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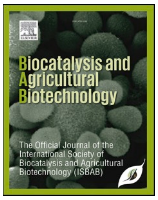

Polyhydroxyalkanoate-based biodegradable coatings for controlled-release urea fertilizers: Effects on oil palm seedling growth and soil microbial communities

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ABSTRACT

Polyhydroxyalkanoate (PHA)-based biodegradable coatings were applied to formulate controlled-release urea fertilizers (CUFs) and evaluated for their effects on fertilizer performance, oil palm nursery growth, and soil microbial diversity. Rice husk was incorporated as a hydrophilic filler to enhance water absorption and nutrient diffusion in the PHA-CUF formulations. The PHA-coated fertilizers showed improved nutrient-release behavior compared with immediate-release fertilizer and supported significantly better seedling growth. The best-performing PHA-CUF treatment resulted in higher plant height, leaf number, chlorophyll content, stem thickness, and fresh biomass than both immediate-release fertilizer and commercial controlled-release fertilizer, while unfertilized plants showed the lowest growth. Soil microbial analysis indicated that PHA-CUF application was associated with increased microbial diversity and shifts in bacterial community composition relative to immediate-release fertilizer, with enrichment of several bacterial groups commonly associated with nutrient cycling in agricultural soils. Preliminary scanning electron microscopy (SEM) observations suggested partial degradation of the polymer coating after soil incubation, supporting the biodegradability of the material. These results demonstrate that PHA-based coatings combined with lignocellulosic fillers can serve as effective biodegradable carriers for controlled-release urea fertilizers, offering potential benefits for plant growth and soil microbial health while reducing reliance on non-degradable polymer coatings.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a class of biodegradable and biocompatible polyesters synthesized by a wide range of microorganisms as intracellular carbon and energy storage compounds, particularly under nutrient-limiting conditions with excess carbon sources (Chen and Jiang, 2018). PHAs are generally categorized into short-chain-length (scl-) and medium-chain-length (mcl-) types (Kourmentza et al., 2017). Owing to their tunable physicochemical properties, such as flexibility, crystallinity, and degradation rates, PHAs are an attractive and sustainable alternative to conventional petroleum-based plastics in both industrial and biomedical

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applications (Chen and Jiang, 2018). In recent years, the potential applications of PHAs have expanded beyond traditional fields, with increasing interest in their use in agriculture. One promising approach involves their application in controlled-release fertilizer (CRFs). CRFs are designed to gradually release nutrients in step with plant uptake, thereby enhancing nutrient use efficiency, minimizing nutrient leaching, and reducing environmental contamination compared with conventional immediate-release fertilizers (IRFs) (Lewu et al., 2021; Govil et al., 2024; Lawrencia et al., 2021). Despite these advantages, the large-scale application of CRFs is limited by the high cost and poor degradability of synthetic polymers, such as polyolefins and polyurethanes, that are commonly used as coating materials (Kegan et al., 2024). Consequently, biodegradable polymers such as PHAs have emerged as environmentally friendly alternatives for sustainable fertilizer formulations (Glibert et al., 2006).

Urea, the most widely used nitrogen fertilizer, accounts for over 70% of global nitrogen fertilizer consumption and is indispensable for ensuring global food security (Kegan et al., 2024). However, most urea fertilizers are used in uncoated forms, leading to rapid nitrogen loss through volatilization and leaching, and consequently low nitrogen use efficiency often only 30-35% (Lawrencia et al., 2021; Voinova et al., 2009). To address this limitation, PHA-based coatings have been explored as biodegradable matrices for urea CRFs. For instance, poly (3-hydroxybutyrate) (PHB) has been used as a coating material in lettuce (*Lactuca sativa*) and creeping bentgrass (*Agrostis stolonifera*) cultivation, demonstrating improved nutrient release control and soil microbial enrichment (Volova et al., 2016a, 2016b; Murugan et al., 2016). However, the high melting temperature of PHB ($T_m \approx 180$ °C) complicates its use in coating urea granules, as fertilizers typically degrade at lower temperatures (Volova et al., 2016b). To overcome this challenge, copolymers with lower T_m values, such as poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) [P (3HB-co-3HHx)], have been developed. Murugan et al. (2020) reported that P (3HB-co-3HHx) combined with oil palm trunk (OPT) fiber effectively reduced nutrient release rates, enhanced nutrient use efficiency, and promoted rhizosphere microbial diversity in oil palm nurseries. Nevertheless, the current production of P (3HB-co-3HHx), which involves extraction from *Cupriavidus necator* using *Tenebrio molitor* larvae, remains costly and limits scalability (Rakkan et al., 2022).

