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Effects of Soil Solarization on the Plant Growth-Promoting Capacity of Soil Bacteria

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Abstract: Soil solarization is a chemical-free, eco-friendly approach that makes use of solar energy trapped under clear polyethylene films to raise the temperature in soil to lethal levels that kill most of the soil-borne pathogens, weed seeds, and pests in the soil. In spite of the proven pesticidal potential of solarization, its effect on plant growth promoting bacteria has not been clearly understood, especially in tropical botanical garden soils. The present study was carried out to determine the effect of two-week soil solarization on the population and diversity of PGPB isolated from University of Benin Botanical Garden soil, Nigeria. Traditional microbiological techniques were used to isolate and identify the bacterial isolates. Five bacterial genera were isolated from the soil pre-solarization and they include *Bacillus mycoides*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter aerogenes* and *Enterobacter cloacae*. After solarization, *S. aureus* was selectively eliminated but the other four bacterial species survived. All the surviving species namely *B. mycoides*, *B. subtilis*, *E. aerogenes* and *E. cloacae* were found to be positive for plant growth-promoting activities such as nitrogen fixation, IAA production and phosphate solubilization. The phosphate solubilization indices were significantly enhanced by solarization for all the surviving taxa. These findings demonstrate that two-week solarization selectively eliminates certain bacteria while enriching a functionally active PGPB community, with implications for sustainable soil management in tropical agricultural systems.

1. Introduction

Soil is the primary structural and nutritional foundation of all terrestrial agricultural systems, providing the biophysical matrix within which microbial communities, plant roots, and soil fauna interact to drive nutrient cycling, organic matter decomposition, and pathogen suppression (Brady and Weil, 2008). Increasingly, the quality and composition of the soil microbiome are recognized as critical determinants of sustainable crop production (Khan *et al*., 2023; Timofeeva *et al*., 2023; Tziros *et al*., 2024). Since soil microbial communities can be influenced by agronomic and soil management practices, approaches that improve soil health while reducing pathogen pressure have become important in sustainable agriculture (Tziros *et al*., 2024; Gebreegziher, 2024). One such approach is soil solarization, a non-chemical hydrothermal technique in which moist soil is covered with transparent polyethylene film and passively heated by solar radiation to temperatures capable of suppressing soil-borne pathogens, weeds, and pests (Abouatallah *et al*., 2012; Stapleton, 2000; Khan

et al., 2023; Tziros *et al*., 2024; Gebreegziher, 2024; Abbas *et al*., 2026). Soil solarization was first reported as an effective disinfestation strategy by Katan *et al*. (1976) in Israel, and has since been employed in tropical and subtropical regions characterized by high solar irradiance and extended warm seasons (Gamliel and Katan, 2012; Gebreegziher, 2024). The soil solarization process has been shown to cause some important chemical changes, including ammonium-N, nitrate-N, Ca^{2+}, Mg^{2+}, and electrical conductivity, as these have proven to be consistently elevated following treatment (post-solarization). These changes have been attributed to the release of nutrients from heat-killed soil biota and thermally-mineralized organic matter (Gebreegziher, 2024; Tziros *et al*., 2024). These nutrient changes may enhance the growth of surviving bacteria, contributing to the enrichment of plant-growth-promoting bacteria (PGPB) populations post-solarization. Solarization also exerts marked non-uniform effects across microbial taxa, depending on the soil types, as heat-sensitive Gram-negative fluorescent pseudomonads and many fungal pathogens may be reduced by over 70% in the upper soil layers (Khan *et al*., 2023; Li *et al*., 2024). In contrast, populations of *Bacillus* species, endospore-forming Gram-positive bacteria, may typically show relative enrichment post-solarization, reflecting both their intrinsic heat resistance and their competitive advantage in pathogen-suppressed soil (Khan *et al*., 2023; Li *et al*., 2024; Zaccadelli *et al*., 2024). Contemporary metagenomic analyses confirm that solarization shifts the dominant bacterial phyla, as Zaccadelli *et al*. (2024) demonstrated that Firmicutes abundance increased significantly in solarized soils, with a concomitant reduction in Proteobacteria and Actinobacteria. The selective preservation of Firmicutes (including *Bacillus* spp.) and *Enterobacteriaceae* has important implications for post-solarization soil PGPB functional capacity. PGPB confer multiple agronomic benefits, including biological nitrogen fixation, phosphate solubilization, the production of phytohormones such as indole-3-acetic acid (IAA), and the suppression of phytopathogens (Omoregie *et al*., 2020; Isagba *et al*., 2023; Timofeeva *et al*., 2023). The capacity of certain *Bacillus* species, heat-tolerant, spore-forming or otherwise thermo-resistant taxa, to persist and even proliferate following heat-treatments has been documented in literature (Berendsen *et al*., 2015; Berendsen *et al*., 2016); nevertheless, evidence of solarization from tropical African soils remains scarce. Moreover, the impact of soil solarization on the plant growth-promoting rhizobacteria (PGPB) community is critically underexplored within the context of the Nigerian agroecosystem.

