fierer *et al.* [72], where nutrient availability and disturbance regime are primary determinants of microbial community composition. The disproportionate contribution of PC F1 (23.74%) relative to PC F2 (13.52%) in the herbicide-treated soil, compared to the more balanced variance distribution in the untreated soil (PC F1 = 15.75%; PC F2 = 15.09%), supports the argument that herbicide application introduces a dominant selective pressure that overrides pre-

existing environmental gradients, effectively collapsing multidimensional community variation onto a single axis of xenobiotic tolerance [96].

The agglomerative hierarchical clustering analysis reinforced these findings by demonstrating greater inter-cluster dissimilarity among herbicide-treated soils (approximately 175 dissimilarity units) than among untreated soils (approximately 142 units). This amplification of beta-diversity under herbicide treatment is consistent with the stress-divergence hypothesis articulated by Chase [35], which predicts that stochastic processes become more influential in community assembly under conditions of environmental stress, driving communities toward alternative compositional states. In this study, the divergence among microbial communities in herbicide-treated soil probably reflects interactions between herbicide chemistry, local soil properties, and pre-existing microbial community composition, all of which influence the trajectory and extent of community restructuring, following herbicide disturbance [97]. The consequence of this divergence is that the effects of herbicide application on soil microbial communities, and on PGP services, cannot be reliably predicted from knowledge of one site alone but require site-specific assessment.

The identification of cosmopolitan genera such as *Bacillus*, *Sphingomonas*, and *Modestobacter* at central positions in the PCA ordination space for treated soils suggests that these taxa constitute core community members that are functionally resilient to herbicide effect. The ecological resilience of *Bacillus* is well-established and attributable to its endospore-forming capacity, metabolic versatility, and production of biosurfactants that may facilitate herbicide degradation [45]. However, functional resilience at the genus level does not necessarily equate to functional equivalence, as herbicide exposure may alter the strain-level composition within genera, potentially

shifting the balance from PGP-proficient strains to less beneficial or non-functional variants [98,99].

The distinct microbial signatures associated with specific sampling sites across both treated and untreated soils, such as the nitrifier-enriched community at Saan and the xerotolerant Actinobacteria-dominated community at Baazu, highlight the enduring influence of local edaphic properties on soil microbial community assembly. This is consistent with the environmental filtering paradigm of community ecology [100] and with empirical evidence that soil type, pH, and organic matter are probably the strongest predictors of microbial community composition at landscape scales [101,102]. The practical implication is that herbicide management strategies aiming to preserve PGP microbial functioning must be tailored to local soil conditions rather than applied uniformly across diverse edaphic environments.

4.3 Implications for sustainable agriculture and soil health

The collective findings of this study raise major concerns about the long-term sustainability of herbicide-dependent weed management in legume and cereal cropping systems in Northern Ghana and similar tropical agroecosystems. The suppression of key nitrogen-fixing genera, particularly *Bradyrhizobium*, *Burkholderia*, and *Clostridium*, when combined with the decrease in biocontrol agents such as *Streptomyces* and organic matter decomposers such as *Planctomyces*, suggests that frequent herbicide application could progressively erode the biological capital upon which smallholder crop production systems resides [103,104]. In systems where N fertiliser use is low due to high cost and in-availability, BNF by rhizobia and free-living diazotrophs is the option for ecosystem functioning, and its disruption by herbicides could have negative effects on soil nitrogen supply, crop yield, and food security [105].

Furthermore, the sequence abundance of xenobiotic-degrading and stress-tolerant genera under herbicide stress at the expense of PGP taxa could represent a form of microbial community decline analogous to the "functional erosion" described by Griffiths *et al.* [106], where taxonomic shifts that appear modest in magnitude carry disproportionately large consequences for ecosystem functioning. The observed community shifts are particularly concerning in the context of cowpea and soybean production, where the efficacy of rhizobial inoculants depends on the compatibility of introduced strains with the resident soil microbiome [107]. If herbicide application alters the competitive landscape of the rhizosphere in ways that disadvantages an introduced *Bradyrhizobium* strain, the economic returns on inoculant investment could be substantially diminished.

Conclusion

In conclusion, herbicide application significantly altered soil microbial community composition at both phylum and genus level, with profound implications for the functioning of plant growth-promoting (PGP) microbes in legume- and cereal-based cropping systems of Northern Ghana. At the phylum level, herbicide treatment was consistently associated with reduced taxonomic richness and a decline in the relative abundance of key PGP phyla, particularly *Firmicutes*, *Actinobacteria* (specifically PGP-associated lineages), and *Planctomycetes*, while concurrently favouring stress-tolerant and oligotrophic phyla such as *Acidobacteria* and *Chloroflexi*. At the genus level, herbicide application resulted in a decrease or disappearance of agronomically important taxa, especially *Bradyrhizobium*, (the principal microsymbiont for soybean and cowpea nodulation), as well as *Burkholderia*, *Streptomyces*, *Clostridium*, *Conexibacter*, and *Planctomyces*. However, it also selectively enriched xenobiotic-degrading and stress-tolerant genera including *Rhodoplanes*, *Sphingomonas*,

Geodermatophilus, and *Nocardioides*. The near-absence of *Bradyrhizobium* from the most abundant genera in herbicide-treated soils at several sites is of agronomic concern, as it poses a direct threat to biological nitrogen fixation, the cornerstone of sustainable legume production in nitrogen-limited tropical soils.

Table 1: GPS coordinates for sampled sites, with herbicide treatment status and the herbicide product names plus active ingredients.

Location	GPS Coordinates of Herbicide-treated Sites		Trade name of Herbicides	Active ingredient	GPS Coordinates of untreated Sites	
Legume-based soils						
Gbarikong	10.372 N	2.654 W	Vezir 240 SL &Agil	Imazethapyr & Propaquizafop	10.370 N	2.650 W
Lambussie	10.847 N	2.694 W	Nwura wura & Target	Glyphosate & Imazethapyr	10.847 N	2.944 W
Saan	10.346 N	2.827 W	Sunphosate & Seadasate	Glyphosate & Glyphosate	10.362 N	2.827 W
Sangu	10.180 N	2.562 W	Vezir 240 SL & Agil	Imazethapyr & Propaquizafop	10.180 N	2.567 W
Baazu	10.418 N	2.889 W	Winner	Glyphosate	10.433 N	2.535 W
Dapuoh	10.429 N	2.625 W	Sunphosate & Nicoking	Glyphosate & Nicosulferon	10.430 N	2.620 W
Konzokalaa	10.424 N	2.577 W	Sunphosate & Seadasate	Glyphosate & Glyphosate	10.424 N	2.578 W
Nanvile	10.231 N	2.713 W	Sunphosate & Nicoking	Glyphosate & Nicosulferon	10.230 N	2.713 W
Cereal-based soils						
Tangbonne	10.382 N	2.673 W	Nnorboa & Nicoking	Glyphosate & Nicosulferon	10.385 N	2.669 W
Nator	10.266 N	2.651 W	Nicoking	Nicosulferon	10.273 N	2.654 W
Chaang	10.241 N	2.493 W	Winner & Herbaking	Glyphosate & 2, 4-D amine	10.241 N	2.493 W
Gbierung	10.367 N	2.689 W	Sunphosate & Nicoking	Glyphosate & Nicosulferon	10.367 N	2.691 W
Nanga	10.378 N	2.728 W	Sunphosate & Nicoking	Glyphosate & Nicosulferon	10.369 N	2.767 W
Takpo	10.199 N	2.657 W	Sunphosate & Alligator	Glyphosate & Pendimethalin	10.221 N	2.651 W
Dakyie	10.441 N	2.570 W	Sunphosate & Seadasate	Glyphosate & Glyphosate	10.440 N	2.570 W

Table 2: List of active compounds and their chemical formulae in herbicide identified which were used in the study sites in 2023 cropping season.

S/N	Active Ingredient	Chemical Formulae
1	Glyphosate/Isopropylamine salt	$C_3H_8NO_5P$/ $C_6H_{17}N_2O_5P$ /$C_6H_{17}N_2O_5P$
2	Nicosulfuron	$C_{15}H_{18}N_6O_6S$
3	Pendimethalin	$C_{13}H_{19}N_3O_4$
4	2,4-D amine	$C_8H_6Cl_2O_3$
5	Propaquizafop	$C_{22}H_{22}ClN_3O_5$
6	Imazathapyr	$C_{15}H_{19}N_3O_3$

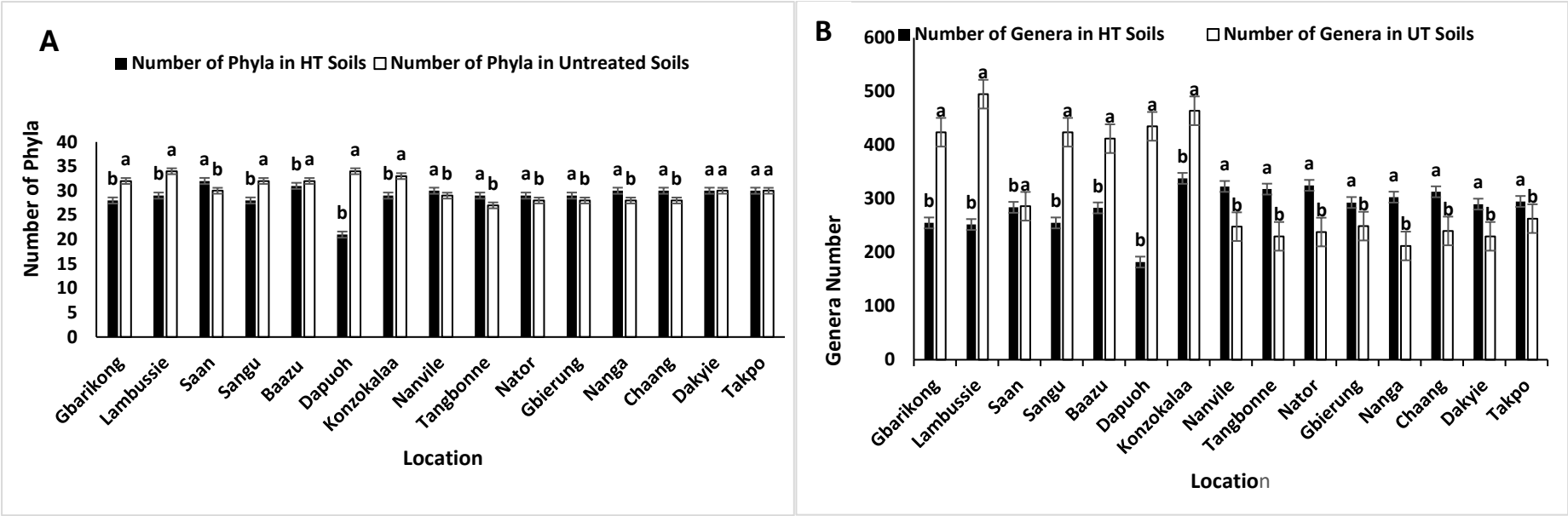
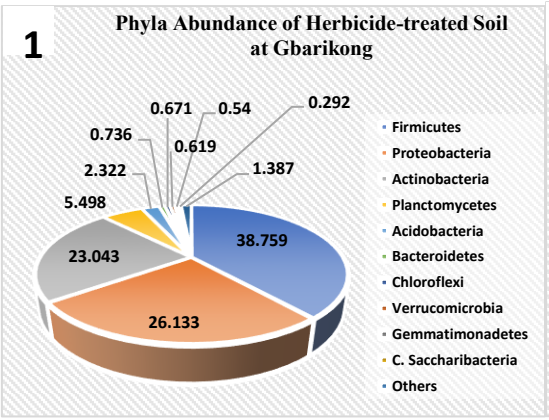
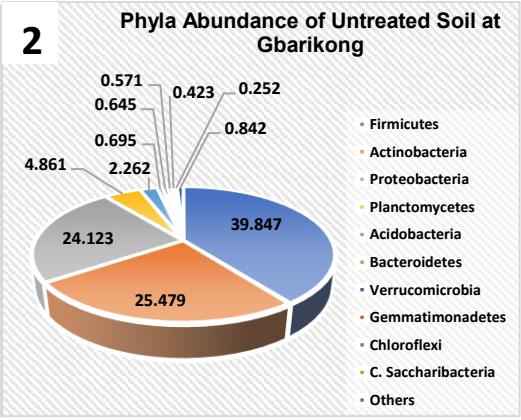


Figure 1: Comparisons of number of A) phyla, B) genera in herbicide-treated and untreated soils.

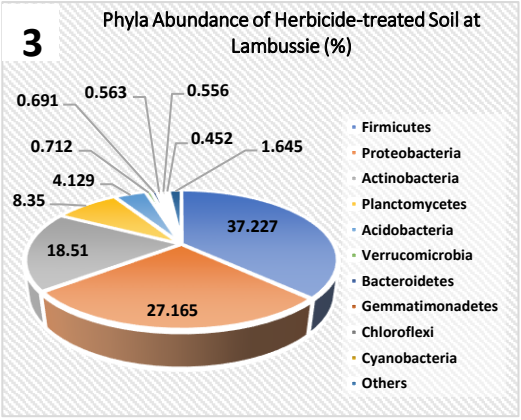
1


Phyla Abundance of Herbicide-treated Soil at Gbarikong
38.759
26.133
23.043
5.498
2.322
0.736
0.671
0.619
0.54
0.292
1.387
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Bacteroidetes
Chloroflexi
Verrucomicrobia
Gemmatimonadetes
C. Saccharibacteria
Others

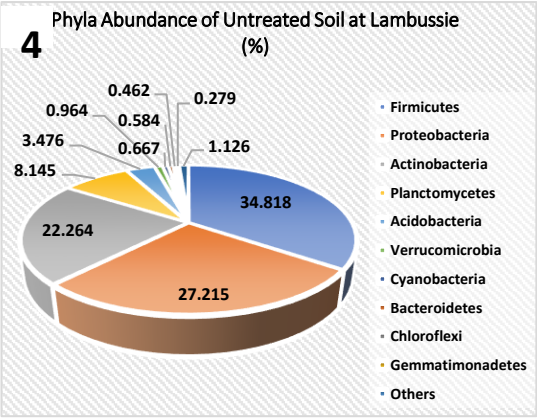
2


Phyla Abundance of Untreated Soil at Gbarikong
39.847
25.479
24.123
4.861
0.695
0.645
0.571
0.423
0.252
2.262
0.842
Firmicutes
Actinobacteria
Proteobacteria
Planctomycetes
Acidobacteria
Bacteroidetes
Verrucomicrobia
Gemmatimonadetes
Chloroflexi
C. Saccharibacteria
Others

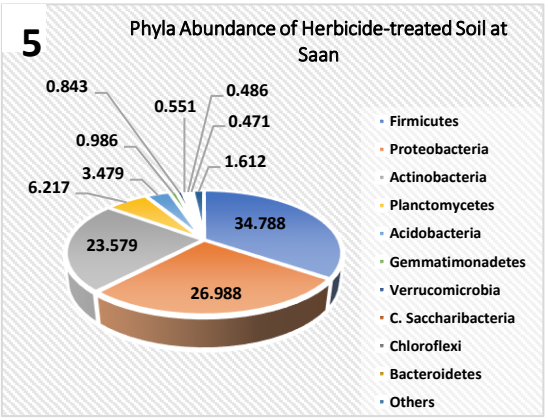
3


Phyla Abundance of Herbicide-treated Soil at Lambussie (%)
37.227
27.165
18.51
8.35
4.129
0.712
0.691
0.563
0.556
0.452
1.645
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Verrucomicrobia
Bacteroidetes
Gemmatimonadetes
Chloroflexi
Cyanobacteria
Others

