

Integrated organic amendments and plant growth-promoting rhizobacteria improve soil fertility and sugarcane growth and yield components under rainfed conditions in Northeast Thailand

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ARTICLE INFO

Received on: 10 March 2026
Accepted on: 14 July 2026
Available Online: XX

Key words:

Plant growth-promoting rhizobacteria,
Organic amendments, Sugarcane,
Rainfed agriculture, Soil fertility,
Northeast Thailand.

ABSTRACT

Sandy soils and unpredictable rainfall are the primary constraints on sugarcane productivity in the Tung Kula Ronghai region of Northeast Thailand. This field study examined the effects of integrated organic amendments combined with plant growth-promoting rhizobacteria (PGPR) on soil fertility, sugarcane growth, and yield components under rainfed conditions. Seven treatments were arranged in a randomized complete block design with three replications during the 2023–2024 growing season (June 2023–March 2024). The integrated treatment (T4) combining poultry manure (3 t/ha), rock phosphate (500 kg/ha), rice bran (100 kg/ha), agricultural lime (200 kg/ha), leonardite (200 kg/ha), and indigenous PGPR inoculant (presumptive *Bacillus* isolate BS-01 and presumptive *Azotobacter* isolate AZ-03 at 10^8 CFU/ml; genus-level identification only; see Section 4) achieved an estimated theoretical yield of 69,911 kg/ha in variety Suphanburi 50. A key finding was a twofold difference in PGPR-associated yield response between the two manure substrates: yield was 91.0% higher with PGPR than without in the chicken manure system (T4 vs. T5, $p < 0.001$, Cohen's $d = 10.35$), compared with a 45.3% increase in the buffalo manure system (T6 vs. T7, $p = 0.015$, $d = 3.36$). Notably, PGPR-associated soil chemistry improvements were nearly identical across both manure systems (available nitrogen: +22.6 vs. +22.0 mg/kg; pH: +0.333 vs. +0.433), suggesting that the differential yield response may be related to substrate-dependent differences in how soil fertility gains were converted into biomass, rather than from differential soil chemical effects of PGPR. Soil chemical properties also improved substantially under T4: pH rose from 4.20 to 5.53, organic matter from 4.10 to 4.73 g/kg (pre-treatment plot mean), available phosphorus by 115%, and available potassium by 56%. The estimated theoretical yield under T4 (derived from stalk density and mean stalk weight) was 13.6% greater than that with chemical fertilizer (61,553 kg/ha) and 185.5% greater than that with the unfertilized control (24,490 kg/ha), supporting further evaluation of this integrated treatment under degraded tropical sandy-soil conditions. Confirming economic viability and regional applicability across the broader 3,370 km² Tung Kula Ronghai area will require multi-location, multi-season validation and formal cost-benefit analysis.

1. INTRODUCTION

Sugarcane (*Saccharum* spp.) is one of Thailand's most important agricultural commodities, with production concentrated in the Central and Northeast regions [1]. Thailand is the world's second-largest sugar exporter, with sugarcane grown on ~1.4 million hectares and annual production exceeding 100 million tonnes [2]. The sector supports around 300,000 farm families and is a major contributor to rural incomes and national economic development [3].

Northeast Thailand covers ~170,000 km² and is home to roughly one-third of the national population, most of whom depend on agriculture

[4]. Productivity is constrained by poor soil quality, erratic rainfall, and limited irrigation infrastructure [5]. These constraints are particularly acute in the Tung Kula Ronghai area, a 3,370 km² lowland spanning Roi Et, Maha Sarakham, Surin, Sisaket, and Yasothon provinces, where highly weathered sandy soils exhibit low water retention, poor nutrient-holding capacity, and strongly acidic pH [6].

Soils in this region typically contain 60%–80% sand with little clay, which accelerates nutrient leaching and reduces cation exchange capacity [7]. pH values commonly fall below 5.5, organic matter rarely exceeds 1%, and both available phosphorus and potassium are deficient [8]. Average annual rainfall is 1,200–1,400 mm, but 80%–85% falls within the monsoon season (May–October), leaving crops exposed to prolonged dry periods from November through April [9].

Farmers in Northeast Thailand have traditionally depended on chemical fertilizers to offset inherent soil fertility deficits [10], but

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prolonged synthetic fertilizer use without organic matter inputs has degraded soil biological activity and eroded long-term productivity [11]. High input costs can further constrain smallholders' ability to apply fertilizers at recommended rates.

Organic amendments offer a practical alternative by improving soil physical, chemical, and biological properties simultaneously [12]. Poultry manure is readily available in Northeast Thailand, where commercial broiler and layer operations generate large volumes of manure that are widely used as organic amendments by smallholder farmers in the region. Poultry production is substantial in Northeast Thailand [13], providing a potential regional source of poultry manure for agricultural use. Poultry manure supplies appreciable quantities of nitrogen, phosphorus, and organic matter. Rock phosphate provides a slowly released phosphorus source well suited to acidic soils [14]. Leonardite (oxidized lignite) is a natural source of humic and fulvic acids that enhance cation exchange capacity, nutrient availability, and soil aggregate formation [15].

Plant growth-promoting rhizobacteria (PGPR) are a functionally diverse group of root-associated bacteria that stimulate plant growth through mechanisms including biological nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and induced systemic resistance [16]. Among PGPR genera, *Bacillus* is considered particularly suitable for field inoculant formulation because its ability to form metabolically dormant endospores confers resistance to heat, desiccation, UV radiation, and other environmental stresses that would otherwise limit inoculant survival in the field and during storage [17]. Field studies confirm that *Bacillus*-based PGPR inoculants can improve sugarcane growth and yield under practical agronomic conditions [18]. *Azotobacter* contributes complementary nitrogen fixation capacity that can reduce reliance on synthetic N inputs [19].

Combining organic amendments with PGPR inoculation has produced synergistic growth responses across a range of cropping systems, including studies that explicitly compared PGPR-alone, organic amendment-alone, and combined treatments, and Combining organic amendments with PGPR inoculation has been reported to provide agronomic benefits across a range of cropping systems [16]. Organic amendments may improve the soil environment for PGPR establishment by providing carbon sources and favorable microsites, whereas PGPR can contribute to nutrient cycling through mechanisms that vary with amendment type, microbial community composition, and soil conditions. These interactions have been documented in studies that compared PGPR-alone, organic amendment-alone, and combined application treatments under field conditions [20,21]. Despite the expanding global literature on PGPR–organic amendment combinations, however, three important knowledge gaps remain. First, based on our review of the available literature, we did not identify any field trial that has simultaneously tested poultry manure, buffalo manure, rock phosphate, leonardite, and lime as a combined input package—with and without PGPR—in rainfed sugarcane; the closest study from Northeast Thailand [22] evaluated PGPR inoculation under rainfed conditions but did not include this complete amendment combination. Second, most PGPR–sugarcane research has been carried out under irrigation or on more fertile soils, leaving the performance of these inputs on the degraded sandy Ultisols of the Tung Kula Ronghai region largely unknown. Third, although a recent study in neighboring Khon Kaen Province showed that bio-organic amendments combined with phosphate-solubilizing bacteria can support competitive sugarcane yields [23], that trial did not include lime-based pH amelioration or leonardite—inputs likely to be particularly important on the strongly acidic ($\text{pH} < 4.5$) sandy soils of this region. Together, these gaps hamper the ability of extension

services to develop evidence-based management recommendations for smallholder sugarcane farmers in the Tung Kula Ronghai region.

The present study addresses these gaps with a seven-treatment randomized complete block field experiment conducted in the Tung Kula Ronghai region under natural rainfed conditions. The design included targeted pairwise comparisons between PGPR-inoculated and non-inoculated treatments within each amendment background (T4 vs. T5; T6 vs. T7), allowing the PGPR-associated response to be estimated against an identical amendment background. The test cultivar was Suphanburi 50, bred specifically for rainfed conditions in Northeast Thailand and widely grown by smallholders [24]. Both PGPR isolates were sourced from sugarcane rhizosphere soils in Sisaket Province, ensuring ecological relevance to the target environment. All organic inputs evaluated—poultry manure, buffalo manure, rock phosphate, agricultural lime, rice bran, and leonardite—are commercially available through agricultural supply channels serving Northeast Thailand [25], so that positive findings can translate directly into actionable extension recommendations.