This study therefore aimed to comprehensively characterize the effect of two-week soil solarization on the identity, abundance, and functional PGPB activity of soil bacterial communities from the University of Benin Botanical Garden, Nigeria. The specific objectives were: (i) to isolate and identify bacterial communities from solarized and non-solarized soils; (ii) to evaluate PGPB traits including nitrogen fixation, IAA production, and phosphate solubilization; and (iii) to quantify changes in phosphate solubilization indices before and after solarization.

2. Methodology

2.1 Study Site and Sample Collection

Soil samples were collected from the University of Benin Botanical Garden, Benin City, Nigeria, a site characterized by tropical humid climate. Two ridges of loamy topsoil were designated as solarized and non-solarized treatment plots, each approximately 1 m^2 in area. For the solarization treatment, soil was moistened to approximately 70–80% field capacity and covered with clear transparent polyethylene film (thickness 25 µm) anchored at the edges. Solarization was maintained for 14 days (Day 0 to Day 14) during the dry-warm season in the month of November 2023, to

maximize solar irradiance capture. Samples were collected from both plots at Day 0 (pre-solarization baseline) and Day 14 (post-solarization) using a sterile soil auger and transferred into pre-sterilized sampling polythene bags. All samples were processed within 24 hours of collection under aseptic conditions.

2.2 Preparation of Culture Media

All bacteriological media were prepared in accordance with the manufacturer's instructions (Oxoid Ltd., UK). Nutrient Agar (NA) and MacConkey Agar (MCA) were prepared, autoclaved at 121°C for 15 minutes, cooled to 45–50°C, and dispensed aseptically into sterile Petri dishes. Differential and selective media included: Chromogenic Bacillus cereus Agar with selective supplement, Sorbitol MacConkey Agar with Cefixime-Tellurite Supplement, Eosin Methylene Blue (EMB) Agar, Pseudomonas Cetrimide Agar, Salmonella-Shigella Agar, Mannitol Salt Agar, and Triple Sugar Iron (TSI) Agar (Omoregie *et al*., 2025; Ngozi *et al*., 2025).

2.3 Serial Dilution, Enumeration, and Isolation

Twenty-five grams of each soil sample was suspended in 225 mL sterile saline water (0.85% NaCl, sterile saline water (SSW)) and homogenized by mixing to yield a stock of 10^{-1} dilution. Serial ten-fold dilutions were prepared by transferring 1 mL aliquots sequentially into 9 mL SSW to obtain dilutions of 10^{-1} to 10^{-6}. Inocula (0.1 mL) from the 10^{-4} dilution were plated onto Nutrient Agar (for total heterotrophic bacteria) using the pour plate technique. Plates were incubated at 28 ± 2°C for 24–48 hours. Colony-forming units per gram of dry soil (CFU/g) were calculated according to Willey *et al*. (2008) using the formula below:

$$\frac{CFU}{g}\ of\ Soil\ = \frac{Number\ of\ colonies\ counted\ x\ Dilution\ used}{volume\ plated\ or\ volume\ of\ inoculum}$$

Morphologically distinct colonies were sub-cultured onto Tryptone Soya Agar (TSA) and incubated at 28 ± 2°C for 24 hours to obtain pure cultures for further characterization (Omoregie *et al*., 2020).