A medium-chain-length (mcl) PHA has been reported in previous studies for PHA produced by *Enterobacter* sp. and *Bacillus thermoamylovorans*, characterized by substantially lower melting temperatures than PHB and P (3HB-co-3HHx) (Rakkan et al., 2023; Choonut et al., 2020). Such thermal properties enhance suitability for coating applications by better matching the heat tolerance of urea-based fertilizers and facilitating melt-processing during fertilizer coating and pelletization. Moreover, biodegradation studies have indicated that these PHAs can be mineralized without the accumulation of toxic intermediates (Chang et al., 2023). In addition to environmental compatibility, PHA-based coatings have been reported to offer agronomic advantages, such as improved soil moisture retention, reduced erosion risk, and good oxidative stability (Glibert et al., 2006).

Therefore, this study aimed to develop a PHA-based controlled-release urea fertilizer using microbially produced PHA as a biodegradable coating material. The PHA-based formulation was characterized for its physicochemical properties and evaluated in oil palm (*Elaeis guineensis*) seedlings during the nursery stage, with plant growth performance, biodegradation behavior, and soil bacterial diversity compared to conventional IRF and a commercial CRF.

2. Materials and methods

2.1. Production and characterization of PHA

Two PHA samples produced by different bacterial strains were used in this study. Both polymers had been previously produced and purified as described in earlier studies (Rakkan et al., 2023; Choonut et al., 2020). The polymers were determined by gas chromatography–mass spectroscopy (GC–MS) as mcl-PHA (Rakkan et al., 2023; Choonut et al., 2020). Both polymers were selected based on their distinct physicochemical behaviors, particularly differences in melting temperature and mechanical properties, which are relevant to their suitability as coating materials for controlled-release fertilizer formulations.

The first polymer was produced by *B. thermoamylovorans* using sodium octanoate as the sole carbon source (Choonut et al., 2020), while the second polymer was produced by *Enterobacter* sp. cultivated with textile wastewater as the carbon source (Rakkan et al., 2023). In the present study, both polymers were used as biodegradable matrices for controlled-release urea fertilizer (CUF) formulation and are hereafter referred to as PHA-A and PHA-B, respectively. Their melting temperatures were previously determined by differential scanning calorimetry (DSC) and were within the range of approximately 60–70 °C, which is suitable for fertilizer coating processes.

These two PHA samples were subsequently used to formulate controlled-release urea fertilizers (PHA-CUF-A and PHA-CUF-B) to evaluate differences in pellet properties, nutrient release behavior, plant growth response, biodegradation, and soil microbial communities.

2.2. Production of PHA-controlled release urea fertilizer (PHA-CUF)

The PHA-controlled release urea fertilizer (PHA-CUF) was produced under optimal conditions reported by Murugan et al. (2020). Isolated PHA, rice husk, and urea (46–0–0) fertilizer were used as raw materials for the preparation of PHA-CUF. The rice husk was ground in a laboratory-scale hammer mill and sieved through a 0.5 mm (100-mesh) sieve before use. An internal mixer was used to mix the CUF formulation comprising 120 g of PHA, 60 g of ground rice husk, and 30 g of urea fertilizer (Brand: ICP Fertilizer Ltd., Thailand) at 130 °C for 15 min. The resulting mixture was then cast into a mould and dried overnight in an oven at 50 °C. The dried pellets were subsequently stored in a desiccator until further analysis.