The present study differs from prior work in three specific ways. First, it was conducted under rainfed conditions after a 2-week establishment irrigation period at a farm-representative site within the Tung Kula Ronghai region, using locally relevant soils, rainfall patterns, and varieties. Second, the seven-treatment field design incorporated pre-specified within-amendment PGPR contrasts (T4 vs. T5; T6 vs. T7), allowing PGPR-associated responses to be estimated within identical amendment backgrounds; such paired within-amendment contrasts remain limited in the published sugarcane literature for this soil environment. From those of the amendment substrate through targeted pairwise comparisons (T4 vs. T5; T6 vs. T7) using independent-samples t-tests the first such comparison reported for sugarcane on this soil type. Third, the entire amendment package consists of commercially available inputs, so positive outcomes can be translated into extension advice without materials beyond the reach of smallholder farmers. To our knowledge, field trials for sugarcane in this agroclimatic zone that combine all three of these features remain limited. Two specific hypotheses were tested: (1) that integrated organic-PGPR management would improve soil chemical properties (pH, OM, AN, AP, and AK) relative to chemical fertilization and non-inoculated organic amendment controls; and (2) that the PGPR-associated yield response would differ between poultry and buffalo manure substrates even at identical PGPR inoculant rates, thereby testing whether substrate quality modulates PGPR field performance a question that requires within-amendment paired contrasts and cannot be resolved by simpler experimental designs. The specific objectives were to: (1) evaluate how integrated organic-PGPR treatments affect soil chemical properties under field conditions; (2) quantify sugarcane growth and yield component responses to different management approaches; and (3) estimate the yield response associated with PGPR inoculation within each amendment system through planned contrasts (T4 vs. T5; T6 vs. T7), with the caveat that estimate the response associated with PGPR inoculation within each amendment system through planned contrasts (T4 vs. T5; T6 vs. T7), while recognizing the absence of direct colonization and mechanistic measurements (Section 4).

2. MATERIALS AND METHODS

2.1. Experimental Site and Design

The experiment was carried out during the 2023–2024 growing season (June 2023–March 2024) at the Agricultural Research Station

of Sisaket Rajabhat University, Yang Chum Noi District, Sisaket Province, northeast Thailand (15°6'N, 104°19'E; elevation 142 m a.s.l.). The experimental site is located in Yang Chum Noi District, near the boundary with Rasi Salai District, within the broader area administratively associated with Tung Kula Ronghai and has coarse-textured soils and strongly seasonal rainfall representative of the broader northeast Thailand agroclimatic zone. Treatments were arranged in a randomized complete block design with three replications, giving 21 experimental units in total. Each plot measured 6 × 8 m (48 m²), with 2 m buffer strips between adjacent plots to reduce inter-plot movement of nutrients and water.

2.2. Soil Characterization

Before treatment application and planting, composite soil samples were collected from 0 to 30 cm depth across the experimental area to characterize baseline soil chemical properties. The soil was classified as sandy loam (64% sand, 24% silt, and 12% clay by hydrometer method) and is classified, according to the USDA Soil Taxonomy, as a with low organic matter and low available nutrient status, indicating a highly weathered, nutrient-poor soil at the study site. This classification is consistent with the highly weathered, low-fertility sandy soils widely described for the Tung Kula Ronghai zone [6]. Baseline chemical properties were: pH 4.22 (1:2.5 soil:water; plots later assigned to T4 averaged 4.20, consistent with the treatment-specific pH change reported in Section 3.1), organic matter 4.11 g/kg (Walkley-Black chromic acid oxidation, OM = SOC × 1.724), available nitrogen 39.4 mg/kg (alkaline permanganate extraction), available phosphorus 5.5 mg/kg (Bray-2 extraction), and exchangeable potassium 45.4 mg/kg (1N ammonium acetate extraction with flame photometry). These values are means of pre-treatment measurements across all 21 plots before randomized treatment assignment. These baseline values are consistent with those widely reported for degraded sandy soils in the Tung Kula Ronghai zone, confirming that the site is representative of the broader regional context.

2.3. Experimental Treatments

The seven treatments were: T1, unfertilized control; T2, chemical fertilizer (15-15-15 NPK at 312.5 kg/ha + urea 46-0-0 at 125 kg/ha + muriate of potash 0-0-60 at 62.5 kg/ha); T3, commercial organic fertilizer at the manufacturer's recommended rate; T4, integrated organic-mineral-biological package (poultry manure 3 t/ha + rock phosphate 500 kg/ha + rice bran 100 kg/ha + agricultural lime 200 kg/ha + leonardite 200 kg/ha + PGPR inoculant); T5, identical to T4 but without PGPR; T6, buffalo manure 3 t/ha + rock phosphate 500 kg/ha + rice bran 100 kg/ha + agricultural lime 200 kg/ha + leonardite 200 kg/ha + PGPR inoculant; and T7, identical to T6 but without PGPR. This structure allowed both the effect of manure source (poultry vs. buffalo) and the specific contribution of PGPR inoculation to be examined through targeted pairwise comparisons (T4 vs. T5; T6 vs. T7). Treatment names, application rates, and the chemical fertilizer sentence. Chemical fertilizers were purchased from commercial suppliers according to standard formulations widely available in the region.

2.4. PGPR Strains and Inoculum Preparation

The PGPR inoculant comprised two bacterial isolates: BS-01 (provisionally designated *Bacillus subtilis* based on morphological and biochemical characterization, pending 16S rRNA confirmation) and AZ-03 (provisionally designated *Azotobacter* sp. on the same basis), both originally isolated from sugarcane rhizosphere soil in Sisaket

Province and maintained in the Applied Microbiology Laboratory at Sisaket Rajabhat University. Species-level molecular identification (16S rRNA sequencing), GenBank submission, public culture collection deposition, biosafety classification, and direct quantification of plant growth-promoting traits were not performed for BS-01 or AZ-03; this constitutes a substantive methodological limitation that restricts the reproducibility of the inoculation component and is addressed in Section 4. Genus-level selection was based entirely on published literature for *Bacillus* and *Azotobacter*; no trait data specific to BS-01 or AZ-03 were measured or confirmed in this study. These genera were selected on the basis of well-documented plant growth-promoting functions reported among PGPR [16]; these functions were not directly demonstrated for BS-01 or AZ-03 genus-level evidence for each trait in sugarcane-associated and other agricultural contexts is reviewed in those cited sources. Compatibility between the two strains was assessed by a cross-streak antagonism assay on nutrient agar (30°C, 48 hours). No zone of inhibition was observed at the streak intersection, indicating no detectable antagonism under the assay conditions.

Each strain was cultured separately in nutrient broth at 30°C for 48 hours on an orbital shaker at 150 rpm, reaching exponential-phase cell densities exceeding 10⁹ CFU/ml. After independent verification of growth and purity, equal volumes of the two cultures were combined and diluted with sterile distilled water to a final working concentration of 10⁸ CFU/ml. This combined consortium was used for all field applications (seed treatment, in-furrow drench, and foliar spray). Quality control comprised viable cell counts by serial dilution plating on nutrient agar, microscopic examination for morphological confirmation and contamination, and purity verification on selective media.

PGPR was applied using multiple delivery routes intended to increase inoculum exposure to the planting material and surrounding soil. First, planting material (setts) was immersed in inoculant suspension (10⁸ CFU/ml) for 30 minutes immediately before planting. Second, 200 ml of concentrated inoculant was applied as an in-furrow drench per planting station at the time of planting. Third, foliar sprays of diluted inoculant (1:100, 500 l/ha) were applied at 30 and 60 days after planting. Foliar sprays of diluted inoculant (1:100, 500 l ha⁻¹) were applied at 30 and 60 days after planting as an additional inoculum-delivery route. Although applied simultaneously at planting, sett immersion and in-furrow drench serve distinct purposes: sett immersion delivers inoculant directly onto the cane surface to facilitate early inoculum contact of emerging bud roots, while the larger-volume drench inoculates the surrounding to distribute inoculum into the surrounding rhizosphere soil as the root system expands.