2.4 Identification of Bacterial Isolates

The isolates were subjected to cultural, morphological, and biochemical characterization using standard protocols specified in the Oxoid Manual (Bridson, 2006) and according to Holt *et al*. (1994). Gram and spore staining were performed according to standard procedures. Biochemical tests included: catalase (3% H_2O_2), oxidase (1% tetramethyl-p-phenylenediamine), urease (Christensen's Urea Agar), indole formation (spot indole test with paradimethylaminocinnamaldehyde reagent), citrate utilization (Simmons Citrate Agar), sugar fermentation (glucose, sucrose, lactose, mannitol), 3% KOH string test, motility, gas and H_2S formation on Triple Sugar Iron Agar, and esculin hydrolysis. Identification was confirmed against the biochemical profiles described by Ngozi *et al*. (2025); Meziane *et al*. (2025) and Omoregie *et al*. (2025).

2.5 Plant Growth-Promoting Activity Assays

2.5.1 Indole Acetic Acid (IAA) Production

IAA production was evaluated using the Salkowski colorimetric method (Patten and Glick, 2002; Kumar *et al*., 2012). Briefly, the isolates were inoculated into Tryptic Soy Broth supplemented with 500 mg/L L-tryptophan (pH 6.0, buffered with 1 g/L MES hydrate) and incubated at 30°C for 72 hours

on a rotary shaker at 150 rpm. Cultures were centrifuged at 3000 rpm for 15 minutes. One millilitre of supernatant was mixed with 2 mL Salkowski reagent (50 mL 35% perchloric acid + 1 mL 0.5 M $FeCl_3$) and incubated at room temperature for 25 minutes. Development of a pink-to-red colour was scored as positive for IAA production.

2.5.2 Nitrogen Fixation Activity

The ability to fix atmospheric nitrogen was assessed by streaking a 24-hour culture onto Jensen's Nitrogen-Free Medium (NFM) comprising: 20 g/L sucrose, 1 g/L K_2HPO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L NaCl, 0.1 g/L $FeCl_3$, 0.005 g/L $Na_2MoO_4 \cdot 2H_2O$, 2 g/L $CaCO_3$, and 15 g/L agar. Plates were incubated at 28°C for up to 7 days. Growth on the nitrogen-deficient medium was recorded as positive for nitrogen fixation (Omoregie *et al*., 2020; Weselowski *et al*., 2016).

2.5.3 Phosphate Solubilization and Solubilization Index (SI)

Phosphate solubilization was assessed using Pikovskaya's agar (Micromaster). Isolates were point-inoculated in triplicate and incubated at 30°C for 3 days. A clear halo-zone around the colony indicated tricalcium phosphate solubilization. The Phosphate Solubilization Index (SI) was calculated according to Doilom *et al*. (2020) using the formula below:

$$Solubilization\ Index\ (SI) = \frac{Colony\ diameter + Halozone\ diameter}{Colony\ diameter}$$

Where SI values greater than 1 indicate positive solubilization activity; higher values represent greater solubilization capacity (Omoregie *et al*., 2020).

2.6 Molecular identification (16S rRNA gene sequencing)

Genomic DNA was extracted by the boiling method (Chakravorty *et al*., 2007). Briefly, a 2-ml aliquot of an overnight broth culture was centrifuged, the pellet resuspended in 200 µl of sterile distilled water, heated at 100 °C for 15 min and clarified by centrifugation; the supernatant served as the DNA template. The 16S rRNA gene was amplified using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1540R (5′-TACGGYTACCTTGTTACGACT-3′) on a GeneAmp 9700 thermal cycler (Applied Biosystems). Cycling comprised initial denaturation at 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 50 °C for 60 s and 72 °C for 90 s; and a final extension at 72 °C for 10 min. Amplicons (1.5 kb) were resolved on 1.5% agarose gel, purified by ethanol precipitation, and sequenced on a 3130xl Genetic Analyzer (Applied Biosystems). The sequences obtained were compared with the NCBI GenBank database using the BLAST algorithm, and each isolate was assigned to the species giving the highest query coverage and sequence identity.