This procedure was applied to produce CUF using the two different PHA samples, referred to hereafter as PHA-CUF-A and PHA-

CUF-B, corresponding to PHA-A and PHA-B described in Section 2.1. Additionally, a control sample containing PHA and rice husk without urea was prepared.

2.3. *Palm plants and fertilizer*

One-month-old oil palm seedlings were obtained from a local farm in Phatthalung (Thailand). The experimental procedure was conducted following the method described by Murugan et al. (2020). The effects of PHA-CUF-A and PHA-CUF-B on plant growth parameters were investigated and compared with those of commercial CRF and IRF. Cultivated soil, collected from a local oil palm plantation in Phatthalung (Thailand), was used as the control treatment without fertilizer application. The fertilizer was applied at an equivalent nitrogen rate per standard nursery recommendations for oil palm seedlings. IRF are applied weekly for a period of 3 months, while CRF and PHA-CUF are applied following the manufacturer's instructions at a rate of 3 g per seedling for 3 months.

2.4. *Characterization of PHA-CUF*

2.4.1. *Determination of water absorption and fertilizer release properties*

To evaluate water absorption, 1 g of PHA-CUF was immersed in 50 mL of distilled water in a Falcon tube, and the sample's weight was measured daily over a period of seven consecutive days. Concurrently, fertilizer release from the PHA-CUF was monitored by measuring changes in the electrical conductivity of the distilled water using a conductivity meter, following the method described by Messa et al. (2020). The results were compared with a control treatment.

To determine the release behavior of urea from the fertilizer in water, the following experiment was carried out: a fertilizer sample (0.5 g) was immersed in 100 mL of distilled water in a glass beaker, properly covered at 25 °C. A quantity of 5.0 mL of solution was removed to estimate the nitrogen content after a certain interval, and then the same volume of fresh water was replenished. According to the Ehrlich reaction, the amount of urea released from the fertilizer was measured at 440 nm by UV−vis spectrophotometry (Niu and Li, 2012). All release experiments were performed in triplicate, and the results were averaged.

2.4.2. *Scanning electron microscope (SEM) analyses*

SEM was used to determine the surface morphology of PHA-CUF before and after three months of application. Samples were mounted on aluminium stubs and sputter-coated with gold for 15 s prior to observation. For SEM operation, an accelerating voltage of 10 kV and magnifications ranging from 500× to 5000 × (FEI Quanta 450 FEG) were used (Choonut et al., 2020). In addition, SEM were used to preliminarily evaluate the degradation of PHA-CUF before and after three months of application.

2.5. *Effect of PHA-CUF on soil and palm nursery*

Ten grams of soil samples from each treatment were collected monthly over the three months. Then, the soil sample was dispersed in 50 mL of distilled water, and the EC value was measured using an EC meter (Mettler Toledo, F3-LE7O3-IP67) (Messa et al., 2020). The results were compared with those from IRF (Brand: ICP fertilizer Ltd., Thailand) and CRF (Brand: Osmocoate Ltd., Thailand), both containing nitrogen: phosphorus: potassium ratios of 46:0:0 and 13:13:13, respectively. The pH meter (Mettler Toledo, AG-CH-8630) was used to determine soil pH.

The PHA-CUF formulations were tested on one-month-old oil palm seedlings. The experiment was arranged in a randomized complete block design (RCBD) to minimize environmental variation. Each treatment, including the control, was replicated ten times per treatment (one seedling per replicate). Plant height was measured from the soil surface to the tip of the tallest leaf using a ruler. Stem thickness was measured with a digital Vernier caliper (Mitutoyo, Japan). The fresh weight was then measured using an electronic balance (Shimadzu, ATY224) (Murugan et al., 2020). The palm samples were carefully washed to remove adhered soil particles, and excess moisture was removed using absorbent paper before measuring.

In the present study, chlorophyll content was also evaluated. Palm leaves were collected after 3 months of cultivation. Leaf chlorophyll content was initially assessed using chlorophyll meter values (CM), which indicate leaf greenness, measured with a chlorophyll meter (MINOLTA™ SPAD-502). The actual chlorophyll concentration was then estimated based on a standard calibration curve relating CM values to chlorophyll content, as proposed by Amir et al. (2005).