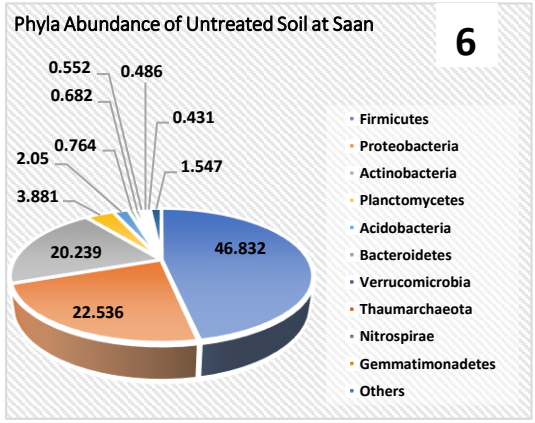
4


Phyla Abundance of Untreated Soil at Lambussie (%)
34.818
27.215
22.264
8.145
3.476
0.964
0.462
0.584
0.279
0.667
1.126
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Verrucomicrobia
Cyanobacteria
Bacteroidetes
Chloroflexi
Gemmatimonadetes
Others

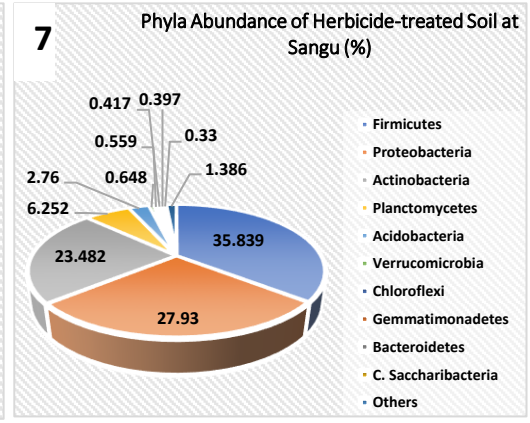
5


Phyla Abundance of Herbicide-treated Soil at Saan
34.788
26.988
23.579
6.217
3.479
0.986
0.843
0.551
0.486
0.471
1.612
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Gemmatimonadetes
Verrucomicrobia
C. Saccharibacteria
Chloroflexi
Bacteroidetes
Others

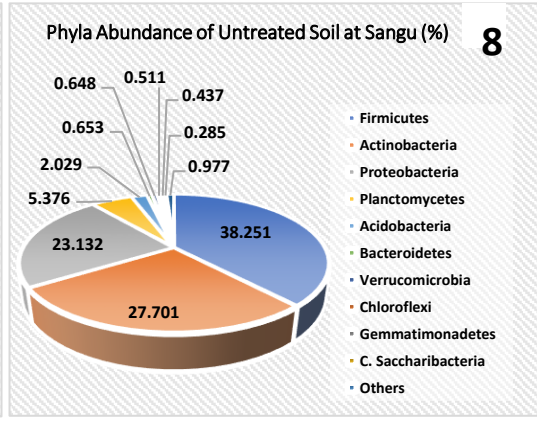
6


Phyla Abundance of Untreated Soil at Saan
46.832
22.536
20.239
3.881
2.05
0.764
0.682
0.552
0.486
0.431
1.547
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Bacteroidetes
Verrucomicrobia
Thaumarchaeota
Nitrospirae
Gemmatimonadetes
Others

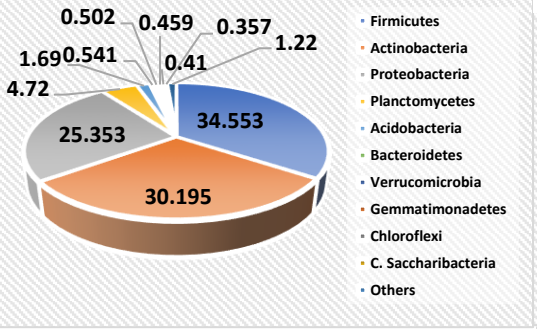
7


Phyla Abundance of Herbicide-treated Soil at Sangu (%)
35.839
27.93
23.482
6.252
2.76
0.559
0.417
0.397
0.33
0.648
1.386
Firmicutes
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Verrucomicrobia
Chloroflexi
Gemmatimonadetes
Bacteroidetes
C. Saccharibacteria
Others

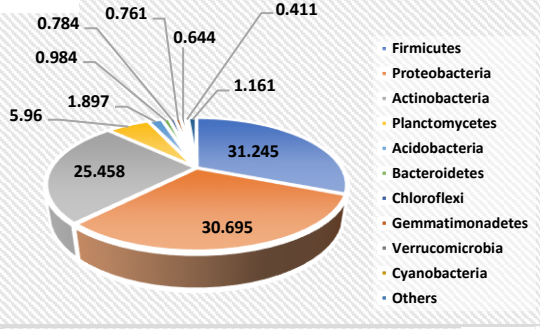
8


Phyla Abundance of Untreated Soil at Sangu (%)
38.251
27.701
23.132
5.376
2.029
0.653
0.648
0.511
0.437
0.285
0.977
Firmicutes
Actinobacteria
Proteobacteria
Planctomycetes
Acidobacteria
Bacteroidetes
Verrucomicrobia
Chloroflexi
Gemmatimonadetes
C. Saccharibacteria
Others

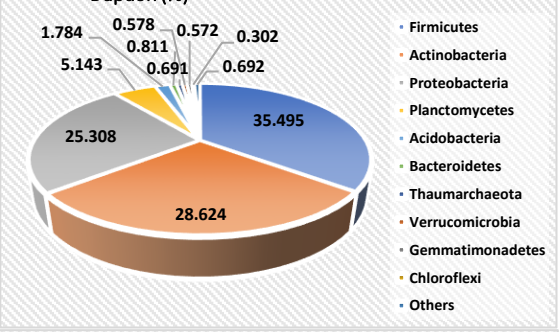
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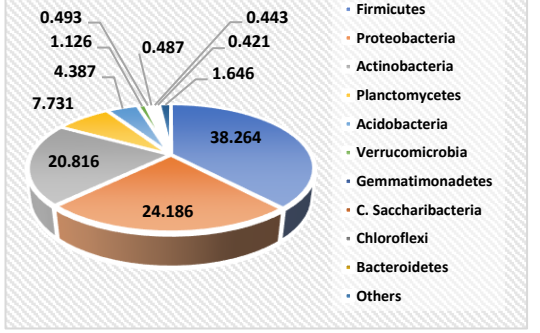
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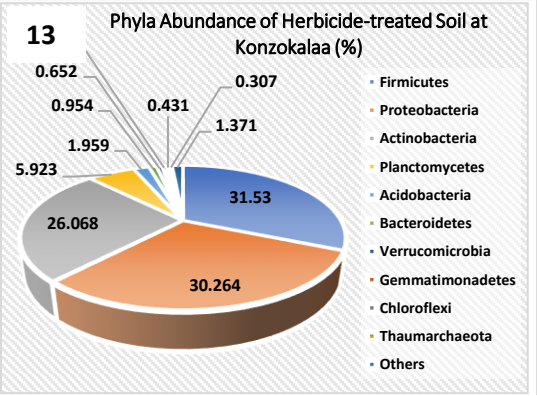
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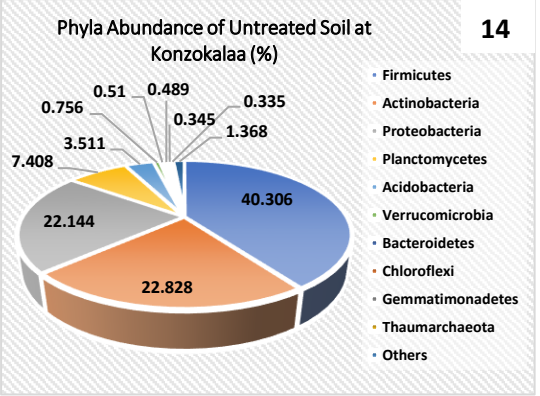
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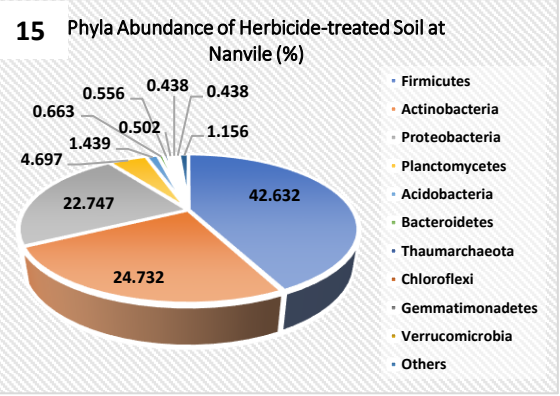
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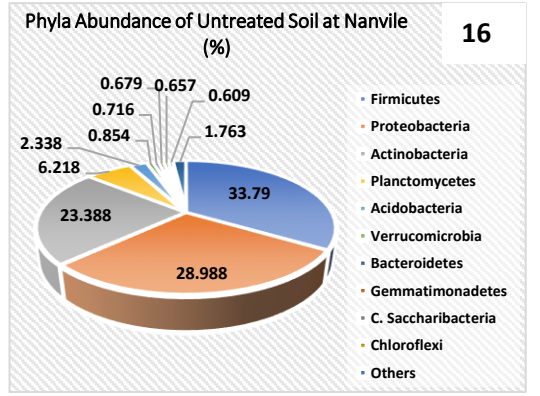
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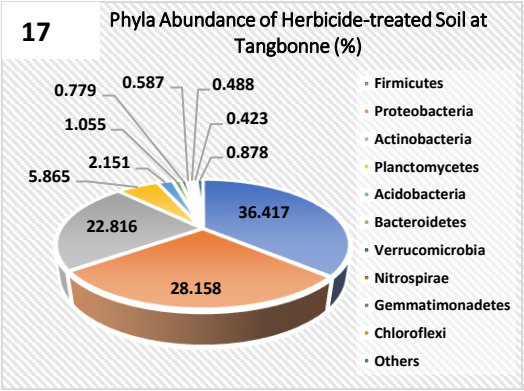
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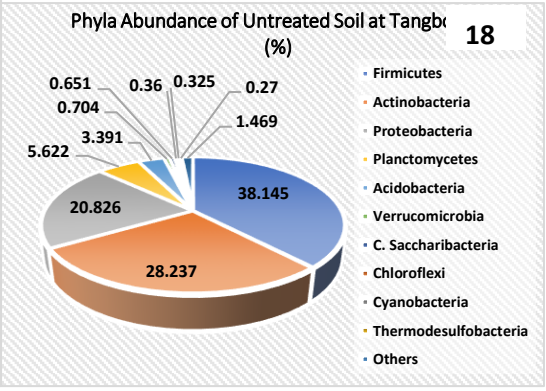
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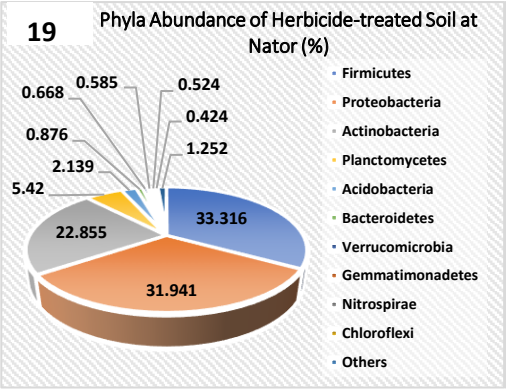
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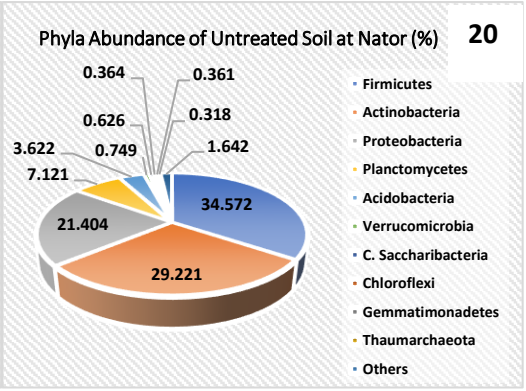
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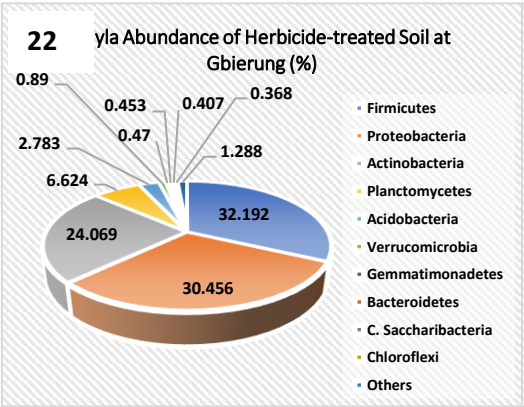
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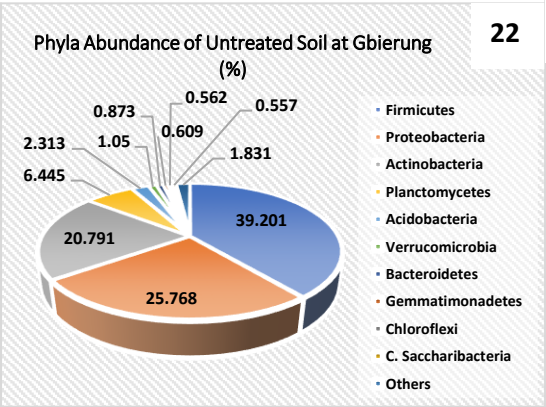
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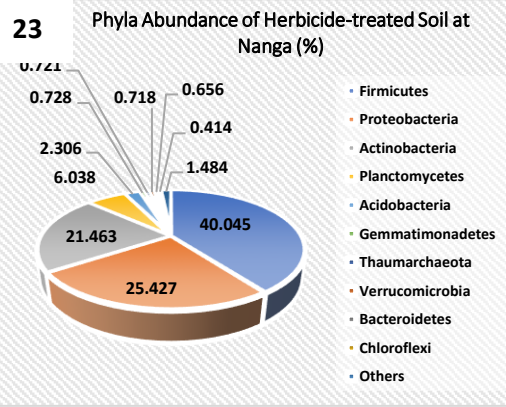
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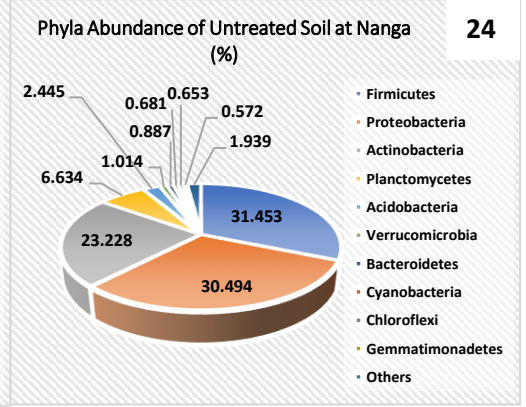
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23