2.7 Statistical Analysis

Phosphate solubilization index (PSI) data from 16 paired pre- and post-solarization samples were compared using the Wilcoxon signed-rank test. The test sample size (n = 16) refers to paired soil samples and is independent of the number of microbial taxa identified during community characterization. Statistical significance was set at $\alpha = 0.05$. All analyses were performed in R version 4.5.1. Visualization of distribution patterns was produced using ggplot2 (Wickham, 2016) and ComplexHeatmap (Gu *et al*., 2016) packages within R-Studio, following the visualization patterns employed for microbiological data (Ogofure and Green, 2025; Ogofure *et al*., 2025a; Ogofure *et al*., 2025b; Serepa-Dlamini *et al*., 2025).

3. Results and Discussion

3.1 Biochemical and Molecular Identification of Bacterial Isolates

The cultural, morphological, and biochemical characteristics of the bacterial isolates recovered from non-solarized soil (Day 0) revealed five taxa: *Bacillus mycoides*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Enterobacter cloacae* (Table 1). Gram-positive isolates (*B. mycoides*, *B. subtilis*, *S. aureus*) appeared as purple-staining rod (for the *Bacillus* spp.) or cocci (*Staphylococcus* spp.) forms under the microscope, with positive endospore staining recorded for the two Bacillus taxa. Gram-negative isolates (*E. aerogenes*, *E. cloacae*) produced pink-staining rods with negative spore staining.

Table 1a. Cultural, morphological and biochemical characteristics of bacterial isolates from non-solarized soil (Day 0)

Parameters					
Elevation	Flat	Flat	Raised	Flat	Flat
Margin	Coarse	Undulate	Smooth	Undulate	Undulate
Colour	Milk white	Cream	Cream	Cream	Cream
Size	Large	Large	Small	Large	Large
Selective agar	BCA	BCA	MSA	EMB	EMB
Gram stain	+	+	+	–	–
Cell type	Rod	Rod	Cocci	Rod	Rod
Arrangement	Dispersed	Dispersed	Clusters	Dispersed	Dispersed
Spore staining	+	+	–	–	–
KOH/String Test	–	–	–	+	+
Catalase	+	+	+	+	+
Indole	–	–	–	–	–
Citrate	–	+	+	+	+
Oxidase	–	–	–	–	–
Motility	+	+	–	+	+
Urease	–	–	+	–	–
Glucose	+	+	+	+	+
Sucrose	–	+	+	+	+
Lactose	–	+	+	+	+
Mannitol	–	+	+	–	–
Gas formation	–	–	–	–	+
H_2S formation	–	–	–	–	–
TSI (Slant/Butt)	K/A	A/A	A/A*	A/A(K*)G*	A/AG
Esculin hydrolysis	+	–	–	+	*(+/–)*
Tentative Identity	*B. mycoides*	*B. subtilis*	*S. aureus*	*E. aerogenes*	*E. cloacae*

BCA = *Bacillus cereus* agar; MSA = Mannitol salt agar; EMB = Eosin methylene blue agar; K = alkaline; A = acid; G = gas; * = reaction variable.

The identities of all isolates were confirmed using 16S rRNA with query coverage and percentage identity exceeding 97%.

Table 1b. Molecular identification of bacterial isolates from soil before solarization.