2.5. Plant Material and Crop Management

The test cultivar was Suphanburi 50, a commercial variety officially released by Thailand's Sugarcane and Sugar Research Institute and selected for its adaptation to the rainfed production conditions of northeast Thailand. The variety was chosen for its rainfed adaptability, moderate drought tolerance, and acceptable commercial sucrose content under the variable rainfall of northeast Thailand [24]. Planting material (stem cuttings) was obtained from a local planting-material source and pre-sprouted under ambient shade to ensure uniform establishment across plots.

Field preparation consisted of primary moldboard plowing to 30 cm followed by secondary harrowing to produce a well-structured seedbed. Organic amendments and mineral supplements were broadcast uniformly within each treatment plot and mechanically

incorporated to ~15–20 cm depth 2 weeks before planting to allow initial decomposition and chemical equilibration. Planting began on 15 June 2023, coinciding with the onset of the monsoon season. Two-bud stem cuttings were placed manually in furrows at 1.5 m between rows and 0.5 m within rows, giving a theoretical density of ~13,333 setts per hectare. This density is consistent with plant-cane densities used in research trials in this region.

After a 2-week establishment period with supplemental irrigation, the crop received no further artificial water inputs and relied entirely on natural monsoon rainfall for the remainder of the growing season (~8.5 months to the March 2024 harvest). Because soil moisture was not instrumentally monitored, growing conditions are best described as rainfed-managed rather than as a quantified drought or stress treatment (Section 4). Meteorological data were obtained from the Sisaket Meteorological Station of the Thai Meteorological Department (TMD), located ~15 km from the experimental site. Official daily rainfall records were obtained from the Sisaket Meteorological Station, Thai Meteorological Department. Official daily rainfall records from the TMD were used to calculate cumulative rainfall during the experimental period (June 2023–March 2024): 1,247 mm over 87 rain days. On-site monitoring of soil moisture, rainfall intensity, and within-field spatial variability of rainfall was not conducted; consequently, while reliable daily rainfall totals were available, the effects of short-term rainfall events and soil water dynamics during the growing season could not be evaluated. This limitation is addressed in Section 4. Standard agronomic practices were applied uniformly across all plots to minimize confounding effects. Weeds were removed by hand at 30, 60, and 90 days after planting. Pest and disease incidence were assessed visually at 30, 60, 90, and 120 days after planting; no chemical interventions were necessary, as No pest or disease outbreaks requiring chemical intervention were observed during routine visual assessments.

2.6. Sampling Procedures and Analytical Methods

Post-harvest soil samples for chemical analysis were collected in March 2024, at approximately 9 months after planting when the crop had reached physiological maturity. From each plot, five randomly located points were cored to 30 cm depth using a bucket auger. The five subsamples per plot were composited, mixed, air-dried at ambient temperature, and sieved to 2 mm to remove coarse fragments and plant debris.

Corresponding 1N ammonium acetate extraction procedures were used for both baseline and post-harvest K measurements; detection instrumentation differed as described above: baseline K was measured by flame photometry; post-harvest K was measured by atomic absorption spectrophotometry. Specific analytical protocols included: soil pH determination in 1:2.5 soil:water suspension with glass electrode potentiometry [26]; soil organic matter (OM) quantification by Walkley-Black chromic acid wet oxidation method, with the measured organic carbon value converted to organic matter using the van Bemmelen factor ($OM = SOC \times 1.724$) [27]; available nitrogen by alkaline permanganate extraction with titration (as described for baseline characterization in Section 2.2); available phosphorus by Bray-2 dilute acid-ammonium fluoride extraction with molybdenum blue colorimetric determination [28]; and exchangeable potassium by neutral 1N ammonium acetate extraction with atomic absorption spectrophotometry [29].

Crop measurements were taken at physiological maturity (approximately 9 months after planting, March 2024). From each plot, 10 randomly selected stalks were measured for height (soil surface to uppermost dewlap node), mid-height diameter, and fresh stalk

weight using calibrated equipment. Juice Brix (%) was measured by a digital refractometer on fresh juice pressed from the 10 sampled stalks. Because pol analysis and fiber determination required for CCS calculation were beyond the scope of this study, Brix is used here as a proxy for juice soluble solids content only and should not be interpreted as equivalent to CCS. Theoretical cane yield per hectare was estimated from two components: stalk density (stalks m^{-2}), determined by complete stalk counts across the full plot, and mean fresh stalk weight from the 10-stalk sample. These were multiplied and scaled to a per-hectare basis. No whole-plot harvested yield was recorded; all yield comparisons therefore refer to estimated theoretical yield calculated from stalk density and stalk weight, not directly measured harvested biomass. This limitation is discussed in Section 4. Stalk density and mean stalk weight are primary yield components in their own right and are reported as such throughout.

2.7. Statistical Analysis

Treatment effects were evaluated using one-way ANOVA with treatment as the single fixed factor. The field experiment was arranged as a randomized complete block design with seven treatments and three blocks; however, block was not included as a factor in the fitted model corresponding to the reported omnibus results. Treatment means were separated using Tukey's HSD test at $p \leq 0.05$.

Targeted pairwise comparisons between the PGPR-inoculated and corresponding non-inoculated treatments within each amendment background (T4 vs. T5 and T6 vs. T7) were additionally evaluated using independent-samples t-tests based on the three plot-level replicates per treatment ($df = 4$). These pairwise tests were distinct from the omnibus ANOVA and did not use the ANOVA residual error term.

The targeted pairwise tests were not adjusted for multiplicity; consequently, their p-values should be interpreted cautiously together with the magnitude of the observed effects. were examined for all eleven response variables prior to ANOVA. Levene's and Shapiro-Wilk diagnostic statistics are reported in Supplementary Table S5. No correction for multiple comparisons was applied across the eleven response variables because each was treated as an independent, pre-specified agronomic outcome; within each variable, Tukey's HSD controls the family-wise error rate for all pairwise comparisons. Complete ANOVA outputs, targeted pairwise comparison results (independent-samples t-tests), and residual diagnostic statistics are provided in Supplementary Tables S3, S4, and S5, respectively. Note: the AP T6 vs. T7 pairwise p-value has been omitted from S4 as it could not be verified from the original output. The underlying datasets for Figures 1 and 2 are provided in Supplementary Tables S1–S3, and a full summary of statistical assumptions and modelling decisions is given in Supplementary Table S9.

3. RESULTS AND DISCUSSION

3.1. Effects on Soil Chemical Properties

All measured soil chemical properties responded significantly to treatment ($p < 0.001$; Table 1, Fig. 1). Soil pH was the most strongly affected parameter ($F[6,14] = 86.96$, $p = 2.9 \times 10^{-10}$, $\eta^2 = 0.974$). T4 (chicken manure + PGPR) reached 5.533 ± 0.033 , significantly higher than all treatments except T6 (5.400 ± 0.058 , buffalo manure + PGPR); these two formed the highest Tukey group. Independent-samples t-test comparisons showed higher AN in PGPR-inoculated treatments within both manure systems (chicken-manure system, T4 vs. T5: 92.3 vs. 69.7 mg kg^{-1} , $+22.7$

mg kg^{-1} , $p = 0.002$, $d = 5.70$, $df = 4$; buffalo-manure system, T6 vs. T7: 87.7 vs. 65.7 mg kg^{-1} , $+22.0 \text{ mg kg}^{-1}$, $p < 0.001$, $d = 7.62$, $df = 4$). Independent-samples t-test comparisons showed higher post-treatment pH in the PGPR-inoculated treatments than in their corresponding non-inoculated treatments in both amendment backgrounds. The mechanism underlying these differences was not directly investigated. Soil organic matter (OM = Walkley-Black $\text{SOC} \times 1.724$) was significantly affected by treatment ($F[6,14] = 24.69$, $p = 1.1 \times 10^{-6}$, $\eta^2 = 0.914$). Buffalo manure treatments

achieved the highest OM values (T6: 5.27 ± 0.15 , T7: $4.93 \pm 0.07 \text{ g kg}^{-1}$), exceeding chicken manure treatments (T4: 4.73 , T5: 4.50 g kg^{-1}). Differences in post-harvest OM between the buffalo- and poultry-manure treatments may reflect differences in manure composition and decomposition dynamics, including differences in C:N ratio and N availability [30,31]. However, manure C:N ratio and decomposition characteristics were not directly measured; this interpretation remains hypothetical. PGPR effects on OM were non-significant in both manure systems (chicken: $p = 0.091$; buffalo: $p = 0.105$), although the large effect sizes ($d = 1.70\text{--}1.81$) suggest