24



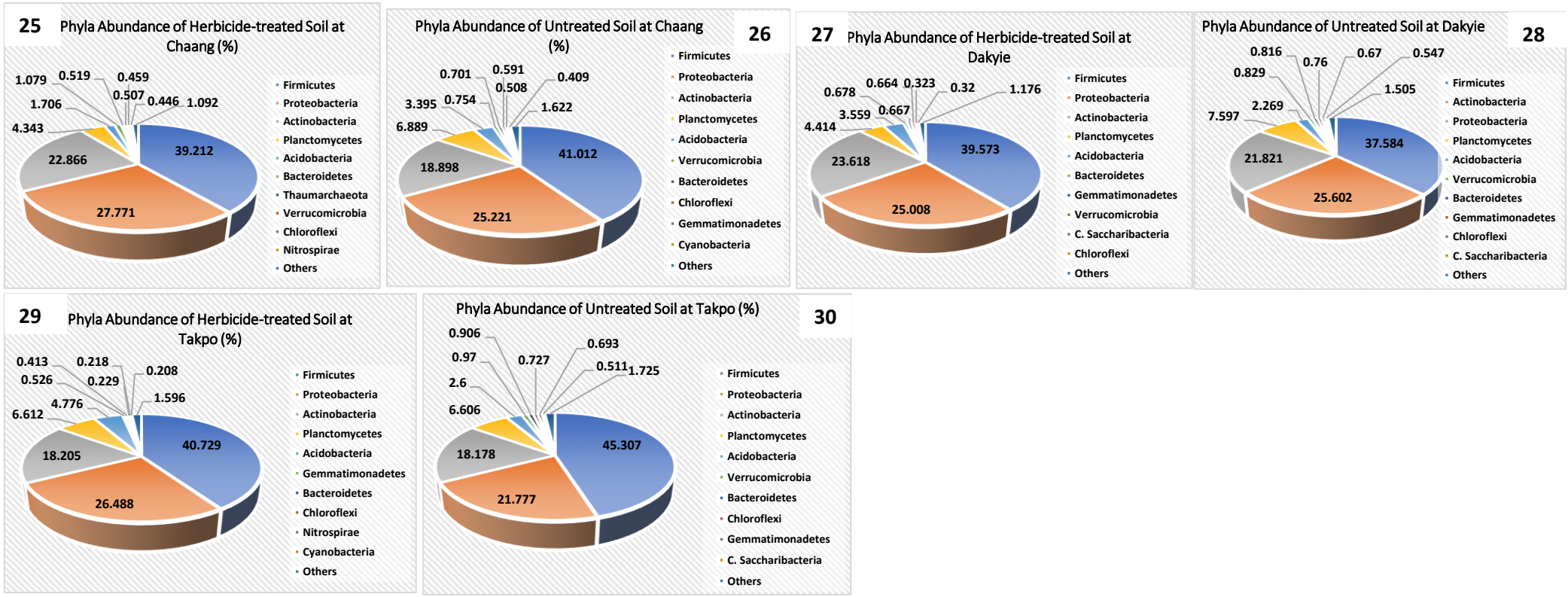
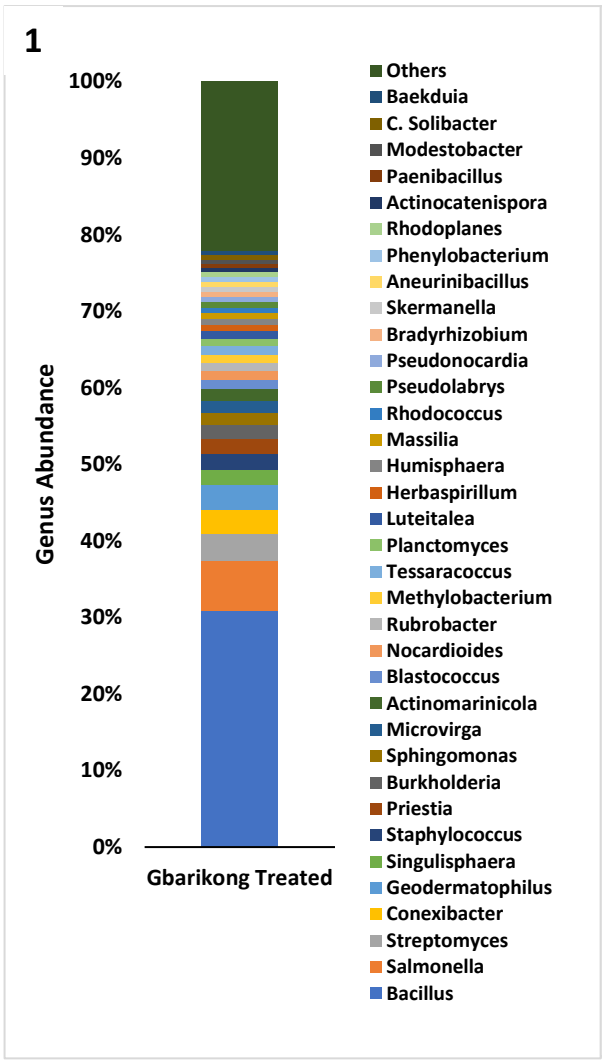
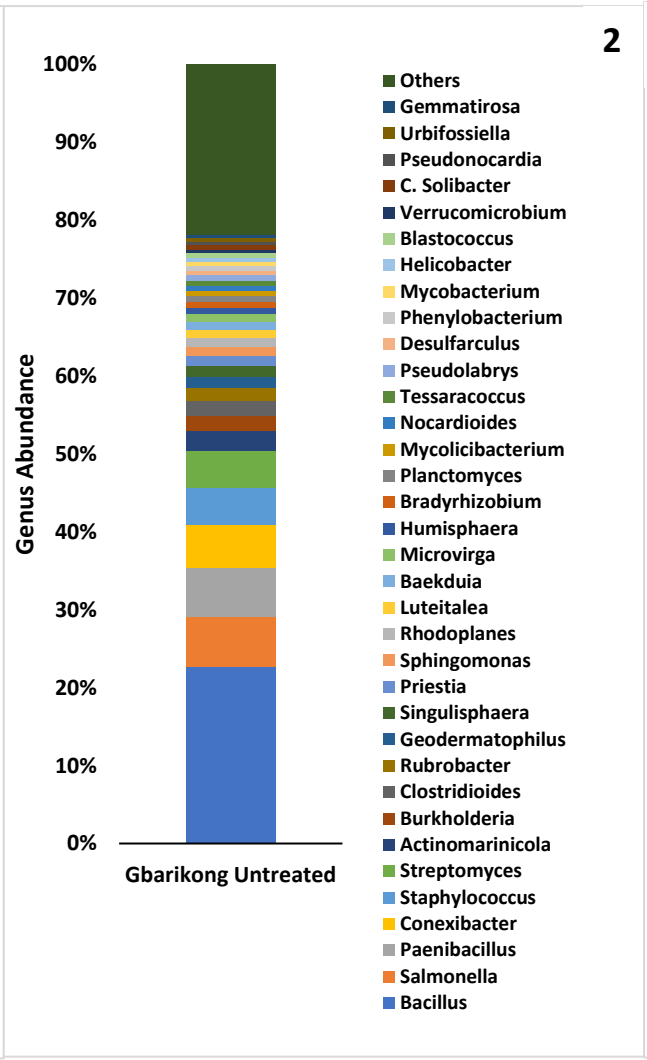
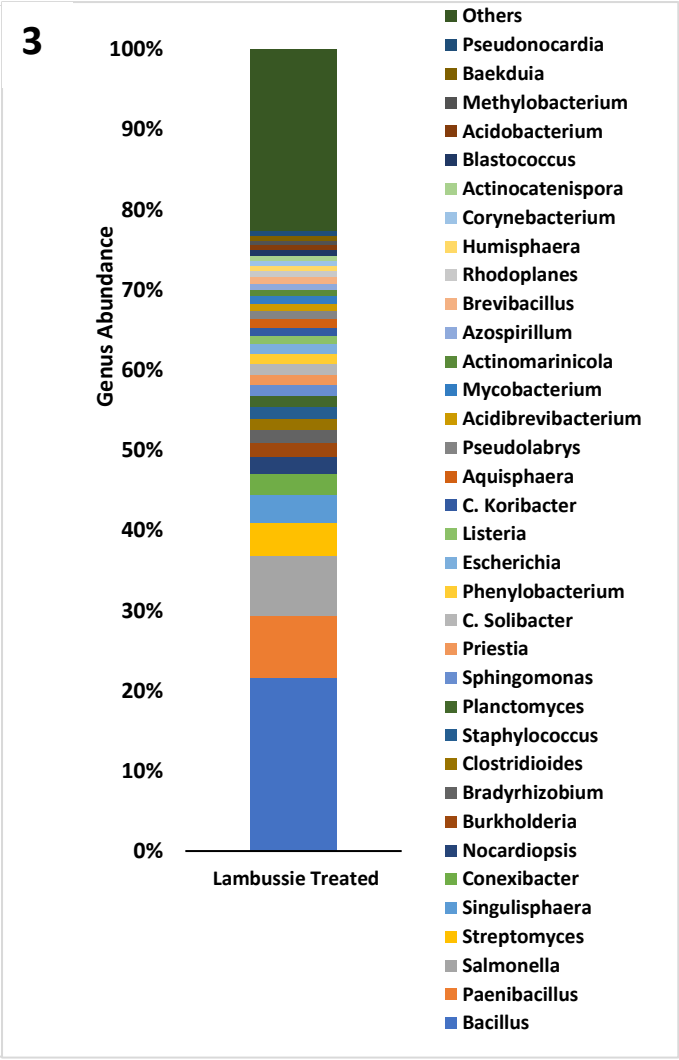
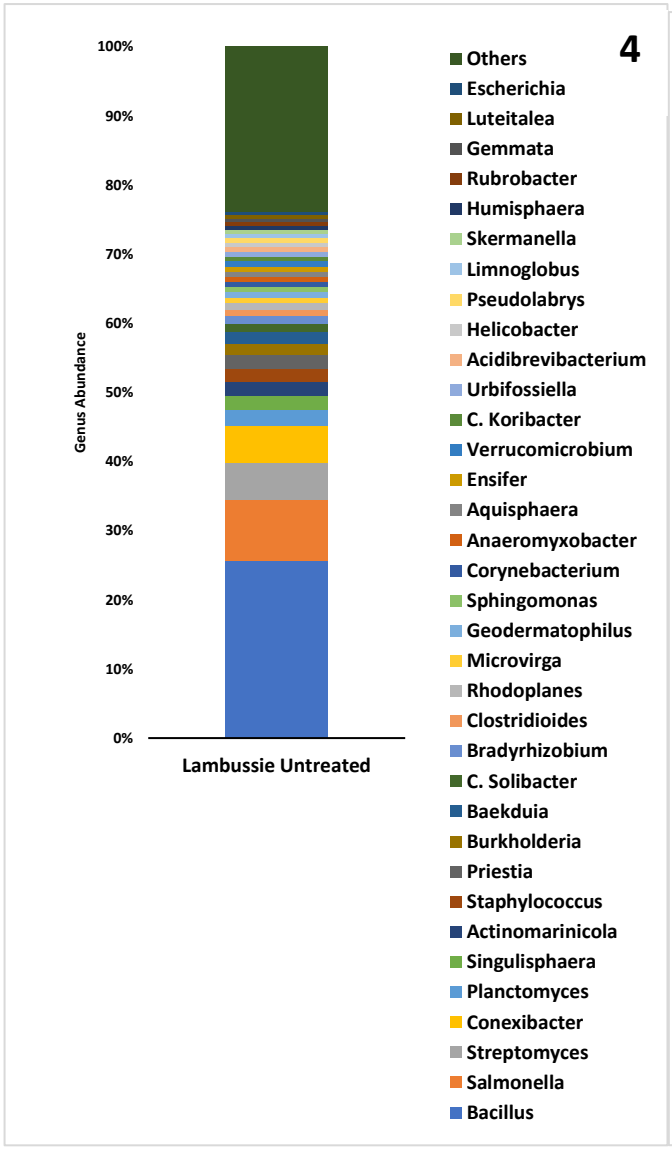


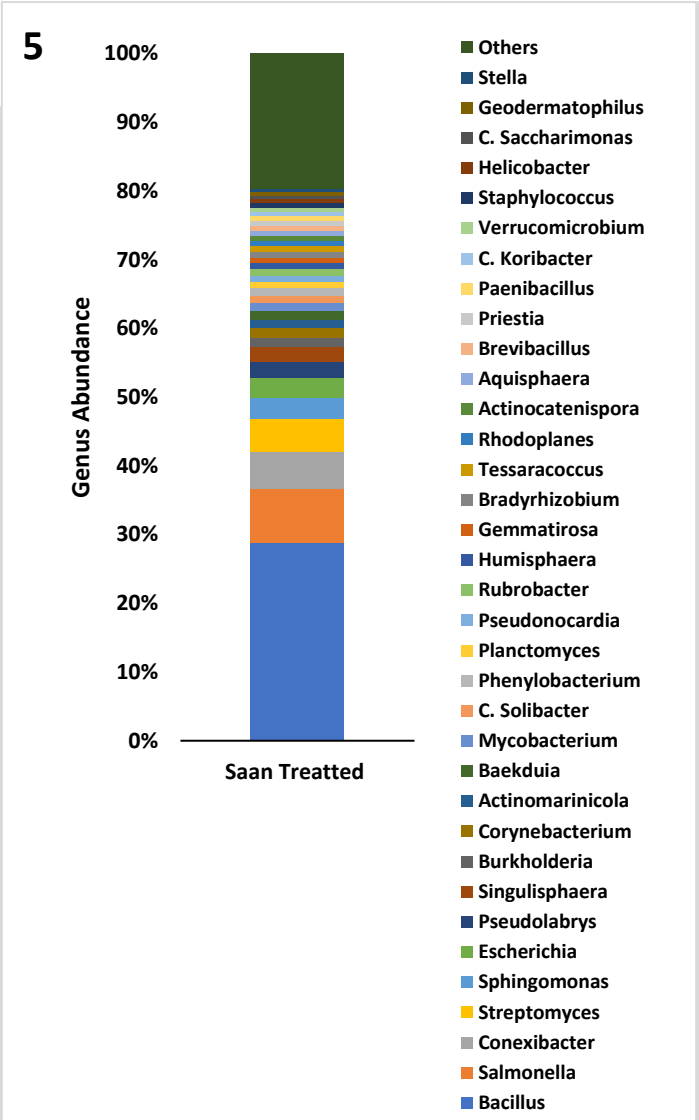
Figure 2 Comparison of Phylum level genomic abundance in herbicide-treated (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29), and untreated (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30) soils across 15 locations.