Code	Bacterial Identity	Query Cover (%)	Identity (%)	Accession Number
Usp1	*Staphylococcus aureus*	99.00	99.86	EF463060.1
Ssp3	*Enterobacter aerogenes*	99.00	99.87	MN190246.1
Ssp4	*Enterobacter cloacae*	99.00	99.67	NR_118011.1
Ssp2	*Bacillus mycoides*	99.00	98.79	EU660365.1
Ssp1	*Bacillus subtilis*	99.00	99.23	MK14580.1

3.2 Plant Growth Promoting Properties of Isolates

All five isolates were screened for nitrogen fixation, IAA production, and phosphate solubilization pre-and post-solarization (Figure 1). *B. mycoides, B. subtilis, E. aerogenes,* and *E. cloacae* were positive for all three PGPB traits. *Staphylococcus aureus* was negative for all three assays, consistent with its classification as a human-adapted pathogen rather than a soil-adapted PGPB. Following 14-day solarization, only four bacterial taxa were recovered from treated soil: *B. mycoides, B. subtilis, E. aerogenes,* and *E. cloacae* (Figure 1). *Staphylococcus aureus* was absent from all post-solarization plates, representing complete elimination from the detectable community. The biochemical profiles and morphological characteristics of the four surviving taxa were consistent with their pre-solarization identifications, confirming species persistence rather than recolonization.

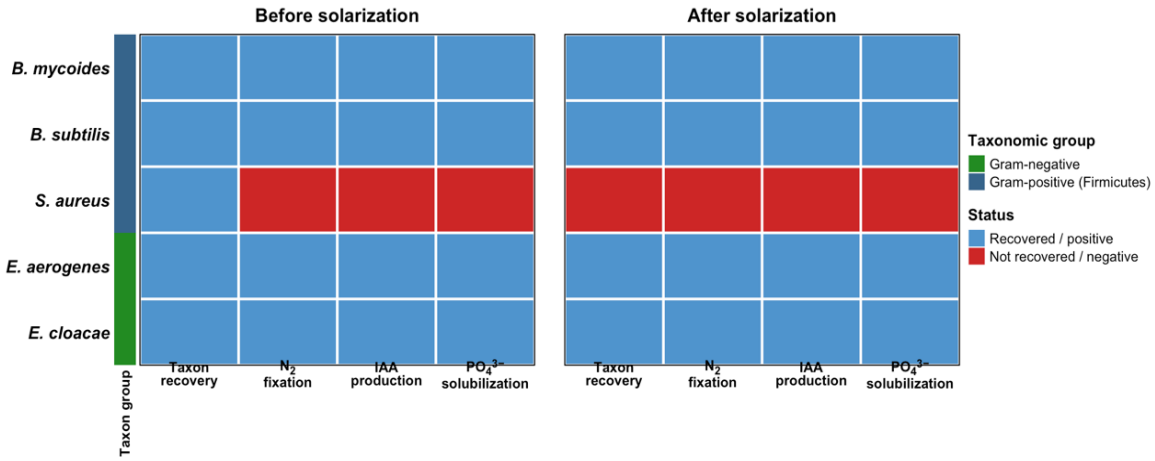

Figure 1. Bacterial recovery and PGP-trait distribution before and after solarization

All four surviving isolates retained full PGPB activity post-solarization (Figure 1) demonstrating that solarization did not remove the functional PGP capacity of surviving taxa. The complete elimination of *S. aureus*, the sole non-PGPB isolate (in our study), from the post-solarization community means that 100% of recoverable bacteria after treatment exhibited all three PGPB traits.

The quantified phosphate solubilization indices (SI) increased for all four PGPB taxa following solarization (Figure 2). *B. subtilis* demonstrated the highest absolute SI in both pre- and post-solarization conditions (SI = 10.0 and 12.0, respectively), followed by *E. cloacae* (4.0 and 5.0), *E. aerogenes* (3.0 and 5.0), and *B. mycoides* (2.0 and 4.0). The proportional increase in SI was greatest

for *B. mycoides* (100% increase) and *E. aerogenes* (67% increase). Wilcoxon signed-rank analysis (W = 10, p = 0.028) confirmed that the increase in SI across all four taxa was statistically significant. Visualization of the distribution of PGPB traits and SI values is shown in Figure 2.