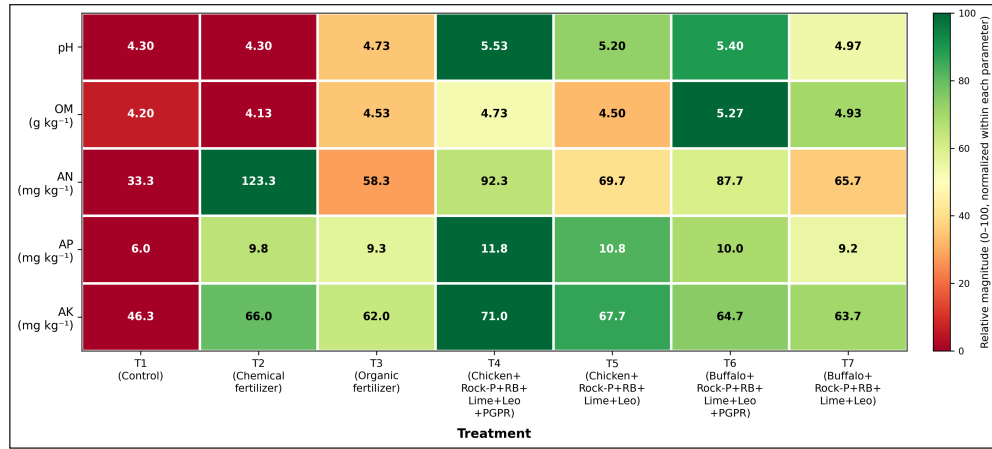


Figure 1. Heatmap visualization of post-treatment soil chemical properties (pH, organic matter [OM], available nitrogen [AN], available phosphorus [AP], and available potassium [AK]) across seven treatments. Cell values are treatment means ($n = 3$); color intensity is normalized independently within each parameter (row-wise, 0–100 scale). Raw data underlying this figure are provided in Supplementary Table S1. T1 = Control (no fertilizer); T2 = Chemical fertilizer (NPK); T3 = Organic fertilizer (commercial); T4 = Chicken manure + rock phosphate + rice bran + lime + leonardite + PGPR; T5 = Chicken manure + rock phosphate + rice bran + lime + leonardite (no PGPR); T6 = Buffalo manure + rock phosphate + rice bran + lime + leonardite + PGPR; and T7 = Buffalo manure + rock phosphate + rice bran + lime + leonardite (no PGPR).

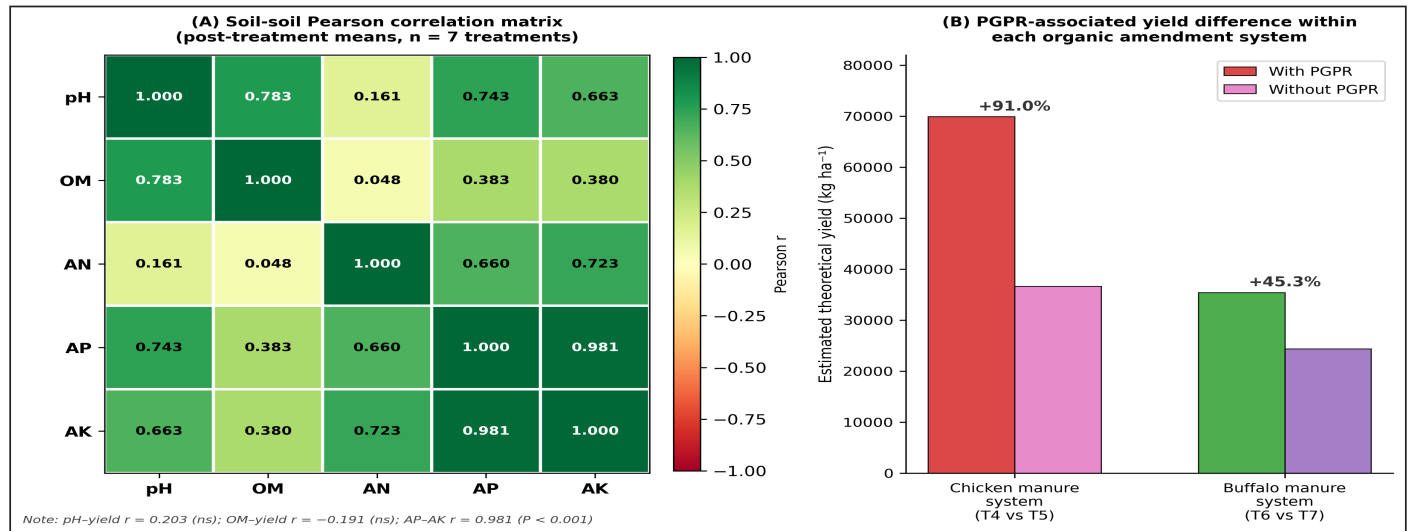


Figure 2. Combined analysis of soil–soil and soil–yield relationships. (A) Pearson correlation matrix among post-treatment soil chemical properties (pH, OM, AN, AP, and AK; $n = 7$ treatment means); raw correlation data are provided in Supplementary Table S2. (B) Bar chart comparing estimated theoretical yield with and without PGPR inoculation within each manure-based amendment system (chicken manure: T4 vs. T5; buffalo manure: T6 vs. T7), illustrating the substantially larger PGPR yield response in the chicken manure system; underlying yield data are provided in Supplementary Table S3. T1 = Control; T2 = Chemical fertilizer (NPK); T3 = Organic fertilizer (commercial); T4 = Chicken manure + rock phosphate + rice bran + lime + leonardite + PGPR; T5 = Chicken manure + rock phosphate + rice bran + lime + leonardite (no PGPR); T6 = Buffalo manure + rock phosphate + rice bran + lime + leonardite + PGPR; and T7 = Buffalo manure + rock phosphate + rice bran + lime + leonardite (no PGPR).

that $n = 3$ may have been insufficient to detect a true biological effect. The post-harvest OM of the unfertilized control (T1: 4.20 g/kg) was marginally above the site-mean baseline (4.11 g/kg). This difference of 0.09 g/kg is within the typical analytical variability for soil OM and most likely reflects natural spatial variation, root turnover, and litter fall over the 9-month growing period rather than a treatment response.

Available nitrogen (AN) showed highly significant treatment effects ($F[6,14] = 40.60$, $p = 4.6 \times 10^{-8}$, $\eta^2 = 0.946$). Chemical fertilizer (T2) yielded the highest AN (123.3 ± 8.8 mg kg⁻¹), reflecting the rapid availability of synthetic ammonium- and urea-based nitrogen compared with mineralization-dependent organic sources. PGPR inoculation significantly raised AN in both manure systems: chicken manure (T4 vs. T5: 92.3 vs. 69.7 mg kg⁻¹, $+22.7$ mg kg⁻¹, $p = 0.002$, $d = 5.70$) and buffalo manure (T6 vs. T7: 87.7 vs. 65.7 mg kg⁻¹, $+22.0$ mg kg⁻¹, $p < 0.001$, $d = 7.62$). Agronomically, the magnitude of these increases is notable: T4 and T6 formed a distinct Tukey group with AN values approaching those under chemical fertilizer, a pattern consistent with, though not direct evidence of, PGPR-stimulated mineralization and/or biological nitrogen fixation.