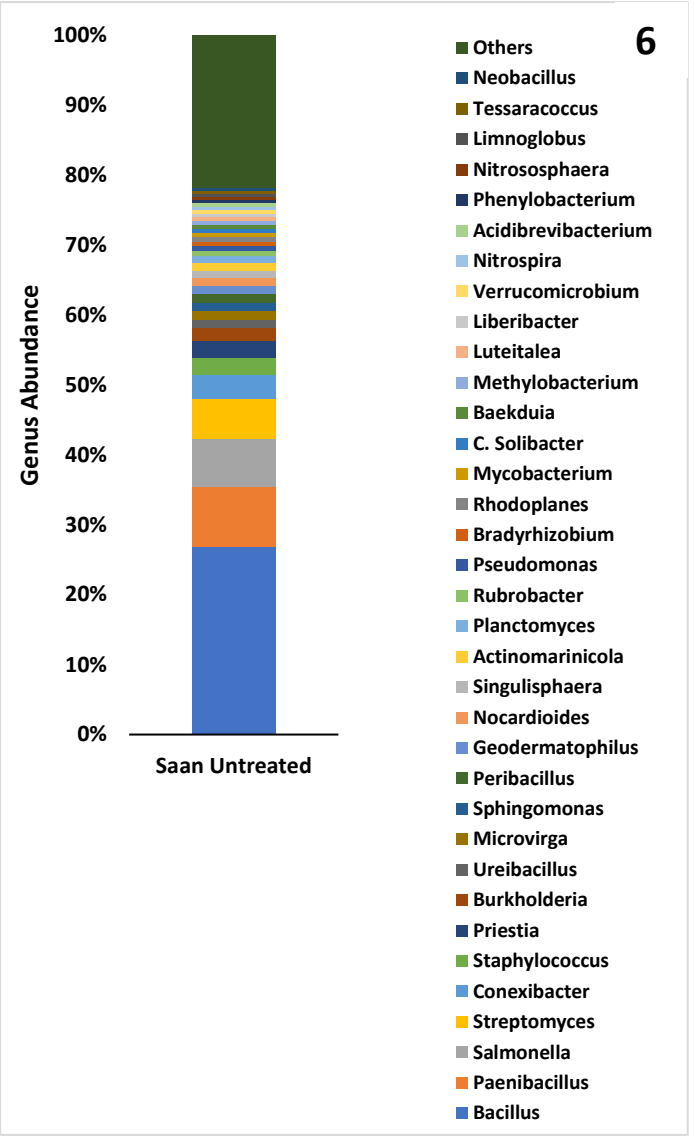

1
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Gbarikong Treated
Others
Baekduia
C. Solibacter
Modestobacter
Paenibacillus
Actinocatenispora
Rhodoplanes
Phenylobacterium
Aneurinibacillus
Skermanella
Bradyrhizobium
Pseudonocardia
Pseudolabrys
Rhodococcus
Massilia
Humisphaera
Herbaspirillum
Luteitalea
Planctomyces
Tessaracoccus
Methylobacterium
Rubrobacter
Nocardioides
Blastococcus
Actinomarinicola
Microvirga
Sphingomonas
Burkholderia
Priestia
Staphylococcus
Singulisphaera
Geodermatophilus
Conexibacter
Streptomyces
Salmonella
Bacillus

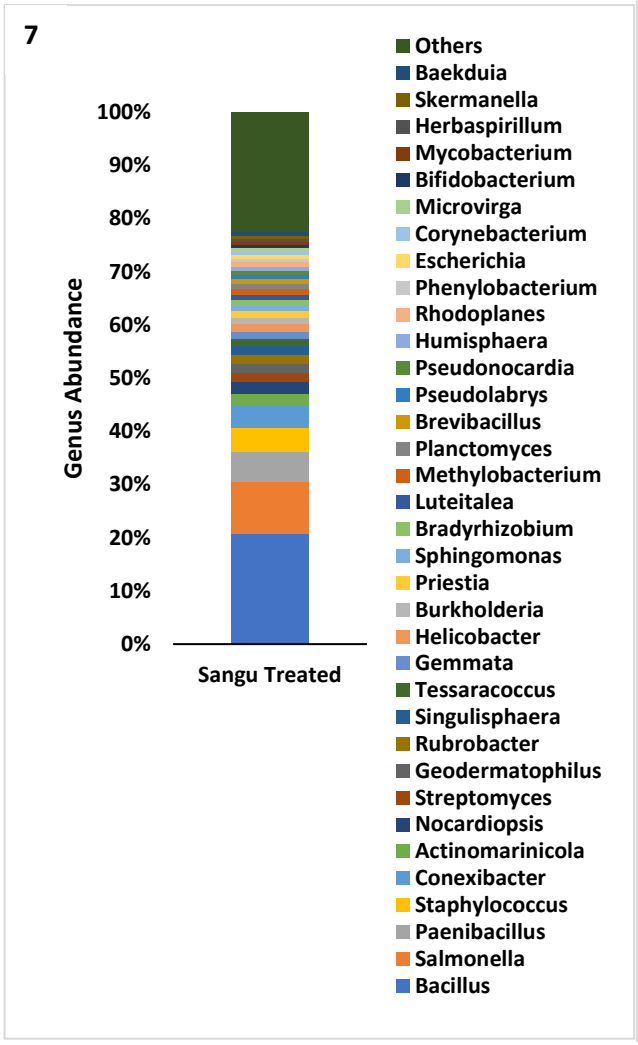

2
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Gbarikong Untreated
Others
Gemmatirosa
Urbifossiella
Pseudonocardia
C. Solibacter
Verrucomicrobium
Blastococcus
Helicobacter
Mycobacterium
Phenylobacterium
Desulfarculus
Pseudolabrys
Tessaracoccus
Nocardioides
Mycolicibacterium
Planctomyces
Bradyrhizobium
Humisphaera
Microvirga
Baekduia
Luteitalea
Rhodoplanes
Sphingomonas
Priestia
Singulisphaera
Geodermatophilus
Rubrobacter
Clostridioides
Burkholderia
Actinomarinicola
Streptomyces
Staphylococcus
Conexibacter
Paenibacillus
Salmonella
Bacillus

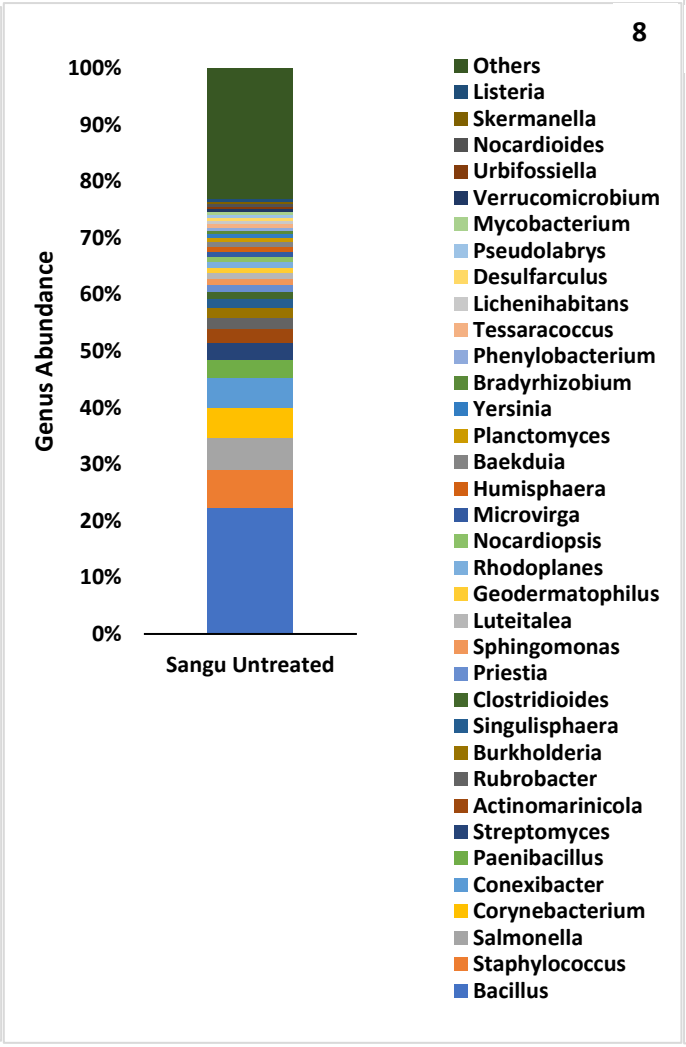

3
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Lambussie Treated
Others
Pseudonocardia
Baekduia
Methylobacterium
Acidobacterium
Blastococcus
Actinocatenispora
Corynebacterium
Humisphaera
Rhodoplanes
Brevibacillus
Azospirillum
Actinomarinicola
Mycobacterium
Acidibrevibacterium
Pseudolabrys
Aquisphaera
C. Koribacter
Listeria
Escherichia
Phenylobacterium
C. Solibacter
Priestia
Sphingomonas
Planctomyces
Staphylococcus
Clostridioides
Bradyrhizobium
Burkholderia
Nocardiopsis
Conexibacter
Singulisphaera
Streptomyces
Salmonella
Paenibacillus
Bacillus

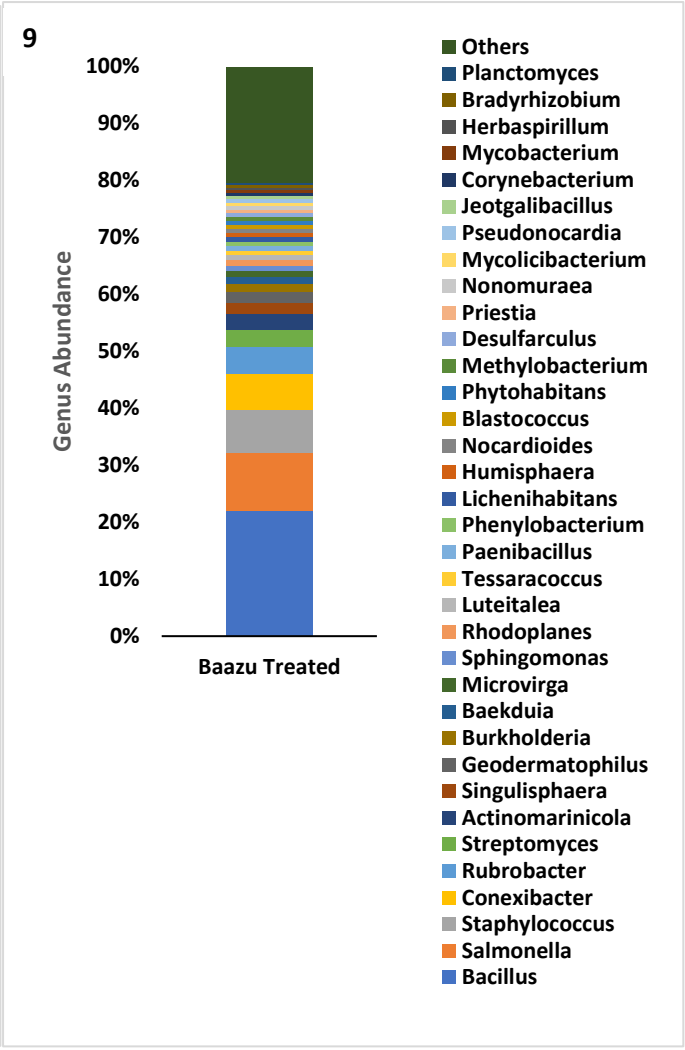

4
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Lambussie Untreated
Others
Escherichia
Luteitalea
Gemmata
Rubrobacter
Humisphaera
Skermanella
Limnoglobus
Pseudolabrys
Helicobacter
Acidibrevibacterium
Urbifossiella
C. Koribacter
Verrucomicrobium
Ensifer
Aquisphaera
Anaeromyxobacter
Corynebacterium
Sphingomonas
Geodermatophilus
Microvirga
Rhodoplanes
Clostridioides
Bradyrhizobium
C. Solibacter
Baekduia
Burkholderia
Priestia
Staphylococcus
Actinomarinicola
Singulisphaera
Planctomyces
Conexibacter
Streptomyces
Salmonella
Bacillus

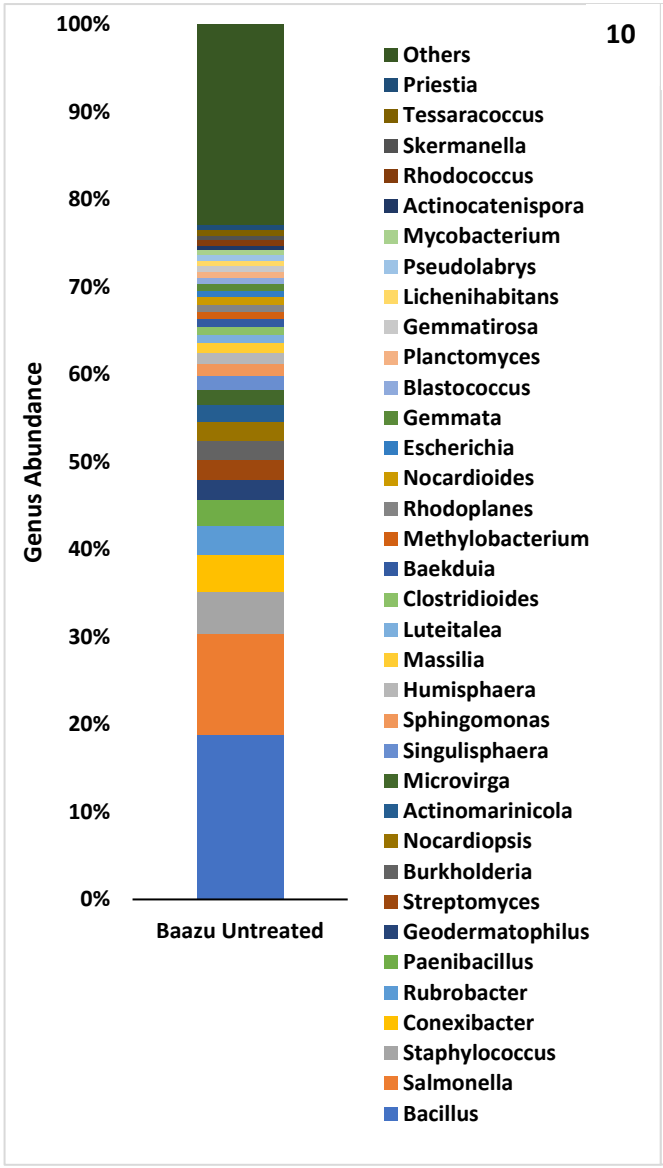

5
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Saan Treatted
Others
Stella
Geodermatophilus
C. Saccharimonas
Helicobacter
Staphylococcus
Verrucomicrobium
C. Koribacter
Paenibacillus
Priestia
Brevibacillus
Aquisphaera
Actinocatenispora
Rhodoplanes
Tessaracoccus
Bradyrhizobium
Gemmatirosa
Humisphaera
Rubrobacter
Pseudonocardia
Planctomyces
Phenylobacterium
C. Solibacter
Mycobacterium
Baekduia
Actinomarinicola
Corynebacterium
Burkholderia
Singulisphaera
Pseudolabrys
Escherichia
Sphingomonas
Streptomyces
Conexibacter
Salmonella
Bacillus

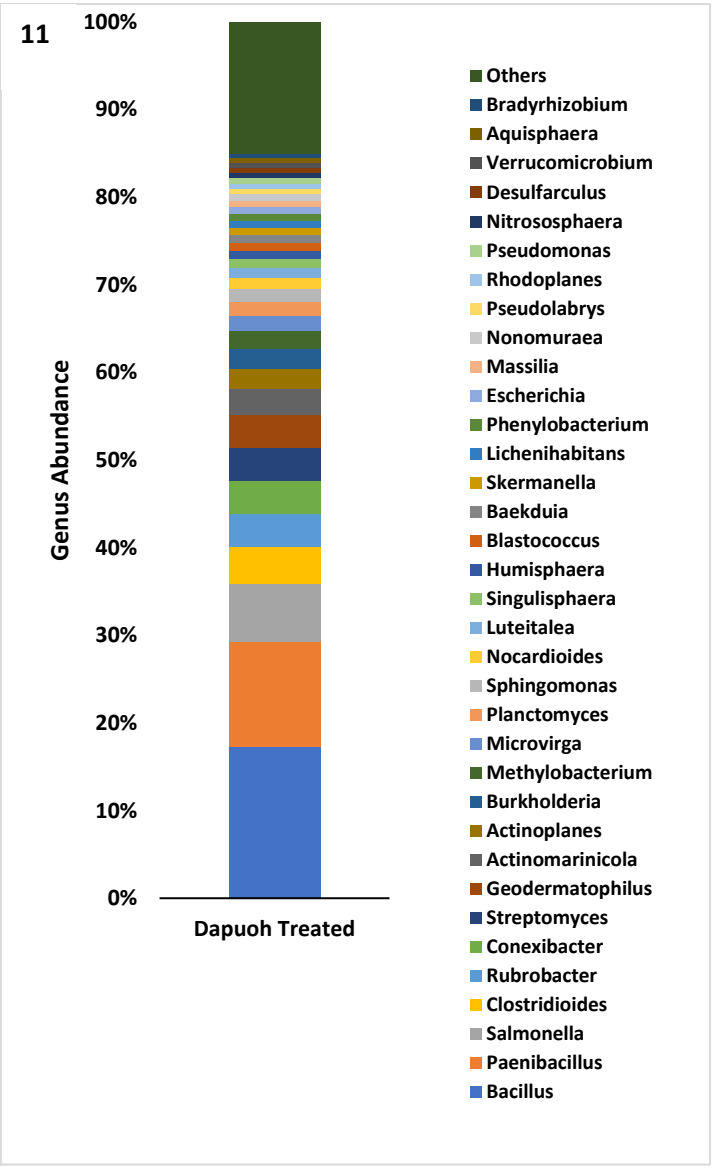

6
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Saan Untreated
Others
Neobacillus
Tessaracoccus
Limnoglobus
Nitrososphaera
Phenylobacterium
Acidibrevibacterium
Nitrospira
Verrucomicrobium
Liberibacter
Luteitalea
Methylobacterium
Baekduia
C. Solibacter
Mycobacterium
Rhodoplanes
Bradyrhizobium
Pseudomonas
Rubrobacter
Planctomyces
Actinomarinicola
Singulisphaera
Nocardioides
Geodermatophilus
Peribacillus
Sphingomonas
Microvirga
Ureibacillus
Burkholderia
Priestia
Staphylococcus
Conexibacter
Streptomyces
Salmonella
Paenibacillus
Bacillus