2.6. *Metagenome sequence analysis*

Three soil samples, including cultivation soil without fertilizer, soil treated with IRF, and soil treated with PHA-CUF-B, were collected for analysis. Total genomic DNA (gDNA) was extracted from each sample using the DNeasy PowerSoil Pro kit (Qiagen, Germany), following the manufacturer's instructions. The extracted DNA was sent to GIBTHAI CO, LTD. (Thailand) for bacterial 16S rDNA and fungal ITS amplicon sequencing. The quality of the gDNA was determined by 1% (w/v) agarose gel electrophoresis, in which 5 μL of each sample was loaded to check for a single intact band. The gels were run at 110 V for 30 min. DNA yield and purity were determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, USA) by measuring absorbance at 260 and 280 nm. Metagenome sequencing of prokaryotic communities was performed on all samples at GIBTHAI CO, LTD. (Thailand), and bioinformatics analysis was conducted using a reference taxonomy database. The results were compared with those of the IRF and control treatment.

2.7. Statistical analysis

All experiments were conducted in triplicate, except the plant study used 10 replicates per treatment. The mean and standard deviation of the triplicate measurements were calculated to present the results. Data analysis was performed using Microsoft Excel 365 and Minitab (Version 21). One-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test was used to assess differences among treatment groups. The exact sample size (n) and p-value were stated in the tables and figure captions.

3. Results and discussion

3.1. Water absorption and PHA-CUF release

The material properties of the PHA-CUF pellets were evaluated to understand their influence on water absorption and nutrient release. The pellets exhibited a density of approximately 1.05 ± 0.05 g/cm^3 and a porosity of about 18%, indicating a compact structure with moderate water permeability. The compressive strength (6 MPa) confirmed good mechanical integrity, preventing cracking or deformation during handling and immersion. Rice husk particles (100–300 μm) were uniformly dispersed within the PHA matrix, enhancing pellet stability and contributing to a gradual release profile. Overall, these properties support the effectiveness of the PHA-CUF as a controlled-release fertilizer system, providing both durability and sustained nutrient delivery. The PHA-CUF samples exhibited particle sizes ranging from 2 to 4 mm (Fig. 1).

DSC, thermogravimetric analysis (TGA), and gel permeation chromatography (GPC) were used to evaluate the thermal and molecular effects of pellet processing at 130 °C on the PHA matrix and PHA-CUF pellets. DSC showed only minor shifts in Tg and a small reduction in melting enthalpy after processing, consistent with slight reorganization of crystalline domains rather than polymer degradation. TGA traces of the pellets revealed no mass-loss event attributable to urea decomposition at 130 °C. In contrast, pure urea exhibits melting near 133–135 °C and decomposition onset at 133–152 °C (Zhu et al., 2021). GPC analysis showed negligible changes in Mn, Mw, and polydispersity index (PDI) after processing, indicating no significant polymer chain scission. Finally, the urea concentration in aqueous extracts of PHA-CUF pellets, analyzed by UV–vis spectrophotometry, showed similar values before and after pellet processing. Together, these data indicate that pellet processing at 130 °C did not cause appreciable urea decomposition or thermal degradation of the PHA matrix.

Water absorption tests were conducted on two different formulations, namely PHA-CUF-A and PHA-CUF-B. In addition, a mixture of PHA and ground rice husk without urea fertilizer was used as the control. Water absorption was determined by measuring the increase in sample weight after immersion in water. In controlled-release fertilizers, water absorption reflects the material's ability to absorb and retain moisture under cultivation conditions and directly influences nutrient solubility and release rates (Naz and Sulaiman, 2016). Among the tested samples, the highest water absorption was observed in the control sample, followed by PHA-CUF-A and PHA-CUF-B (Table 1). These findings suggest that the inclusion of urea fertilizer reduces the water-absorption capacity of the PHA–rice husk matrix.