Available phosphorus (AP) showed the largest treatment effect of all parameters ($F[6,14] = 104.67$, $p = 8.1 \times 10^{-11}$, $\eta^2 = 0.978$). T4 achieved the highest AP (11.8 ± 0.2 mg kg⁻¹), representing a 115% increase over the pre-treatment baseline of 5.5 mg kg⁻¹. This improvement is consistent with lime-driven pH amelioration and, possibly, PGPR-associated phosphate solubilization [16]; however, phosphate-solubilizing activity of BS-01 and AZ-03 was not directly measured the well-documented effect of pH on phosphorus availability [32] is consistent with the observed pH-AP correlation ($r = 0.743$, $p = 0.056$, $n = 7$ treatment means), which did not reach conventional significance at this sample size but is directionally coherent with established pH-P chemistry. The T4 versus T5 pairwise comparison (independent-samples t-test, $df = 4$) showed a significant difference in AP within the chicken-manure system ($+1.0$ mg kg⁻¹, $p = 0.013$, $d = 3.46$). The T6 versus T7 comparison showed a positive but non-significant difference within the buffalo-manure system ($+0.8$ mg kg⁻¹, $p = 0.067$, ns, $d = 2.04$) but not in the buffalo manure system (T6 vs. T7: $+0.8$ mg kg⁻¹, $p = 0.067$), although the effect size was notable ($d = 2.04$). Exchangeable potassium (AK) was also highly significant ($F[6,14] = 98.90$, $p = 1.2 \times 10^{-10}$, $\eta^2 = 0.977$), rising from a baseline of 45.4 to 71.0 mg kg⁻¹ in T4 (+56%). The T4 versus T5 pairwise

Table 1. Soil chemical properties (mean $\pm$ SE, $n = 3$) following organic amendment and PGPR inoculation under rainfed sugarcane, Northeast Thailand.

Treatment	pH	OM (g kg ⁻¹)	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)
T1: Control (no fertilizer)	4.300 $\pm$ 0.058 ^d	4.20 $\pm$ <0.01 ^c	33.3 $\pm$ 3.4 ^d	6.0 $\pm$ <0.01 ^d	46.3 $\pm$ 0.3 ^d
T2: Chemical fertilizer (NPK)	4.300 $\pm$ 0.058 ^d	4.13 $\pm$ 0.09 ^c	123.3 $\pm$ 8.8 ^a	9.8 $\pm$ 0.2 ^c	66.0 $\pm$ 1.0 ^b
T3: Organic fertilizer (commercial)	4.733 $\pm$ 0.067 ^c	4.53 $\pm$ 0.03 ^b	58.3 $\pm$ 6.0 ^c	9.3 $\pm$ 0.2 ^c	62.0 $\pm$ 1.2 ^c
T4: Chicken manure + Rock-P + Rice bran + Lime + Leonardite + PGPR	5.533 $\pm$ 0.033 ^a	4.73 $\pm$ 0.03 ^b	92.3 $\pm$ 1.5 ^b	11.8 $\pm$ 0.2 ^a	71.0 $\pm$ 0.6 ^a
T5: Chicken manure + Rock-P + Rice bran + Lime + Leonardite	5.200 $\pm$ 0.058 ^b	4.50 $\pm$ 0.10 ^b	69.7 $\pm$ 2.9 ^c	10.8 $\pm$ 0.2 ^b	67.7 $\pm$ 0.3 ^a
T6: Buffalo manure + Rock-P + Rice bran + Lime + Leonardite + PGPR	5.400 $\pm$ 0.058 ^a	5.27 $\pm$ 0.15 ^a	87.7 $\pm$ 0.9 ^b	10.0 $\pm$ 0.3 ^b	64.7 $\pm$ 0.9 ^b
T7: Buffalo manure + Rock-P + Rice bran + Lime + Leonardite	4.967 $\pm$ 0.033 ^b	4.93 $\pm$ 0.07 ^a	65.7 $\pm$ 2.2 ^c	9.2 $\pm$ 0.2 ^c	63.7 $\pm$ 0.9 ^c
<i>F</i> -value ($df = 6,14$)	86.96***	24.69***	40.60***	104.67***	98.90***
CV (%)	1.9	3.0	10.3	3.2	2.2
η^2 (effect size)	0.974	0.914	0.946	0.978	0.977

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). AN = available nitrogen; AP = available phosphorus; AK = available potassium; OM = organic matter (Walkley-Black SOC $\times$ 1.724 conversion). *** $p < 0.001$.

Table 2. Sugarcane growth and yield components (mean $\pm$ SE, $n = 3$) under integrated organic amendment and PGPR inoculation treatments, Northeast Thailand.

Treatment	Stalk no. (no. m ⁻²)	Stalk height (cm)	Stalk diameter (cm)	Single stalk weight (g)	Theoretical yield (kg ha ⁻¹)	Brix (%)
T1: Control (no fertilizer)	2.17 $\pm$ 0.09 ^c	277.7 $\pm$ 1.9 ^{bc}	3.53 $\pm$ 0.03 ^b	1130.0 $\pm$ 41.6 ^c	24,490 $\pm$ 1,440 ^d	18.0 $\pm$ 0.6
T2: Chemical fertilizer (NPK)	2.73 $\pm$ 0.07 ^{ab}	300.0 $\pm$ 5.8 ^{ab}	4.33 $\pm$ 0.43 ^{ab}	2256.7 $\pm$ 42.6 ^{ab}	61,553 $\pm$ 470 ^b	18.7 $\pm$ 0.3
T3: Organic fertilizer (commercial)	2.52 $\pm$ 0.06 ^{abc}	279.3 $\pm$ 5.5 ^{bc}	4.37 $\pm$ 0.33 ^{ab}	1600.0 $\pm$ 57.7 ^b	40,200 $\pm$ 600 ^c	17.7 $\pm$ 0.3
T4: Chicken manure + Rock-P + Rice bran + Lime + Leonardite + PGPR	2.85 $\pm$ 0.09 ^a	325.0 $\pm$ 10.4 ^a	4.80 $\pm$ 0.17 ^a	2453.3 $\pm$ 49.1 ^a	69,911 $\pm$ 932 ^a	19.0 $\pm$ 0.6
T5: Chicken manure + Rock-P + Rice bran + Lime + Leonardite	2.42 $\pm$ 0.06 ^{bc}	279.7 $\pm$ 5.5 ^{bc}	3.93 $\pm$ 0.13 ^{ab}	1510.0 $\pm$ 70.2 ^b	36,600 $\pm$ 2,456 ^c	17.3 $\pm$ 0.3
T6: Buffalo manure + Rock-P + Rice bran + Lime + Leonardite + PGPR	2.38 $\pm$ 0.06 ^{bc}	274.7 $\pm$ 2.9 ^{bc}	4.03 $\pm$ 0.09 ^{ab}	1486.7 $\pm$ 21.9 ^b	35,409 $\pm$ 1,422 ^c	18.0 $\pm$ <0.01
T7: Buffalo manure + Rock-P + Rice bran + Lime + Leonardite	2.20 $\pm$ 0.10 ^c	259.7 $\pm$ 5.5 ^c	3.70 $\pm$ 0.06 ^b	1103.3 $\pm$ 57.8 ^c	24,377 $\pm$ 2,268 ^d	17.3 $\pm$ 0.3
<i>F</i> -value ($df = 6,14$)	10.94***	12.86***	3.70*	105.48***	129.32***	2.57 ns
CV (%)	5.4	3.6	9.5	5.3	6.4	3.8
η^2 (effect size)	0.824	0.846	0.614	0.978	0.982	0.524

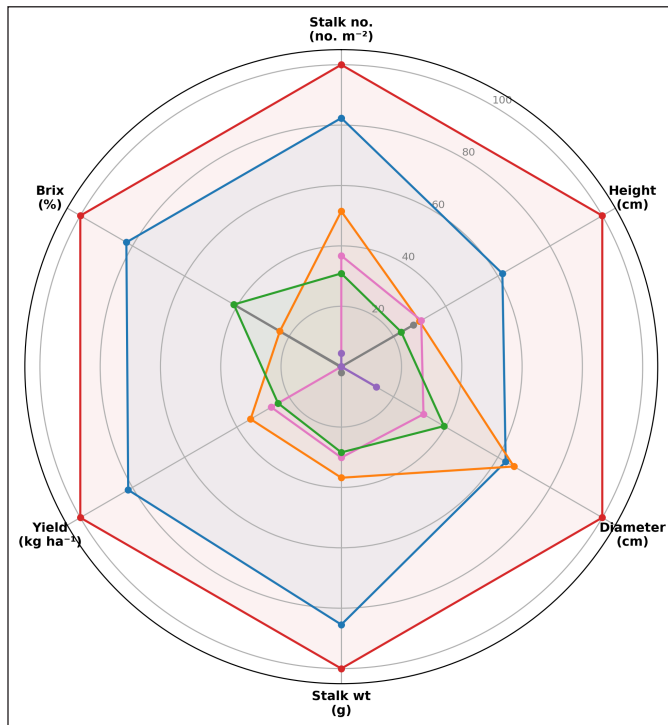


Figure 3. Radar chart depicting multi-dimensional crop performance parameters across treatments. Parameters include theoretical yield, stalk number, stalk height, stalk diameter, single-stalk weight, and juice Brix (%).