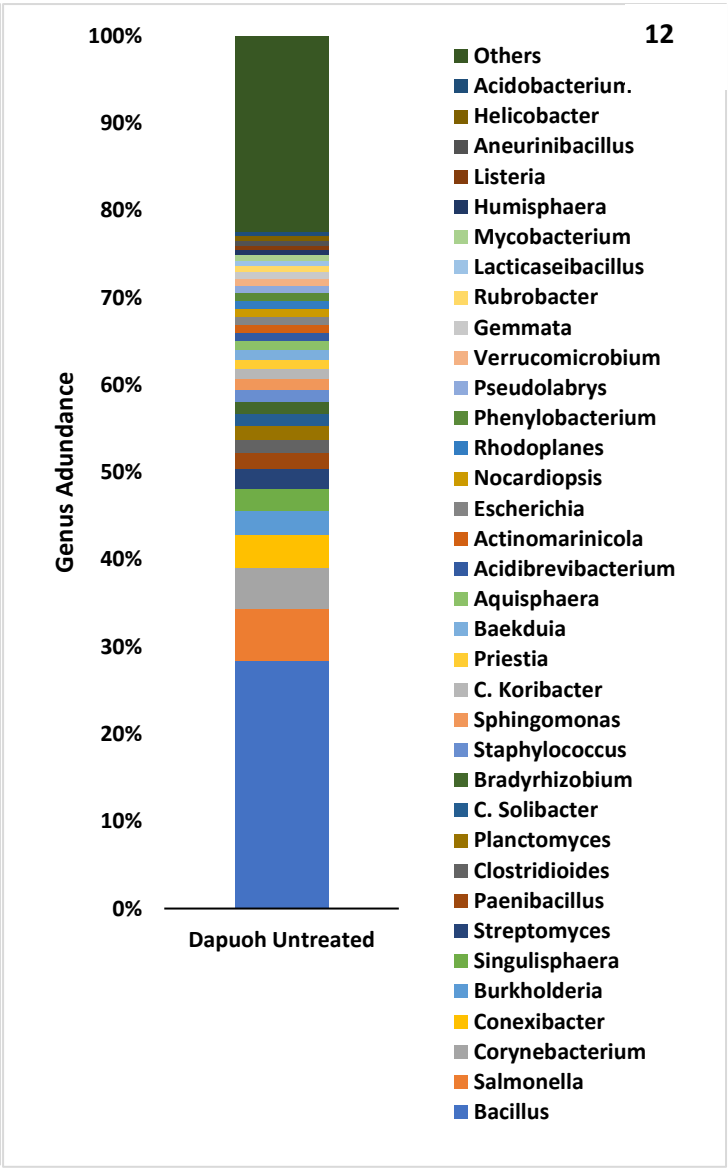

7
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sangu Treated
Others
Baekduia
Skermanella
Herbaspirillum
Mycobacterium
Bifidobacterium
Microvirga
Corynebacterium
Escherichia
Phenylobacterium
Rhodoplanes
Humisphaera
Pseudonocardia
Pseudolabrys
Brevibacillus
Planctomyces
Methylobacterium
Luteitalea
Bradyrhizobium
Sphingomonas
Priestia
Burkholderia
Helicobacter
Gemmata
Tessaracoccus
Singulisphaera
Rubrobacter
Geodermatophilus
Streptomyces
Nocardiopsis
Actinomarinicola
Conexibacter
Staphylococcus
Paenibacillus
Salmonella
Bacillus


8
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sangu Untreated
Others
Listeria
Skermanella
Nocardioides
Urbifossiella
Verrucomicrobium
Mycobacterium
Pseudolabrys
Desulfarculus
Lichenihabitans
Tessaracoccus
Phenylobacterium
Bradyrhizobium
Yersinia
Planctomyces
Baekduia
Humisphaera
Microvirga
Nocardiopsis
Rhodoplanes
Geodermatophilus
Luteitalea
Sphingomonas
Priestia
Clostridioides
Singulisphaera
Burkholderia
Rubrobacter
Actinomarinicola
Streptomyces
Paenibacillus
Conexibacter
Corynebacterium
Salmonella
Staphylococcus
Bacillus

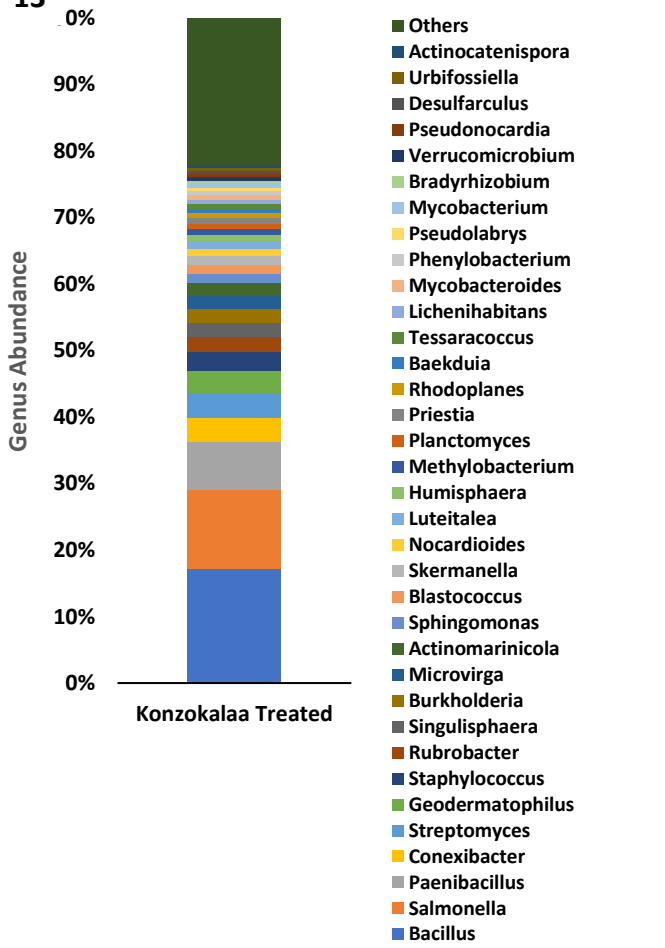

9
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Baazu Treated
Others
Planctomyces
Bradyrhizobium
Herbaspirillum
Mycobacterium
Corynebacterium
Jeotgalibacillus
Pseudonocardia
Mycolicibacterium
Nonomuraea
Priestia
Desulfarculus
Methylobacterium
Phytohabitans
Blastococcus
Nocardioides
Humisphaera
Lichenihabitans
Phenylobacterium
Paenibacillus
Tessaracoccus
Luteitalea
Rhodoplanes
Sphingomonas
Microvirga
Baekduia
Burkholderia
Geodermatophilus
Singulisphaera
Actinomarinicola
Streptomyces
Rubrobacter
Conexibacter
Staphylococcus
Salmonella
Bacillus


10
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Baazu Untreated
Others
Priestia
Tessaracoccus
Skermanella
Rhodococcus
Actinocatenispora
Mycobacterium
Pseudolabrys
Lichenihabitans
Gemmatirosa
Planctomyces
Blastococcus
Gemmata
Escherichia
Nocardioides
Rhodoplanes
Methylobacterium
Baekduia
Clostridioides
Luteitalea
Massilia
Humisphaera
Sphingomonas
Singulisphaera
Microvirga
Actinomarinicola
Nocardiopsis
Burkholderia
Streptomyces
Geodermatophilus
Paenibacillus
Rubrobacter
Conexibacter
Staphylococcus
Salmonella
Bacillus

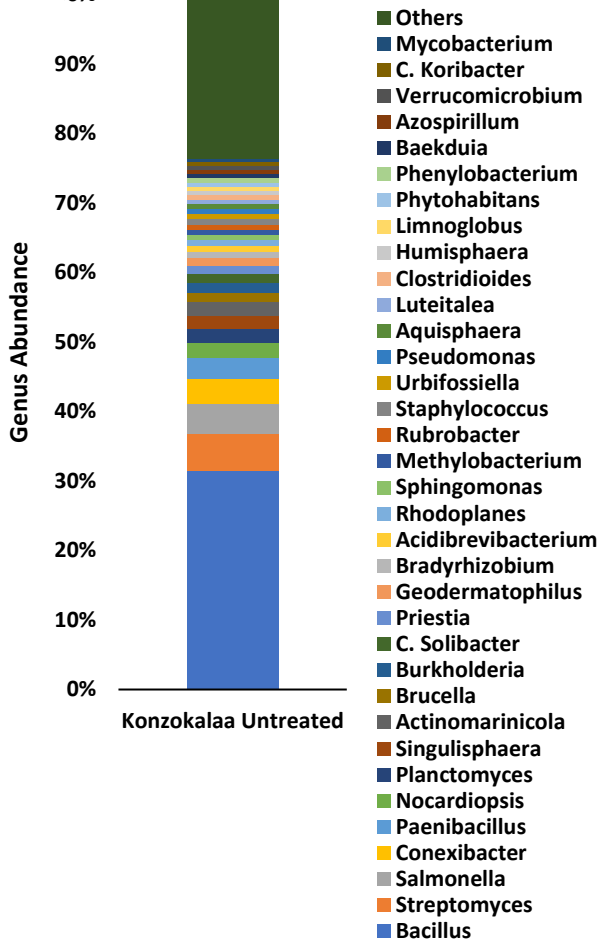

11
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Dapuoh Treated
Others
Bradyrhizobium
Aquisphaera
Verrucomicrobium
Desulfarculus
Nitrososphaera
Pseudomonas
Rhodoplanes
Pseudolabrys
Nonomuraea
Massilia
Escherichia
Phenylobacterium
Lichenihabitans
Skermanella
Baekduia
Blastococcus
Humisphaera
Singulisphaera
Luteitalea
Nocardioides
Sphingomonas
Planctomyces
Microvirga
Methylobacterium
Burkholderia
Actinoplanes
Actinomarinicola
Geodermatophilus
Streptomyces
Conexibacter
Rubrobacter
Clostridioides
Salmonella
Paenibacillus
Bacillus


12
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Adundance
Dapuoh Untreated
Others
Acidobacteriun.
Helicobacter
Aneurinibacillus
Listeria
Humisphaera
Mycobacterium
Lacticaseibacillus
Rubrobacter
Gemmata
Verrucomicrobium
Pseudolabrys
Phenylobacterium
Rhodoplanes
Nocardiopsis
Escherichia
Actinomarinicola
Acidibrevibacterium
Aquisphaera
Baekduia
Priestia
C. Koribacter
Sphingomonas
Staphylococcus
Bradyrhizobium
C. Solibacter
Planctomyces
Clostridioides
Paenibacillus
Streptomyces
Singulisphaera
Burkholderia
Conexibacter
Corynebacterium
Salmonella
Bacillus

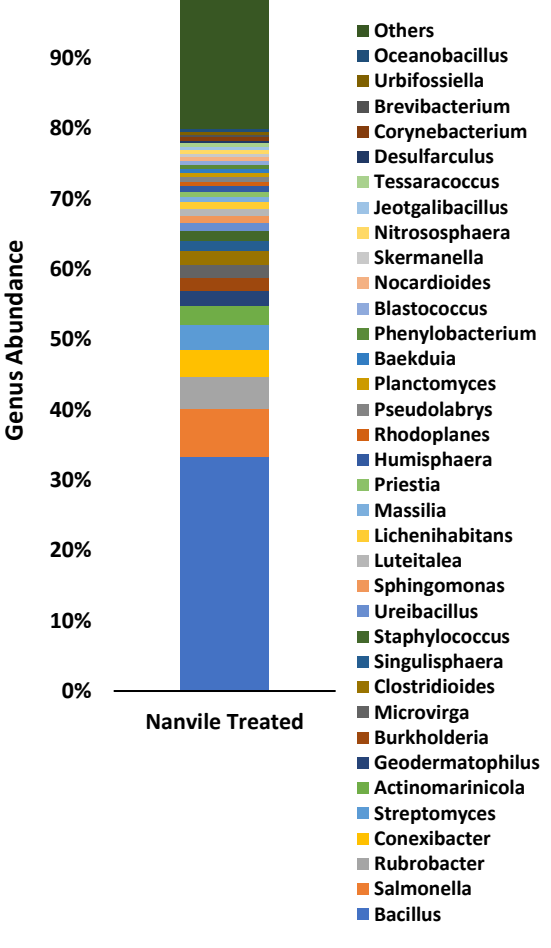
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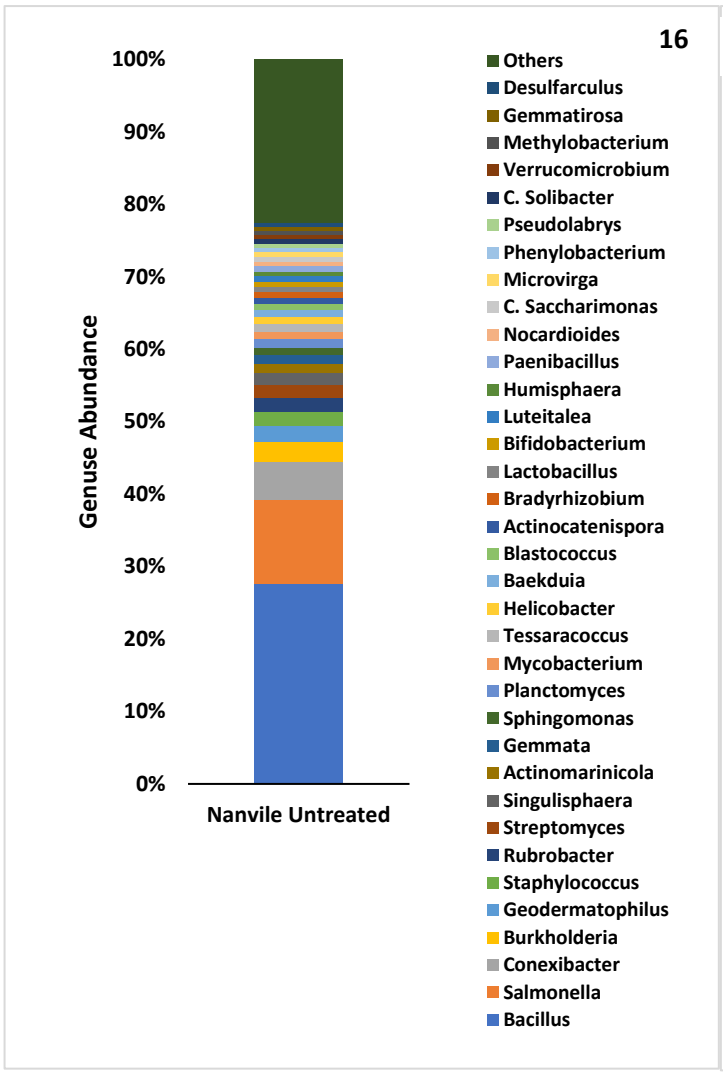