Several interacting mechanisms may contribute to this effect. Solid urea particles can occupy pore spaces within the composite, reducing effective porosity and limiting capillary pathways for water uptake (Ramli et al., 2013). In addition, urea and its dissolution products may alter ionic strength in the surrounding medium, which can reduce swelling of hydrophilic components of the husk and suppress water imbibition (Q and Park, 2001). Urea may also competitively interact with hydroxyl groups of lignocellulosic fibers through hydrogen bonding, thereby decreasing available binding sites for water molecules and further limiting water penetration (Sajeeb et al., 2018). Moreover, partial surface deposition of urea during drying may increase local surface resistance to rewetting, slowing subsequent water absorption (Kumar et al., 2025). Collectively, these physical and interfacial effects can explain the lower

Fig. 1. The PHA-controlled release urea fertilizer (PHA-CUF).

Table 1
Water absorption and urea release in distilled water of PHA-based controlled-release fertilizers (PHA-CUF-A and PHA-CUF-B) and a control (PHA-B and rice husk without urea) over 7 days.

Formulation	Water absorption			Fertilizer release		
	Day1 (g)	Day7 (g)	Difference (g)	Day1 (μS/cm)	Day7 (μS/cm)	Difference (μS/cm)
PHA-CUF-A	1.0 ± 0.1^{a}	2.5 ± 0.1^{a}	1.5 ± 0.1^{a}	160 ± 1.0^{a}	1610 ± 1.0^{a}	1450 ± 1.0^{a}
PHA-CUF-B	1.0 ± 0.1^{a}	2.2 ± 0.1^{b}	1.2 ± 0.1^{b}	157 ± 1.0^{b}	1607 ± 1.0^{b}	1450 ± 1.0^{a}
Control	1.0 ± 0.1^{a}	2.0 ± 0.1^{c}	1.0 ± 0.1^{c}	10 ± 0.1^{c}	10 ± 0.1^{c}	0 ± 0.1^{b}

Values represent mean ± standard deviation ($n = 3$). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

water uptake of PHA–husk composites containing urea fertilizer compared with the fertilizer-free system.

PHA, as a family of biodegradable polyesters, is generally hydrophobic and exhibits low intrinsic water absorption. To improve water uptake and facilitate nutrient diffusion, rice husk was incorporated into the PHA matrix as a filler and water-conducting component. In this study, rice husk also served as an initial conduit for fertilizer release prior to gradual diffusion through the polymer coating. As a byproduct of rice milling, rice husk has been widely explored as a sustainable filler material in agricultural and polymer applications (Lourith and Kanlayavattanakul, 2013). Other lignocellulosic materials have also been reported for similar purposes, including vascular bundles from oil palm trunks (Murugan et al., 2020), sisal fiber (Sreekumar et al., 2009), and rubberwood (Abdullah et al., 2012). Rice husk was selected in this study due to its local availability and its potential contribution to agricultural waste valorization. The husk was homogenized to a fine powder to improve dispersion within the polymer matrix and to promote formation of interconnected diffusion pathways. Differences in water absorption between the two PHA-CUF formulations are therefore likely associated with variations in coating compactness and internal matrix permeability, which influence water penetration and swelling behavior of the pellets.

Fertilizer release was evaluated for both PHA-CUF formulations and the control treatment by monitoring changes in electrical conductivity (EC) of water over 7 days. Distilled water alone exhibited a constant conductivity of approximately 0.9 ± 0.2 μS/cm,