Each axis represents normalized values (0–100 scale) to facilitate visual comparison across different measurement units. T1 = Control; T2 = Chemical fertilizer (NPK); T3 = Organic fertilizer (commercial); T4 = Chicken manure + rock phosphate + rice bran + lime + leonardite + PGPR; T5 = Chicken manure + rock phosphate + rice bran + lime + leonardite (no PGPR); T6 = Buffalo manure + rock phosphate + rice bran + lime + leonardite + PGPR; and T7 = Buffalo manure + rock phosphate + rice bran + lime + leonardite (no PGPR).

comparison (independent-samples t-test, $df = 4$) showed a significant difference in AK within the chicken-manure system ($+3.3 \text{ mg kg}^{-1}$, $p = 0.008$, $d = 4.08$). The T6 versus T7 comparison did not show a significant difference in AK within the buffalo-manure system ($+1.0 \text{ mg kg}^{-1}$, $p = 0.467$). We speculate, as an untested hypothesis, The greater AK response observed in the poultry-manure system may reflect substrate-dependent microbial or nutrient-cycling processes. K-solubilizing rhizobacteria have been reported to enhance K availability [34]. However, K-solubilizing activity of BS-01 and AZ-03 was not measured, and no direct evidence of substrate-specific K solubilization was obtained in this study. The PGPR-AK difference was significant in the chicken-manure system (T4 vs. T5: $+3.3 \text{ mg kg}^{-1}$, $p = 0.008$, $d = 4.08$, independent-samples t-test, $df = 4$) but not in the buffalo-manure system (T6 vs. T7: $+1.0 \text{ mg kg}^{-1}$, $p = 0.467$).

3.2. Sugarcane Growth and Yield Components Response

Treatment significantly affected stalk number, stalk height, stalk diameter, single-stalk weight, and theoretical yield; Brix did not differ significantly among treatments. Effects were $p < 0.001$ except for stalk diameter ($p = 0.020$) (Table 2, Fig. 3). T4 (chicken manure + PGPR) produced the highest theoretical yield ($69,911 \pm 932 \text{ kg ha}^{-1}$), significantly exceeding all other treatments ($F[6,14] = 129.32$, $p = 1.9 \times 10^{-11}$). T5, which received the same amendments as T4 but without PGPR, produced $36,600 \pm 2,456 \text{ kg ha}^{-1}$, giving a 91.0% PGPR-associated yield advantage ($p < 0.001$, $d = 10.35$). This large effect

indicates a strong positive association between PGPR inoculation and yield in the chicken manure system, although the design does not permit attribution to PGPR alone (Section 4). A positive but smaller PGPR effect was also observed in the buffalo manure system: T6 (buffalo manure + PGPR, $35,409 \pm 1,422 \text{ kg ha}^{-1}$) outperformed T7 (no PGPR, $24,377 \pm 2,268 \text{ kg ha}^{-1}$) by 45.3% ($p = 0.015$, $d = 3.36$). The core observation of this study is a dissociation between PGPR-associated soil chemistry gains and yield gains across the two manure substrates. PGPR inoculation produced near-identical improvements in AN (chicken: $+22.6 \text{ mg/kg}$, $p = 0.002$; buffalo: $+22.0 \text{ mg/kg}$, $p < 0.001$) and pH (chicken: $+0.333$, $p = 0.008$; buffalo: $+0.433$, $p = 0.003$) in both systems. Yet yield responses differed by a factor of two: $+91.0\%$ in the chicken manure system versus $+45.3\%$ in the buffalo manure system. Because soil chemistry responses were symmetric but yield responses were not, the observed dissociation between soil chemistry and yield responses suggests that amendment substrate context may influence how PGPR-associated soil changes are translated into crop performance. than through soil chemistry alone. Differences in manure composition and decomposition dynamics may have contributed to the contrasting yield responses; however, C:N ratio and other compositional characteristics of the applied manures were not directly measured, which could partly explain the larger yield response in the chicken manure system; however, colonization was not directly measured, and this interpretation remains hypothetical. The yield advantage observed in the chicken manure system is broadly consistent with PGPR-mediated yield improvements reported in sugarcane [18].

The higher AP in PGPR-inoculated treatments is consistent with PGPR-mediated phosphate solubilization documented in other systems [21,34], a mechanism that would be expected to support early root development and tillering. The increase in available N following inoculation is consistent with altered N cycling associated with PGPR, including biological N fixation reported for sugarcane-associated bacteria [19] and PGPR more broadly [16]; however, neither N fixation nor mineralization was directly quantified in the present study. Elevated AN under PGPR inoculation is broadly consistent with nitrogen fixation and enhanced mineralization, mechanisms well documented for *Bacillus* and *Azotobacter* [34,35], which would have supplemented organic nitrogen throughout vegetative growth. Phytohormone production, particularly auxins and cytokinins, may have further contributed to root proliferation and shoot elongation [36], and induced systemic resistance mechanisms could have enhanced tolerance to biotic and abiotic stresses [35], although neither was directly measured (Section 4). Combined PGPR–organic amendment systems have been reported to improve crop performance across diverse cropping systems [16], lending biological plausibility to the integrated approach evaluated here; direct mechanistic evidence in sugarcane under rainfed tropical conditions remains a priority for future research.

T4 also outperformed chemical fertilizer (T2: $61,553 \pm 470 \text{ kg ha}^{-1}$) by 13.6% ($p = 0.001$, $d = 6.53$) and the unfertilized control (T1: $24,490 \pm 1,440 \text{ kg ha}^{-1}$) by 185.5% ($p < 0.001$, $d = 21.61$). Stalk height showed a similar ranking ($F[6,14] = 12.86$, $p = 5.4 \times 10^{-5}$): T4 reached $325.0 \pm 10.4 \text{ cm}$ compared with $279.7 \pm 5.5 \text{ cm}$ in T5 and $277.7 \pm 1.9 \text{ cm}$ in the control. Stalk diameter was also significantly affected ($F[6,14] = 3.70$, $p = 0.020$), with T4 ($4.80 \pm 0.17 \text{ cm}$) exceeding T5 ($3.93 \pm 0.13 \text{ cm}$) and the control ($3.53 \pm 0.03 \text{ cm}$).

Juice Brix did not differ significantly among treatments ($F[6,14] = 2.57$, $p = 0.069$), ranging from 17.3% in T5 and T7 to 19.0% in T4. This indicates that treatment effects were primarily expressed through biomass accumulation rather than changes in juice quality. The

13.6% yield advantage of T4 over chemical fertilizer provides initial evidence that the integrated organic-PGPR approach warrants further evaluation for resource-constrained smallholders, consistent with a recent Khon Kaen Province study in which bio-organic amendments with phosphate-solubilizing bacteria achieved yields comparable to mineral fertilization while improving soil-microbial attributes [23].

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's HSD test). Theoretical yield was calculated from stalk density and mean individual stalk weight at the replicate level; displayed stalk number means are rounded to two decimal places, which may result in minor discrepancies (up to 120 kg ha⁻¹ for T3; see Supplementary Table S3 for treatment-specific discrepancy values) when recalculating yield from rounded values. * $p < 0.05$; *** $p < 0.001$; ns = not significant.