Genus Abundance
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Konzokalaa Treated
Others
Actinocatenispora
Urbifossiella
Desulfarculus
Pseudonocardia
Verrucomicrobium
Bradyrhizobium
Mycobacterium
Pseudolabrys
Phenylobacterium
Mycobacteroides
Lichenihabitans
Tessaracoccus
Baekduia
Rhodoplanes
Priestia
Planctomyces
Methylobacterium
Humisphaera
Luteitalea
Nocardioides
Skermanella
Blastococcus
Sphingomonas
Actinomarinicola
Microvirga
Burkholderia
Singulisphaera
Rubrobacter
Staphylococcus
Geodermatophilus
Streptomyces
Conexibacter
Paenibacillus
Salmonella
Bacillus

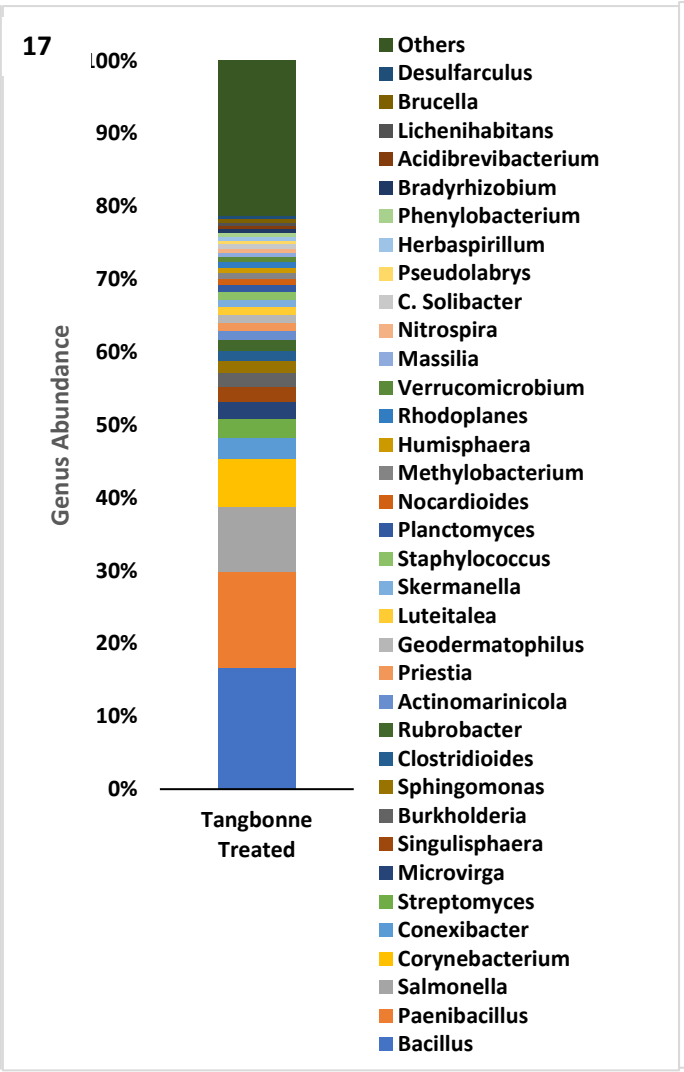
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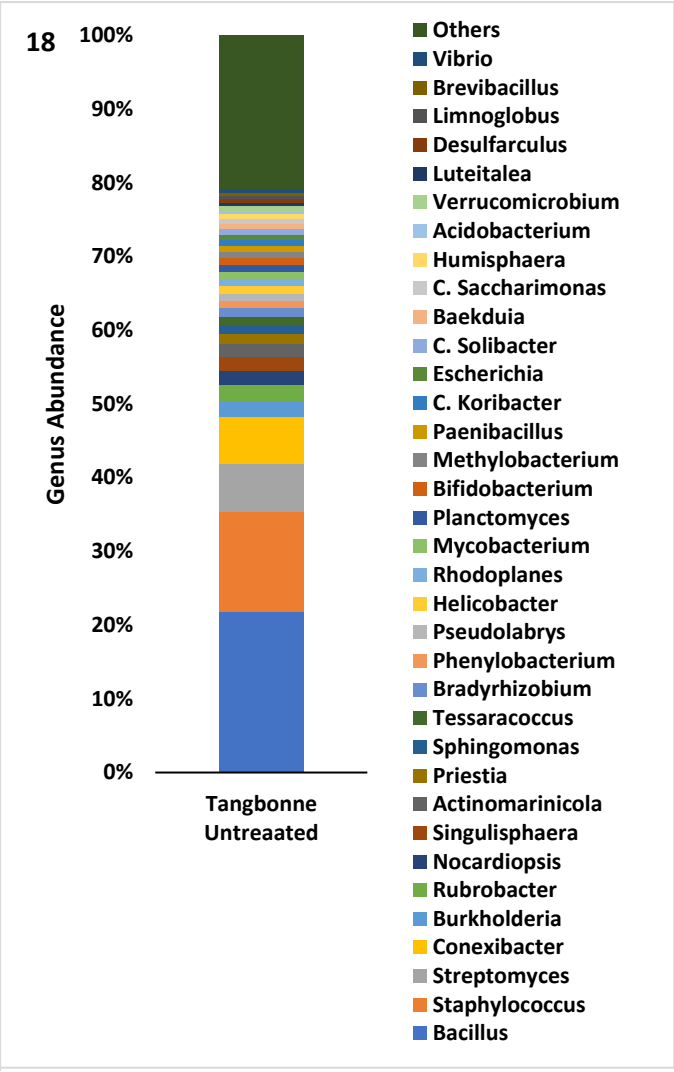

Genus Abundance
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0%
Konzokalaa Untreated
Others
Mycobacterium
C. Koribacter
Verrucomicrobium
Azospirillum
Baekduia
Phenylobacterium
Phytohabitans
Limnoglobus
Humisphaera
Clostridioides
Luteitalea
Aquisphaera
Pseudomonas
Urbifossiella
Staphylococcus
Rubrobacter
Methylobacterium
Sphingomonas
Rhodoplanes
Acidibrevibacterium
Bradyrhizobium
Geodermatophilus
Priestia
C. Solibacter
Burkholderia
Brucella
Actinomarinicola
Singulisphaera
Planctomyces
Nocardiopsis
Paenibacillus
Conexibacter
Salmonella
Streptomyces
Bacillus

15

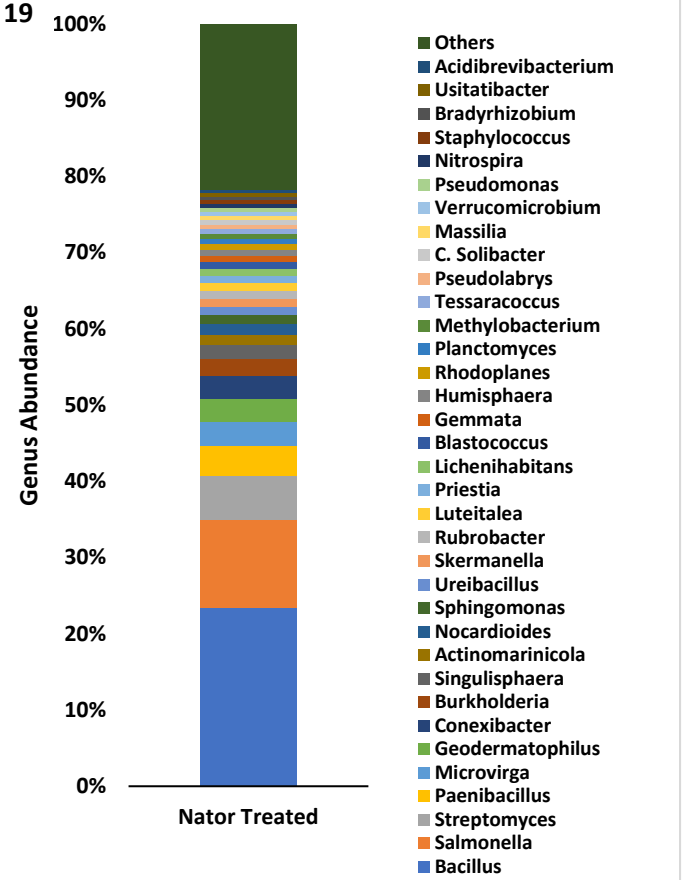

Genus Abundance
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Nanvile Treated
Others
Oceanobacillus
Urbifossiella
Brevibacterium
Corynebacterium
Desulfarculus
Tessaracoccus
Jeotgalibacillus
Nitrososphaera
Skermanella
Nocardioides
Blastococcus
Phenylobacterium
Baekduia
Planctomyces
Pseudolabrys
Rhodoplanes
Humisphaera
Priestia
Massilia
Lichenihabitans
Luteitalea
Sphingomonas
Ureibacillus
Staphylococcus
Singulisphaera
Clostridioides
Microvirga
Burkholderia
Geodermatophilus
Actinomarinicola
Streptomyces
Conexibacter
Rubrobacter
Salmonella
Bacillus


16
Genuse Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Nanvile Untreated
Others
Desulfarculus
Gemmatirosa
Methylobacterium
Verrucomicrobium
C. Solibacter
Pseudolabrys
Phenylobacterium
Microvirga
C. Saccharimonas
Nocardioides
Paenibacillus
Humisphaera
Luteitalea
Bifidobacterium
Lactobacillus
Bradyrhizobium
Actinocatenispora
Blastococcus
Baekduia
Helicobacter
Tessaracoccus
Mycobacterium
Planctomyces
Sphingomonas
Gemmata
Actinomarinicola
Singulisphaera
Streptomyces
Rubrobacter
Staphylococcus
Geodermatophilus
Burkholderia
Conexibacter
Salmonella
Bacillus

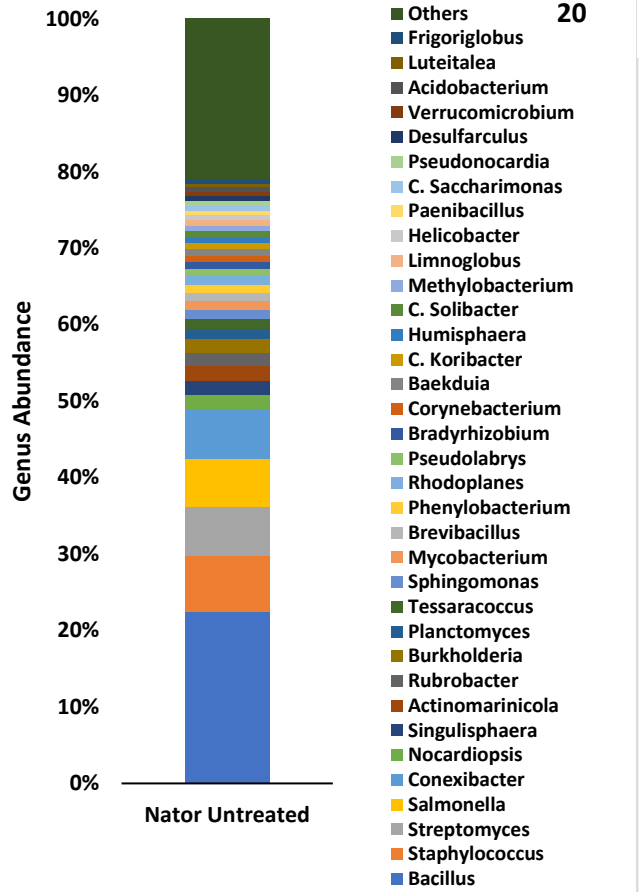

17
Genus Abundance
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Tangbonne
Treated
Others
Desulfarculus
Brucella
Lichenihabitans
Acidibrevibacterium
Bradyrhizobium
Phenylobacterium
Herbaspirillum
Pseudolabrys
C. Solibacter
Nitrospira
Massilia
Verrucomicrobium
Rhodoplanes
Humisphaera
Methylobacterium
Nocardioides
Planctomyces
Staphylococcus
Skermanella
Luteitalea
Geodermatophilus
Priestia
Actinomarinicola
Rubrobacter
Clostridioides
Sphingomonas
Burkholderia
Singulisphaera
Microvirga
Streptomyces
Conexibacter
Corynebacterium
Salmonella
Paenibacillus
Bacillus


18
Genus Abundance
100%
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Tangbonne
Untreaated
Others
Vibrio
Brevibacillus
Limnoglobus
Desulfarculus
Luteitalea
Verrucomicrobium
Acidobacterium
Humisphaera
C. Saccharimonas
Baekduia
C. Solibacter
Escherichia
C. Koribacter
Paenibacillus
Methylobacterium
Bifidobacterium
Planctomyces
Mycobacterium
Rhodoplanes
Helicobacter
Pseudolabrys
Phenylobacterium
Bradyrhizobium
Tessaracoccus
Sphingomonas
Priestia
Actinomarinicola
Singulisphaera
Nocardiopsis
Rubrobacter
Burkholderia
Conexibacter
Streptomyces
Staphylococcus
Bacillus

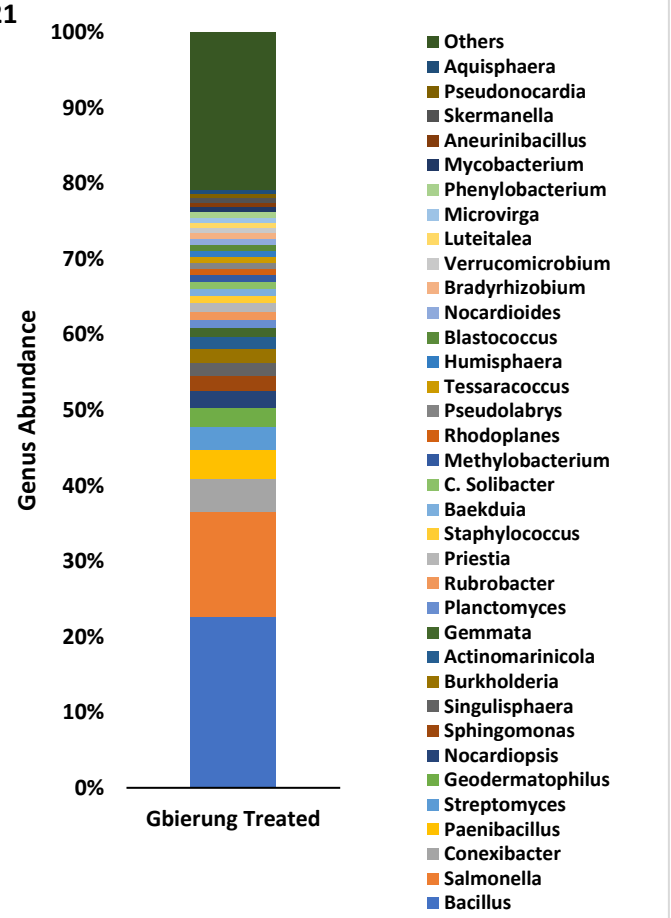
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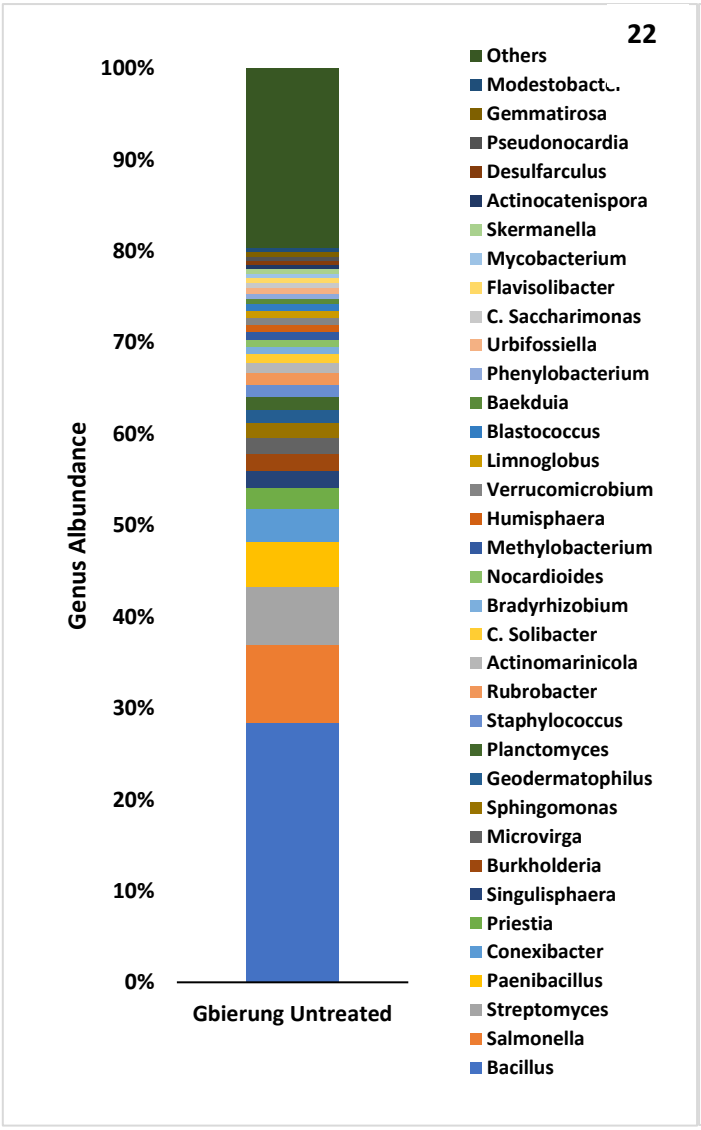