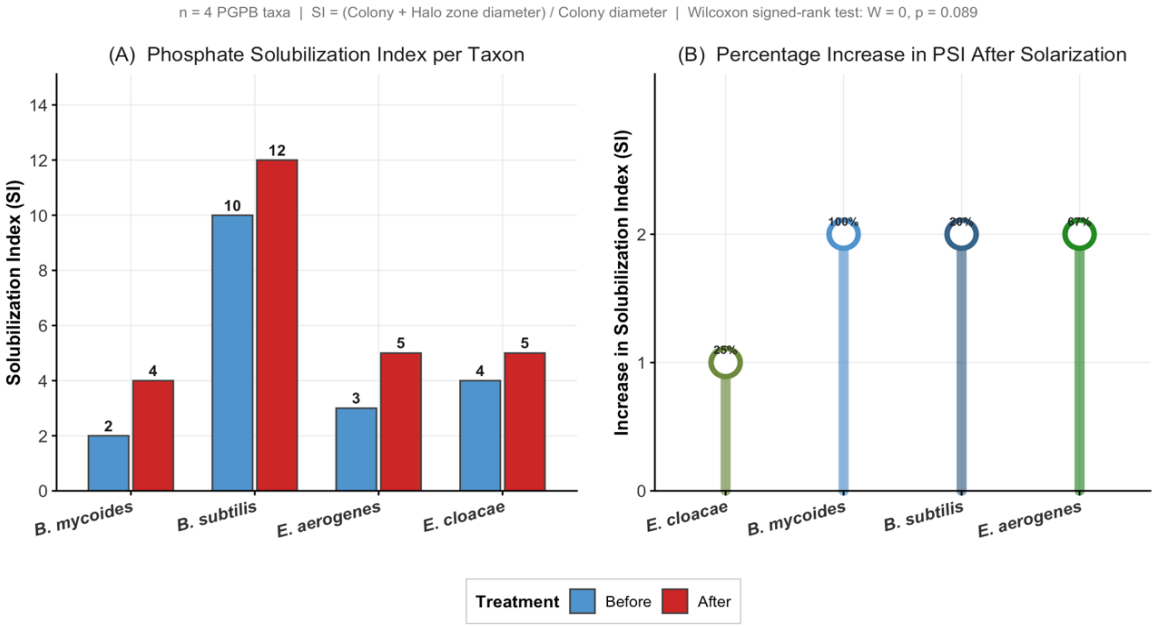

Figure 2 A & B. Phosphate solubilization index before and after soil solarization with solubilization index per taxon (A) and Percentage increase in PSI after solarization.

This study provides proof-of-concept that a two-week soil solarization applied to tropical botanical garden soil selectively eliminates heat-sensitive pathogenic bacteria while preserving and functionally enriching a community of plant growth-promoting taxa. The five pre-solarization isolates, *B. mycoides*, *B. subtilis*, *S. aureus*, *E. aerogenes*, and *E. cloacae*, encompass both thermolabile (*S. aureus*) and thermotolerant (*Bacillus* spp.) taxa, making the system an informative model for examining solarization-mediated community restructuring. The complete elimination of *S. aureus* from post-solarization soil is consistent with the literature, as Wu *et al.* (2009) reported that "sustained soil heating can drive pathogens to undetectable levels, and solar systems become much more lethal once temperatures reach roughly 50–60°C or higher". The thermal death studies of *S. aureus*, as documented by Kennedy *et al.* (2005), specify a time to 90% kill of approximately 3–5 minutes at 60°C and 15–20 minutes at 50°C. Given that soil temperatures during solarization in tropical climates readily exceed 50°C in the uppermost 10 cm for several hours daily, the plausibility of elimination of *S. aureus* over a 14-day period is physiologically expected. The ecological significance of this elimination is twofold: *S. aureus* is a recognised food safety contaminant on crop surfaces (Khan *et al.*, 2023), and its suppression in the plant root environment represents a non-chemical food safety benefit of solarization that has been inadequately highlighted in prior literature.