3.3. Integrated Soil–Crop Relationships and Regional Implications

Correlations between post-treatment soil properties and theoretical cane yield at the treatment-mean level ($n = 7$; full data in Supplementary Tables S6–S7) showed a more nuanced pattern than the soil–soil relationships alone. Available nitrogen was the only soil property to correlate significantly with yield ($r = 0.761$, $p = 0.047$), consistent with N availability showing the strongest observed association with yield in this dataset ($r = 0.761$, nominal $p = 0.047$); however, this association should be regarded as exploratory because it was based on only seven treatment-level observations and no multiplicity correction was applied. Available phosphorus ($r = 0.664$, $p = 0.104$) and available potassium ($r = 0.630$, $p = 0.130$) showed moderate positive associations with yield that did not reach significance at $n = 7$. Soil pH ($r = 0.203$, $p = 0.662$) and organic matter ($r = -0.191$, $p = 0.681$) were weakly and non-significantly associated with yield. Although pH amelioration and organic matter accumulation were strongly associated with the integrated treatments at the soil level (Table 1, Fig. 1), neither translated into a significant soil–yield correlation across the seven treatment means may partly reflect the limited statistical power associated with only seven treatment-level observations; however, the present dataset cannot distinguish limited power from a genuinely weak relationship. The contrast between the strong soil–soil correlations (e.g., AP–AK: $r = 0.981$, $p < 0.001$; Fig. 2A) and the weaker soil–yield associations underscores that yield is determined by multiple interacting factors beyond any single soil chemical property, and that more robust soil–yield relationships will require additional seasons and a greater number of treatment-level observations. The sole significant soil–yield correlation was AN ($r = 0.761$, $p = 0.047$), despite AP showing the largest treatment F-value ($F = 104.67$) of any parameter measured. Available N showed the strongest observed association with yield in this dataset. However, given only seven treatment-level observations and no multiplicity correction, this relationship should be regarded as exploratory rather than evidence that N was the principal yield-limiting factor. This is consistent with the high nitrogen demand during rapid vegetative establishment of plant-cane, and with higher AN in PGPR-inoculated treatments in both manure systems in the targeted pairwise t-test comparisons (chicken-manure system T4 vs. T5: +22.7 mg/kg, $p = 0.002$; buffalo-manure system T6 vs. T7: +22.0 mg/kg, $p < 0.001$, $df = 4$ for both) (chicken: +22.7 mg/kg, $p = 0.002$; buffalo: +22.0 mg/kg, $p < 0.001$). Together, these patterns suggest that PGPR-mediated nitrogen cycling, whether through fixation, enhanced mineralization, or both, may have been a central mechanism in this system, although direct confirmation was not obtained.

The integrated T4 package addressed multiple soil constraints simultaneously: acidity through lime, nutrient deficiency through

manure and rock phosphate, organic matter quality through leonardite, and rhizosphere biology through PGPR. The resulting yield advantage over both chemical fertilizer (nutrient supply only) and uninoculated organic amendments (nutrient and pH correction without biological enhancement) is consistent with the added value of addressing these constraints in combination, even though individual soil–yield correlations did not all reach significance at $n = 7$.

The yield responses observed here are broadly consistent with, though somewhat larger than, those from comparable integrated organic-PGPR field trials under similar agroclimatic conditions. In a sugarcane trial from neighboring Khon Kaen Province, the best bio-organic treatment achieved only a ~1% yield advantage over mineral fertilization (48.8 vs. 48.3 t ha⁻¹) [23], a much smaller margin than the 13.6% advantage of T4 observed in the present study. One possible explanation lies in the inclusion of lime and leonardite, both absent from the Khon Kaen trial. Lime elevated soil pH from 4.20 to 5.53 in T4, moving the system out of the strongly acidic range (pH < 4.5) where aluminum and manganese toxicity, together with phosphorus fixation, impair root function and constrain PGPR activity [36]. Leonardite, supplying humic and fulvic acids, contributes cation exchange capacity and promotes aggregate formation—both critically limited in the coarse sandy soils of this site [15]. One possible explanation is the inclusion of lime and leonardite, both of which were absent from the comparator study. However, because these components were applied together with other amendments, their independent contributions cannot be separated in the present design. The observed response suggests that pH amelioration and organic matter management may be relevant components of integrated PGPR systems on acidic sandy soils, but factorial experiments are required to test their individual and interactive effects. Substrate-dependent PGPR responses have been reported across contrasting soil and crop environments [18,35]; the present study contributes observations from acidic tropical sandy soils under rainfed conditions. Taken together, the present results quantify a management combination, soil type, and crop context that have not been extensively investigated in the published literature for this specific soil and climate context.

Beyond the immediate agronomic results, these findings have both scientific and practical implications. The higher pH observed in the integrated treatments suggests that pH amelioration may have contributed to improved crop performance. However, because lime was applied as part of a multi-component package and no lime-only treatment was included, the independent contribution of lime cannot be determined. of integrated organic-PGPR management on strongly acidic sandy soils. The 13.6% yield advantage of T4 over chemical fertilization (T2) was accompanied by a pH shift from 4.20 to 5.53, while T3 (commercial organic fertilizer without lime or PGPR) achieved only pH 4.73 and a yield of 40,200 kg/ha suggesting that The higher pH observed in the integrated treatments suggests that pH amelioration may have contributed to improved crop performance; however, because lime was applied as part of a multi-component package and no lime-only treatment was included, the independent contribution of lime cannot be determined from the present experimental design.. At the practical level, the Tung Kula Ronghai region covers 3,370 km² of broadly similar sandy soils. Sugarcane cultivation is an important agricultural activity in this area, where production is constrained by the same sandy, low-fertility soils and rainfall variability documented here; nationally, approximately 300,000 farming households are engaged in sugarcane production [3]. The materials used in this study are commonly obtainable agricultural inputs; however, their local availability, cost, and accessibility to smallholders were not formally evaluated in the present study. Formal cost-benefit analysis comparing T4 with conventional chemical

fertilizer inputs under realistic smallholder cost and market price scenarios is required before economic recommendations can be made. Do not claim economic feasibility from yield results alone. Multi-season, multi-site validation is still required before confident regional-scale recommendations can be made, but the present results provide the empirical foundation that justifies that investment.

4. STUDY LIMITATIONS

Several aspects of the experimental design limit the strength of the inferences that can be drawn. Yield was not directly harvested but estimated from stalk density and the mean weight of 10 sampled stalks per plot. The approach is common in agronomic screening trials but misses plot-level harvest losses, border effects, and stalk-weight variability outside the sample. The differences reported, including the 91.0% PGPR-associated advantage and the 13.6% advantage over chemical fertilizer, need confirmation with net-plot mechanical harvest weighing. Second, total N, P, and K inputs were not equalized across treatments. T4 and T6 received a multi-component package that supplied substantially more nutrients and soil conditioners than T2 or T3. The superior performance of T4 and T6 therefore cannot be attributed to PGPR inoculation alone and may partly reflect the greater overall nutrient input. Total nutrient inputs were not equalized across all treatments; therefore, comparisons of T4/T6 with T2/T3 cannot distinguish PGPR contribution from nutrient-input differences. In contrast, T4 versus T5 and T6 versus T7 involved identical amendment compositions and differed only in PGPR inoculation, allowing estimation of PGPR-associated responses within each amendment background. However, the absence of a mock-inoculum or heat-killed inoculum control, together with the lack of direct measurements of PGPR survival, rhizosphere colonization, and functional activity, limits mechanistic attribution of these responses. Third, the study provided no direct mechanistic evidence: rhizosphere colonization was not quantified, strain survival was not tracked by re-isolation or qPCR, no heat-killed inoculum control was included, and plant nutrient uptake was not measured. The reported pairwise comparisons (T4 vs. T5; T6 vs. T7, independent-samples t-tests, $df = 4$) should therefore be interpreted as associations between inoculation and improved outcomes, not as proof of a specific mechanism. Fourth, the PGPR strain characterization is a substantive limitation. Specifically: (a) no 16S rRNA sequencing was performed for BS-01 or AZ-03, so species-level identification rests on morphological and biochemical presumption only; (b) neither strain has GenBank accession numbers; (c) neither has been deposited in a recognized public culture collection (e.g., DSMZ, ATCC, NRRL, or TISTR); (d) no formal biosafety or risk-group assessment has been conducted; and (e) the PGP traits attributed to these genera in Section 2.4 are based on published literature, not on direct characterization of the isolates used here. Consequently, the identity, safety, and functional capacity of BS-01 and AZ-03 cannot be independently verified, which represents a reproducibility gap and constrains the mechanistic interpretations in Section 3.2. Molecular identification, public deposition, and direct PGP trait measurement are identified as priority requirements for any future work and are prerequisites for regulatory or commercial application of these strains. Fifth, rainfall and soil moisture were not monitored at sufficient resolution to make quantitative claims about crop performance under rainfed stress. Beyond the initial 2-week irrigation period, the crop relied on natural rainfall recorded by the nearby Sisaket Meteorological Station (Thai Meteorological Department, approximately 15 km from the experimental site); total seasonal rainfall (1,247 mm) was measured, but although complete daily rainfall records were available from the nearby Sisaket Meteorological Station (TMD), the study did not include on-site monitoring of soil moisture