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Genus Abundance
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Others
Acidibrevibacterium
Usitatibacter
Bradyrhizobium
Staphylococcus
Nitrospira
Pseudomonas
Verrucomicrobium
Massilia
C. Solibacter
Pseudolabrys
Tessaracoccus
Methylobacterium
Planctomyces
Rhodoplanes
Humisphaera
Gemmata
Blastococcus
Lichenihabitans
Priestia
Luteitalea
Rubrobacter
Skermanella
Ureibacillus
Sphingomonas
Nocardioides
Actinomarinicola
Singulisphaera
Burkholderia
Conexibacter
Geodermatophilus
Microvirga
Paenibacillus
Streptomyces
Salmonella
Bacillus

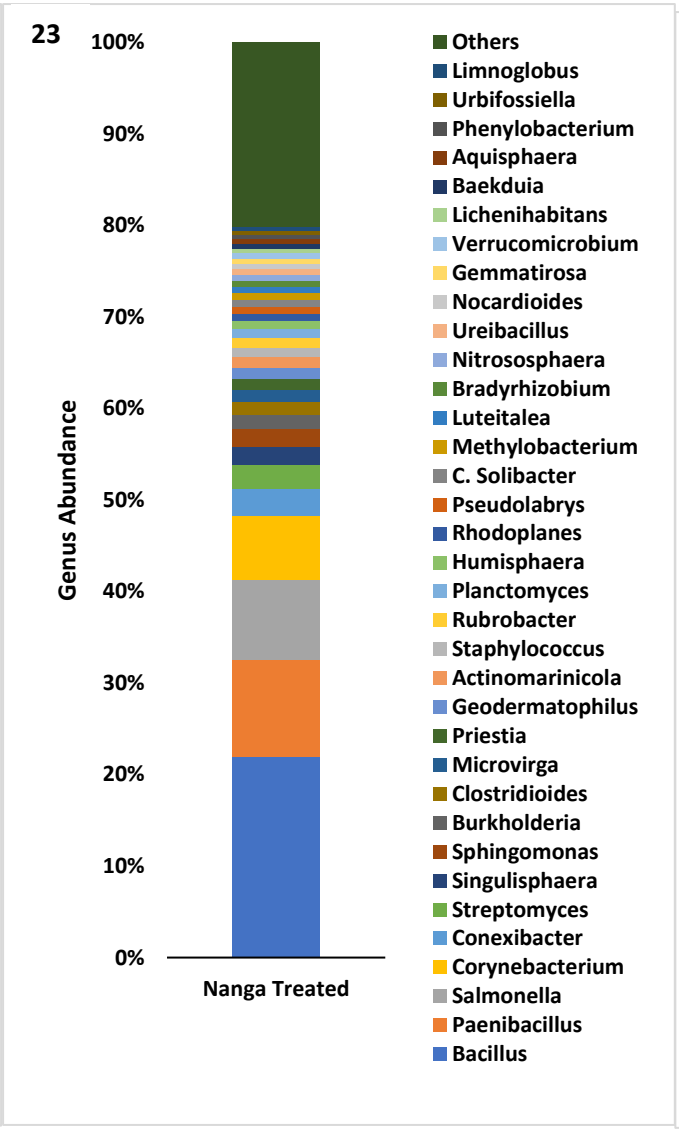
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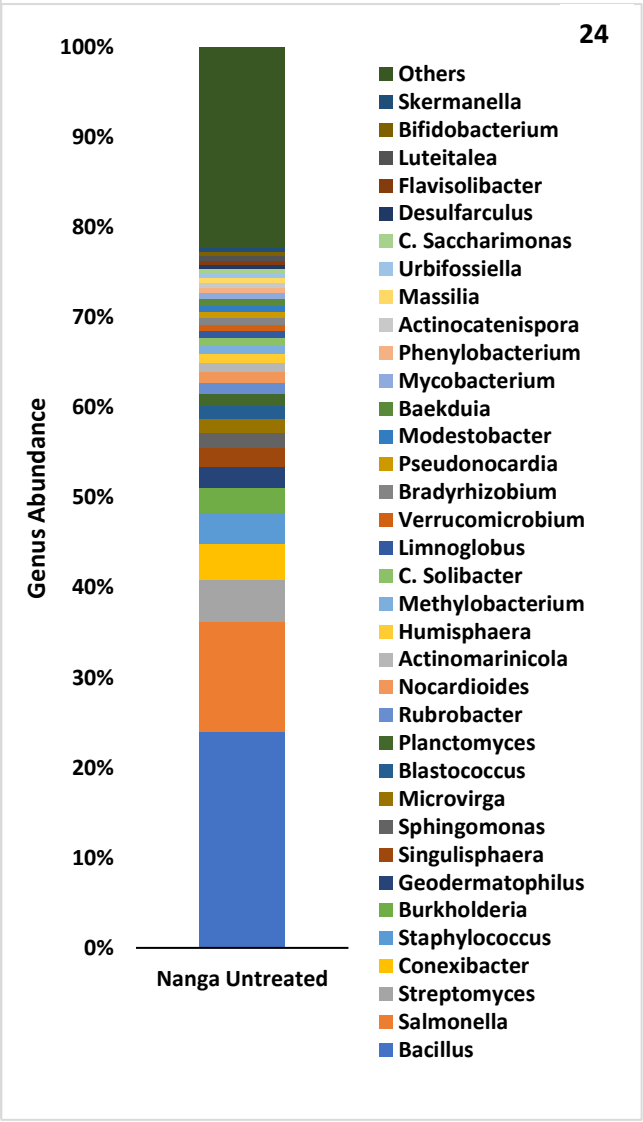

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Genus Abundance
Nator Untreated
Others
Frigoriglobus
Luteitalea
Acidobacterium
Verrucomicrobium
Desulfarculus
Pseudonocardia
C. Saccharimonas
Paenibacillus
Helicobacter
Limnoglobus
Methylobacterium
C. Solibacter
Humisphaera
C. Koribacter
Baekduia
Corynebacterium
Bradyrhizobium
Pseudolabrys
Rhodoplanes
Phenylobacterium
Brevibacillus
Mycobacterium
Sphingomonas
Tessaracoccus
Planctomyces
Burkholderia
Rubrobacter
Actinomarinicola
Singulisphaera
Nocardiopsis
Conexibacter
Salmonella
Streptomyces
Staphylococcus
Bacillus

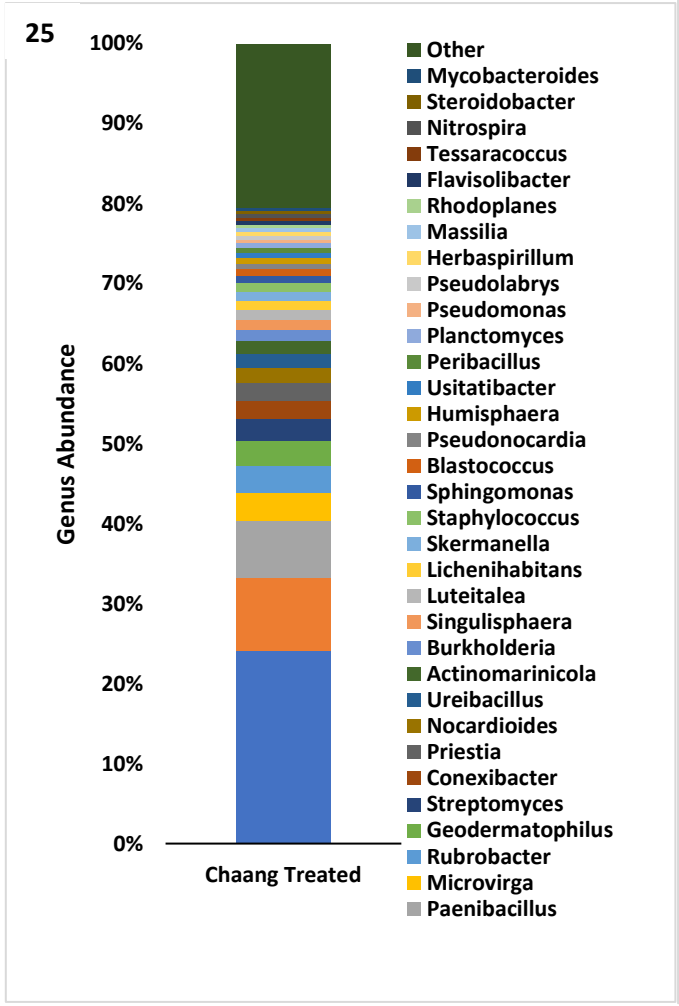
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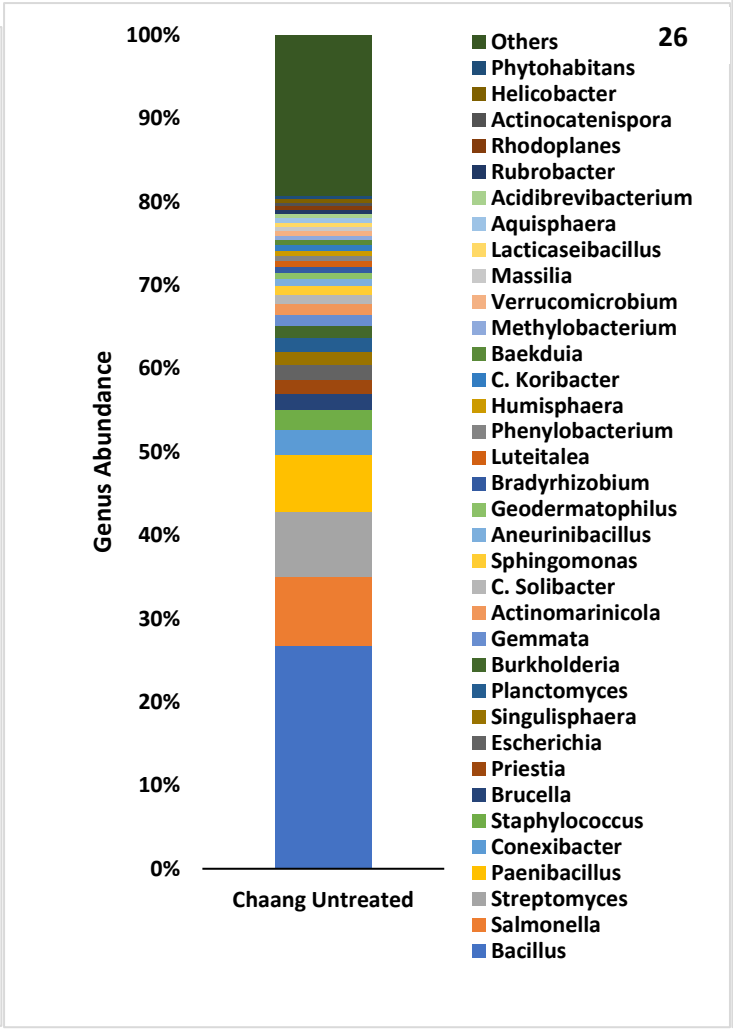

100%
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Genus Abundance
Gbierung Treated
Others
Aquisphaera
Pseudonocardia
Skermanella
Aneurinibacillus
Mycobacterium
Phenylobacterium
Microvirga
Luteitalea
Verrucomicrobium
Bradyrhizobium
Nocardioides
Blastococcus
Humisphaera
Tessaracoccus
Pseudolabrys
Rhodoplanes
Methylobacterium
C. Solibacter
Baekduia
Staphylococcus
Priestia
Rubrobacter
Planctomyces
Gemmata
Actinomarinicola
Burkholderia
Singulisphaera
Sphingomonas
Nocardiopsis
Geodermatophilus
Streptomyces
Paenibacillus
Conexibacter
Salmonella
Bacillus

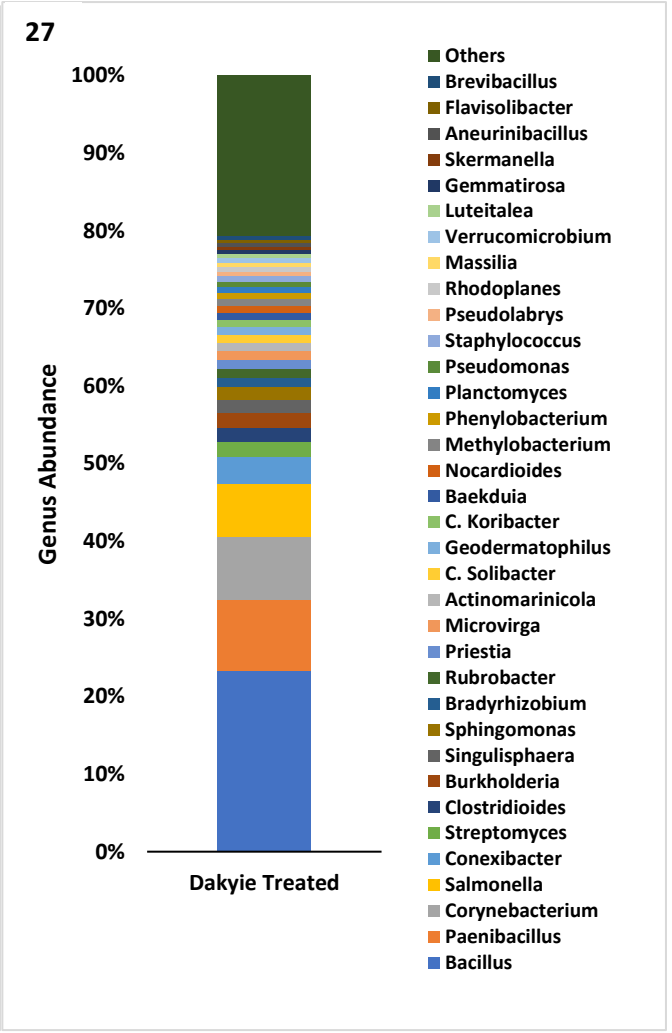

22
Genus Albundance
100%
90%
80%
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60%
50%
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10%
0%
Gbierung Untreated
Others
Modestobacter
Gemmatirosa
Pseudonocardia
Desulfarculus
Actinocatenispora
Skermanella
Mycobacterium
Flavisolibacter
C. Saccharimonas
Urbifossiella
Phenylobacterium
Baekduia
Blastococcus
Limnoglobus
Verrucomicrobium
Humisphaera
Methylobacterium
Nocardioides
Bradyrhizobium
C. Solibacter
Actinomarinicola
Rubrobacter
Staphylococcus
Planctomyces
Geodermatophilus
Sphingomonas
Microvirga
Burkholderia
Singulisphaera
Priestia
Conexibacter
Paenibacillus
Streptomyces
Salmonella
Bacillus