The survival and persistence of *B. mycoides*, and *B. subtilis* are consistent with their characterized heat resistance mechanisms. *Bacillus* species produce dipicolinic acid-containing endospores with decimal reduction temperatures (D_{121}) well above those of vegetative bacteria, conferring survival at temperatures that routinely cause logarithmic kill of non-spore-formers (Setlow, 2006; Setlow *et al.*, 2006). The persistence of *Enterobacter* species in solarized soil is less intuitive, as these Gram-negative organisms do not form endospores. However, *Enterobacter* spp. may occupy deeper, cooler soil micro-niches below the lethal temperature threshold during solarization, and their recovery post-treatment

may reflect both survival and rapid recolonization from protected pockets, a pattern consistent with Omotayo and Eegunranti (2023) who documented the rapid recolonization of solarized soil by Enterobacter following soil solarization on tomato growth. Furthermore, evidence from (Simmons *et al*., 2013) supports the plausible explanation of *Enterobacter* species recolonization following solarization even though it is not a direct demonstration of *Enterobacter* during solarization.

The universal PGPB activity demonstrated by all four surviving taxa is agronomically significant. Nitrogen fixation capacity in free-living soil bacteria reduces dependence on synthetic nitrogen fertilizers; phosphate solubilization increases the bioavailability of the substantial pool of fixed phosphorus in tropical soils; and IAA production directly promotes root proliferation and thereby enhances nutrient and water uptake (Patten and Glick, 2002; Omoregie *et al*., 2020; Timofeeva *et al*., 2023; Isagba *et al*., 2023). The functional redundancy across four PGPB taxa occupying distinct ecological niches (endospore-forming Gram-positive Bacillaceae versus non-sporing Gram-negative Enterobacteriaceae) suggests that the post-solarization PGPB community has robust functional resilience. The statistically significant increase in phosphate solubilization indices following solarization (Wilcoxon $W = 10$, $p = 0.028$) is consistent with mechanistic expectations. Solarization releases soluble mineral nutrients from heat-killed soil biota and thermally mineralised organic matter (Khan *et al*., 2023; Gebreegziher, 2024). The resulting nutrient flush, combined with reduced competition from non-surviving microorganisms, creates conditions that may stimulate metabolic investment in phosphate-solubilizing enzyme systems by surviving PGPB, particularly the synthesis of gluconic acid and organic acid mixtures that drive dissolution of calcium- and iron-bound phosphate complexes. Etubu and Osobase (2018) similarly documented elevated phosphate solubilization activity in post-solarization soils and attributed the increase to competitive release and temperature-driven enhancement of microbial metabolic rates in surviving thermo-tolerant taxa.

One limitation of this study is the relatively short solarization period (14 days). Although this duration represents the minimum effective treatment period typically used in tropical environments, it may have underestimated the extent of microbial community restructuring that could be achieved under the recommended 4–6 weeks of solarization treatment (Stapleton, 2000). Enumeration was limited to culturable bacteria on non-selective media; culture-independent methods (metagenomics) would provide a more complete picture of community diversity changes. The small sample size ($n = 2$ sampling points, one plot per treatment) precludes rigorous inferential statistics on community-level diversity indices; future studies should incorporate replicated plots and multiple sampling time points. Nevertheless, the qualitative consistency of results, elimination of *S. aureus* and retention of all four PGPB taxa across replicate plates provides confidence in the main conclusions.

Conclusion

This study demonstrates that two-week soil solarization applied to tropical garden soil selectively eliminates the plant growth-neutral bacterium *Staphylococcus aureus* while preserving a community of functionally active PGPB including, *Bacillus* and *Enterobacter* species. All four surviving taxa demonstrated positive nitrogen fixation, IAA production, and phosphate solubilization activities. Phosphate solubilization indices increased significantly across all four surviving taxa following solarization ($p = 0.028$), suggesting that solarization not only preserves but functionally enhances the PGPB community. These findings support the integration of soil solarization into sustainable tropical soil management strategies.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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