or high-resolution rainfall characteristics such as intensity and event distribution. Therefore, the influence of short-term water stress on sugarcane performance could not be quantitatively assessed, and the rainfed conditions are described in aggregate terms only. Finally, the experiment was conducted at one site over a single season with three replications. Broader claims regarding regional scalability, economic viability, or applicability to other varieties and soil types should be treated as preliminary until multi-location, multi-season validation and formal cost-benefit analysis have been completed.

Although the field experiment was arranged as a randomized complete block design, block was not included in the statistical model corresponding to the reported omnibus ANOVA results. In addition, the targeted within-amendment comparisons were evaluated using independent-samples t-tests rather than block-aware paired or model-based contrasts. Consequently, the reported inference does not explicitly account for block-to-block variability, and the targeted pairwise p-values should be interpreted cautiously, particularly given the small sample size of three replicates per treatment. No multiplicity adjustment was applied across these targeted pairwise tests.

Because the soil–yield correlations were calculated from only seven treatment means and multiple correlations were examined, these relationships should be interpreted as exploratory. The AN–yield association ($r = 0.761$, nominal $p = 0.047$) therefore provides hypothesis-generating evidence rather than confirmation that N was the principal yield-limiting factor.

5. CONCLUSION

When the same PGPR inoculant was applied within two organic amendment substrates, yield responses differed by a factor of two: +91.0% in the chicken manure system (T4 vs. T5, $p < 0.001$, $d = 10.35$) versus +45.3% in the buffalo manure system (T6 vs. T7, $p = 0.015$, $d = 3.36$). This occurred despite nearly identical PGPR-associated soil chemistry improvements in both systems (AN: +22.6 vs. +22.0 mg/kg; pH: +0.333 vs. +0.433). This dissociation between soil chemistry and yield responses the contrasting yield responses suggest that amendment background may influence PGPR-associated crop responses; however, the underlying mechanisms were not directly evaluated, not merely the soil chemical effects of PGPR, a finding with direct implications for the design and interpretation of PGPR field trials in acidic tropical soils. The integrated T4 amendment package (poultry manure, rock phosphate, rice bran, agricultural lime, leonardite, and PGPR inoculant) also improved all five measured soil fertility parameters and achieved an estimated theoretical yield of 69,911 kg/ha 13.6% higher than chemical fertilization (subject to the limitations discussed in Section 4). Soil improvements in T4 included a pH increase from 4.20 to 5.53, organic matter from 4.10 to 4.73 g/kg, and increases in available nitrogen (+134%), phosphorus (+115%), and potassium (+56%). PGPR-inoculated treatments showed higher available nitrogen and pH than their corresponding non-inoculated treatments in both manure systems, while its effects on estimated yield, phosphorus, and potassium availability were more pronounced in the chicken manure system. Among the soil–yield correlations at the treatment-mean level ($n = 7$), only available nitrogen reached significance ($r = 0.761$, $p = 0.047$). Given the small number of treatment-level observations ($n = 7$), these correlation results should be regarded as exploratory. This replicated field study from Sisaket Province, within the broader agroecological area associated with Tung Kula Ronghai, provides quantitative evidence that an integrated organic amendment package with locally isolated PGPR can simultaneously improve all five measured soil chemical properties and achieve estimated theoretical yield exceeding that of

conventional chemical fertilization under rainfed conditions.—a 3,370 km² area with extensive smallholder sugarcane farming—to show that an integrated organic amendment package with locally isolated PGPR can simultaneously improve all five measured soil chemical properties and achieve estimated theoretical yield exceeding that of conventional chemical fertilization under rainfed conditions following a 2-week establishment irrigation period. Targeted pairwise comparisons using independent-samples t-tests ($df = 4$) showed PGPR-associated yield differences in both manure systems (chicken manure: +91.0%, $p < 0.001$, $d = 10.35$; buffalo manure: +45.3%, $p = 0.015$, $d = 3.36$). These results provide replicated quantitative field data for this crop–soil–management context. Three broader points emerge from this work. The differential yield responses (+91.0% vs. +45.3%) achieved with identical PGPR rates confirm that amendment substrate quality is a key determinant of PGPR field efficacy, a finding that may be related to differences in substrate quality, although this mechanism was not directly tested in the present study. Investigators designing future PGPR–organic amendment trials in tropical soils should treat substrate selection as a primary experimental factor, not merely as context. Second, that nitrogen availability, not phosphorus or potassium, was the sole soil property to correlate significantly with yield ($r = 0.761$, $p = 0.047$), combined with consistently large PGPR–AN contrasts in both manure systems, supports altered N cycling as a hypothesis warranting direct testing, rather than establishing it as the mechanism responsible for the yield response. supports altered N cycling as a hypothesis warranting direct testing, rather than establishing it as the mechanism responsible for the yield response. This is a testable hypothesis amenable to ¹⁵N isotope tracing and rhizosphere colonization assays. Third, the large effect sizes across all five soil parameters ($\eta^2 = 0.914$ – 0.978) on a soil entering the trial at pH 4.20 show that a single coordinated amendment application can simultaneously shift multiple soil quality indicators toward agronomically favorable ranges within one growing season a result that is relevant to rehabilitation programs on degraded Ultisols well beyond the immediate study region. The materials used in this study are commonly obtainable agricultural inputs, supporting practical relevance; however, regional availability and cost were not formally evaluated and both PGPR isolates were obtained from local rhizosphere soils, which strengthens the translational relevance of these results for agricultural extension. These results identify the integrated organic-PGPR approach as a strong candidate for multi-season, multi-location validation in the Northeast Thailand smallholder sugarcane sector. Four research priorities follow directly from these findings: replicating the experiment at multiple sites within the Tung Kula Ronghai region over at least two growing seasons; molecularly characterizing BS-01 and AZ-03, depositing them in a recognized culture collection, and directly measuring their plant growth-promoting traits; replacing the estimated theoretical yield with net-plot mechanical harvest data; and conducting a formal cost-benefit analysis under realistic smallholder input cost and market price scenarios.

6. ACKNOWLEDGMENTS

This research was financially supported by the Sisaket Rajabhat University Research and Innovation Promotion (SRIP) Fund and the Fundamental Fund for fiscal year 2024 provided by the National Science, Research and Innovation Fund (NSRF) Thailand (Project Code 4694885; Proposal Code 2567A15412016). The authors thank the technical staff at the Sisaket Rajabhat University research station for field and laboratory assistance.

7. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in

drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

During manuscript preparation and revision, the authors used Claude and ChatGPT for English-language editing, clarity, and editorial consistency checks. During revision, these tools were also used to assist in identifying internal inconsistencies in the presentation of statistical results. All statistical decisions, calculations retained in the manuscript, scientific interpretations, and final revisions were independently reviewed and approved by the authors, who take full responsibility for the accuracy and integrity of the work. No AI tool generated experimental data. No AI tool has been listed as an author. The authors apologize for not including this disclosure in the original submission.

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How to cite this article:

Sehawong W, Subepang S. Integrated organic amendments and plant growth-promoting rhizobacteria improve soil fertility and sugarcane growth and yield components under rainfed conditions in Northeast Thailand. *J Appl Biol Biotech* 2026. Article in Press. <http://doi.org/10.7324/jabb.2026.313352>