23
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Nanga Treated
Others
Limnoglobus
Urbifossiella
Phenylobacterium
Aquisphaera
Baekduia
Lichenihabitans
Verrucomicrobium
Gemmatirosa
Nocardioides
Ureibacillus
Nitrososphaera
Bradyrhizobium
Luteitalea
Methylobacterium
C. Solibacter
Pseudolabrys
Rhodoplanes
Humisphaera
Planctomyces
Rubrobacter
Staphylococcus
Actinomarinicola
Geodermatophilus
Priestia
Microvirga
Clostridioides
Burkholderia
Sphingomonas
Singulisphaera
Streptomyces
Conexibacter
Corynebacterium
Salmonella
Paenibacillus
Bacillus


24
Genus Abundance
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Nanga Untreated
Others
Skermanella
Bifidobacterium
Luteitalea
Flavisolibacter
Desulfarculus
C. Saccharimonas
Urbifossiella
Massilia
Actinocatenispora
Phenylobacterium
Mycobacterium
Baekduia
Modestobacter
Pseudonocardia
Bradyrhizobium
Verrucomicrobium
Limnoglobus
C. Solibacter
Methylobacterium
Humisphaera
Actinomarinicola
Nocardioides
Rubrobacter
Planctomyces
Blastococcus
Microvirga
Sphingomonas
Singulisphaera
Geodermatophilus
Burkholderia
Staphylococcus
Conexibacter
Streptomyces
Salmonella
Bacillus


25
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Chaang Treated
Other
Mycobacteroides
Steroidobacter
Nitrospira
Tessaracoccus
Flavisolibacter
Rhodoplanes
Massilia
Herbaspirillum
Pseudolabrys
Pseudomonas
Planctomyces
Peribacillus
Usitatibacter
Humisphaera
Pseudonocardia
Blastococcus
Sphingomonas
Staphylococcus
Skermanella
Lichenihabitans
Luteitalea
Singulisphaera
Burkholderia
Actinomarinicola
Ureibacillus
Nocardioides
Priestia
Conexibacter
Streptomyces
Geodermatophilus
Rubrobacter
Microvirga
Paenibacillus


26
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Genus Abundance
Chaang Untreated
Others
Phytohabitans
Helicobacter
Actinocatenispora
Rhodoplanes
Rubrobacter
Acidibrevibacterium
Aquisphaera
Lacticaseibacillus
Massilia
Verrucomicrobium
Methylobacterium
Baekduia
C. Koribacter
Humisphaera
Phenylobacterium
Luteitalea
Bradyrhizobium
Geodermatophilus
Aneurinibacillus
Sphingomonas
C. Solibacter
Actinomarinicola
Gemmata
Burkholderia
Planctomyces
Singulisphaera
Escherichia
Priestia
Brucella
Staphylococcus
Conexibacter
Paenibacillus
Streptomyces
Salmonella
Bacillus


27
100%
90%
80%
70%
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40%
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Genus Abundance
Dakyie Treated
Others
Brevibacillus
Flavisolibacter
Aneurinibacillus
Skermanella
Gemmatirosa
Luteitalea
Verrucomicrobium
Massilia
Rhodoplanes
Pseudolabrys
Staphylococcus
Pseudomonas
Planctomyces
Phenylobacterium
Methylobacterium
Nocardioides
Baekduia
C. Koribacter
Geodermatophilus
C. Solibacter
Actinomarinicola
Microvirga
Priestia
Rubrobacter
Bradyrhizobium
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Singulisphaera
Burkholderia
Clostridioides
Streptomyces
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Salmonella
Corynebacterium
Paenibacillus
Bacillus

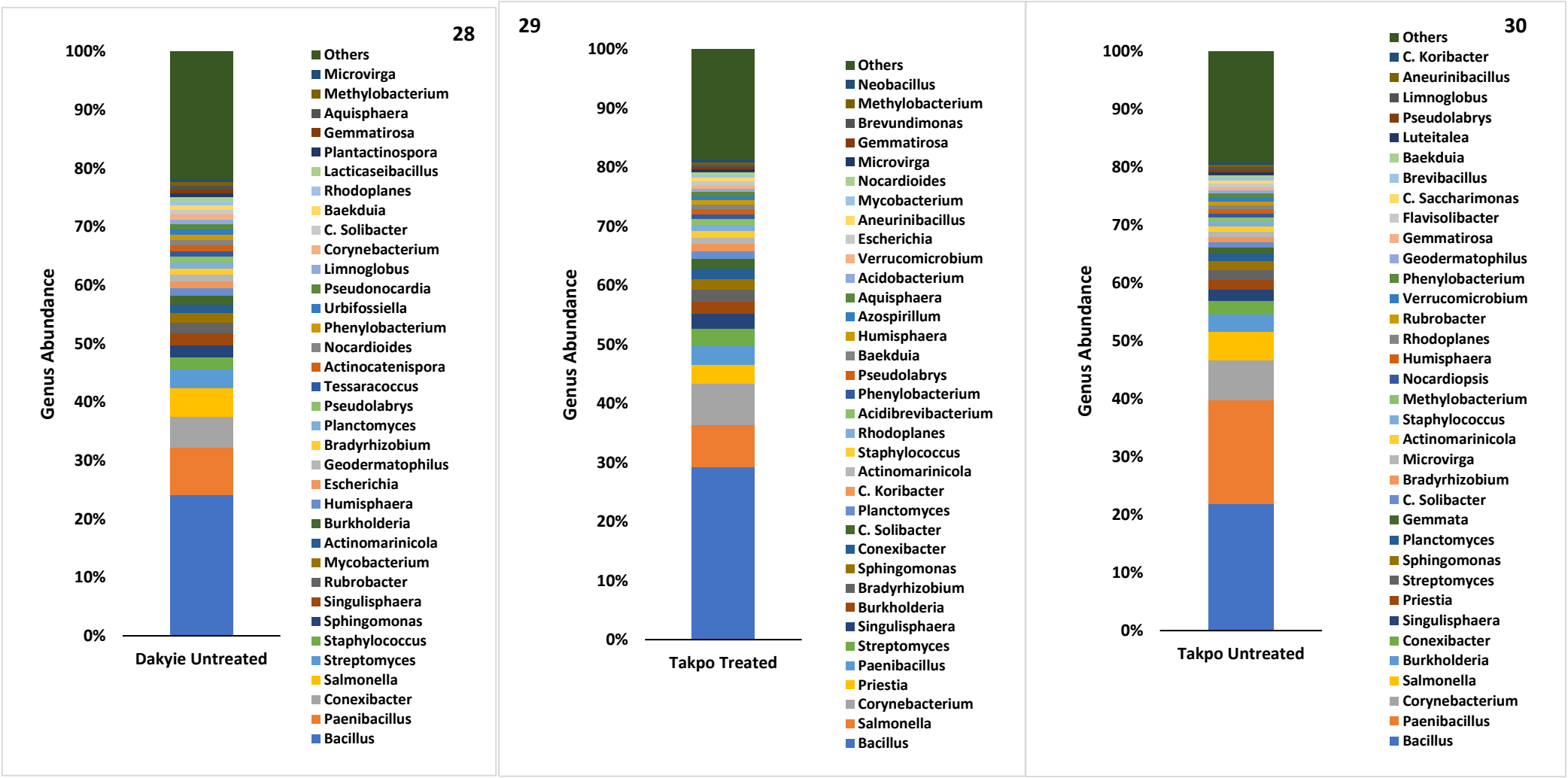


Figure 3: Comparison of genus level genomic abundance in herbicide-treated (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 29), and untreated (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30) soils across 15 locations.

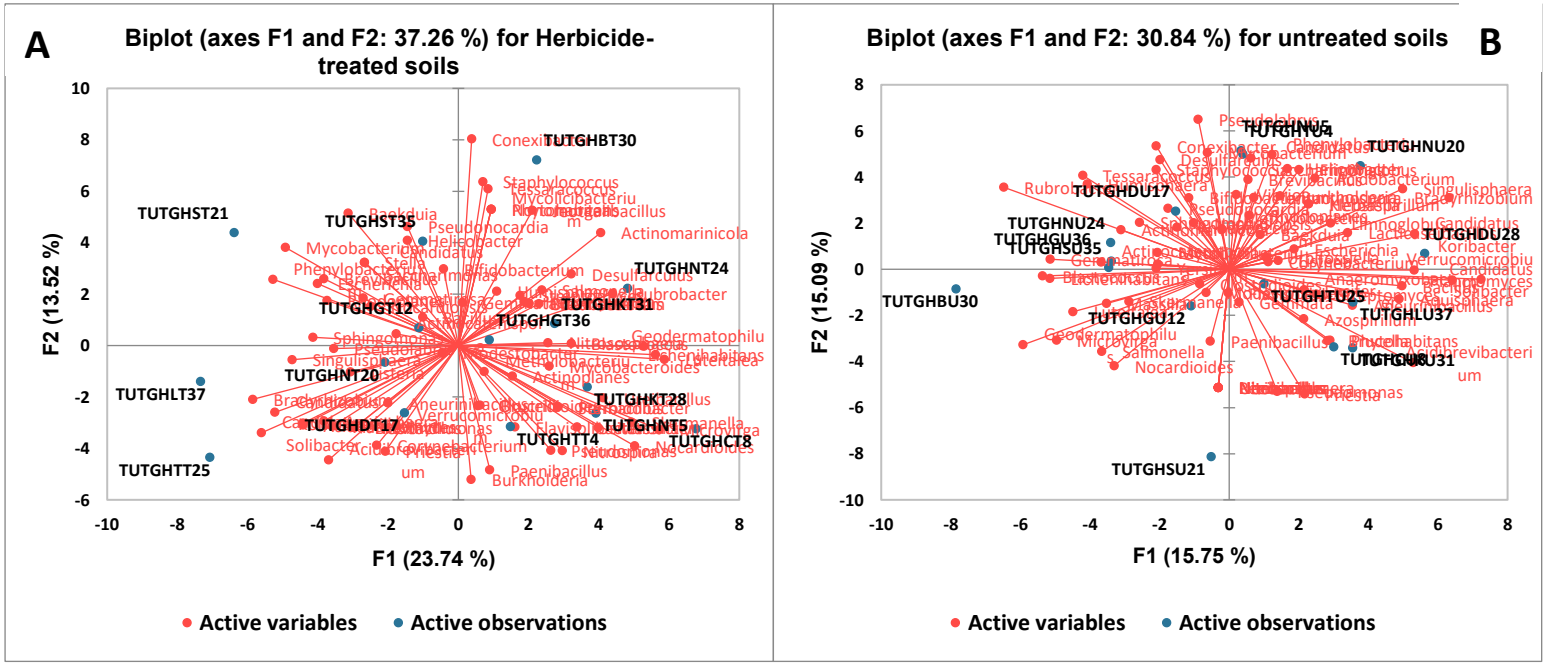


Figure 4. Principal component analysis (PCA)) showing the scoring for the various taxonomic groups of A) herbicide-treated, and B) untreated soil samples.

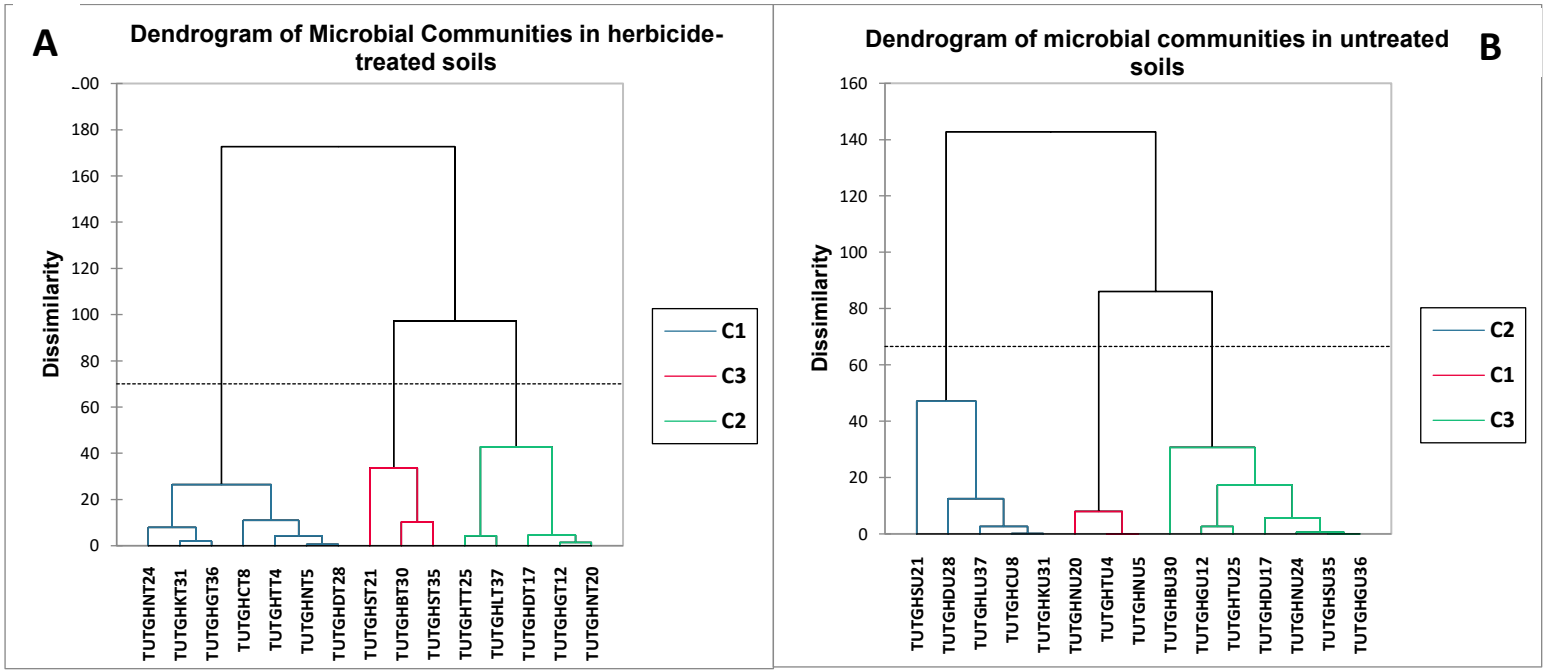


Figure 5. Hierarchical clustering to illustrate a dissimilarity matrix of community's functional diversity in A) herbicide-treated, and B) untreated soil samples. Where C1= Cluster1, C2= Cluster2 and C3= Cluster3.

Abbreviations

PGP: Plant growth promoting
PCA: Principal component algorithm
PCR: Polymerase chain reaction
DNA: Deoxyribonucleic acid

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Authors' contributions

ANK and ROF were involved in soil surveys, sample collection and farmers engagements. TM analysed the data. ANK wrote the first draft. TM., TYN., PS. and FDD. reviewed and edited the manuscript. FDD. conceptualized the study and secured the funding for this study.

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Data availability

The datasets generated and used in this study are available on the NCBI repository and can be accessed at **https://www.ncbi.nlm.nih.gov/sra/PRJNA1514777**

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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