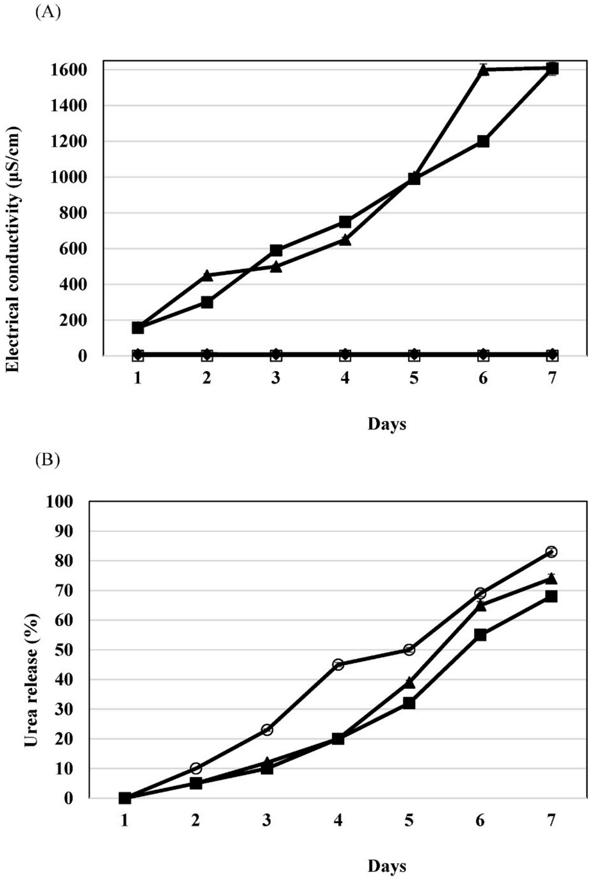

Fig. 2. Fertilizer release (A) in distilled water using PHA-CUF-A (▲), PHA-CUF-B (■), rice husk alone (◆), distilled water alone (□), and rice husk + PHA without fertilizer (control, ○). Urea release (B) in distilled water using urea-IRF (○), PHA-CUF-A (▲), and PHA-CUF-B (■).

while rice husk alone produced slightly higher values (9.7 ± 0.5 μS/cm), attributable to the presence of soluble mineral residues. In contrast, EC values for PHA-CUF treatments increased substantially with time, indicating progressive release of fertilizer ions from the pellets (Fig. 2A). The control sample showed no significant EC increase, confirming that conductivity changes primarily originated from fertilizer release rather than from polymer or husk components. Among the tested formulations, formulation A exhibited higher EC values than formulation A (Table 1), suggesting a higher nutrient release rate. This behavior can be attributed to higher water permeability and more accessible diffusion pathways within the composite matrix, which facilitate faster dissolution and transport of urea through the coating layer. The initial rapid increase in EC is likely caused by dissolution of fertilizer particles located near the pellet surface rather than by polymer degradation (Murugan et al., 2020).

The influence of the PHA coating on urea release was further evaluated using UV–vis spectrophotometry. Pure urea dissolved completely in water within one day (Fig. 2B), whereas both PHA-CUF formulations exhibited significantly slower release profiles. This retardation effect is mainly attributed to the barrier function of the PHA coating, which limits direct contact between urea and water and restricts rapid diffusion of dissolved nutrients. Water is gradually absorbed into the polymer matrix and diffuses through microscale pathways formed by the polymer–husk structure, enabling sustained nutrient release over time. Although formulation A showed slightly higher release rates than formulation B, the overall differences were not statistically significant. These results demonstrate that both PHA-CUF formulations effectively regulate nutrient availability and have potential to reduce nutrient losses compared with uncoated urea fertilizers (Naz and Sulaiman, 2016).

3.2. Effect of PHA-CUF formulations on nursery palm growth

Growth parameters of palm seedlings, including plant height, leaf number, chlorophyll content, stem diameter, and biomass, were used to evaluate fertilizer performance. Overall, all fertilizer treatments, including PHA-CUF formulations, commercial CRF, and IRF, significantly enhanced plant growth compared with the fertilizer-free control (Table 2). Among the treatments, PHA-CUF formulation A consistently promoted greater plant height and biomass accumulation, followed closely by formulation B. The comparable performance of both PHA-CUF formulations suggests that both were able to supply nitrogen effectively during the nursery stage through controlled nutrient release.

Chlorophyll content, which reflects plant nitrogen status, was substantially higher in PHA-CUF and IRF treatments than in CRF and the control. This result indicates that these formulations were able to maintain sufficient nitrogen availability to support chlorophyll synthesis and photosynthetic activity during growth. Because chlorophyll concentration is closely linked to nitrogen uptake, the observed increase suggests that nutrient release from PHA-CUF formulations was well aligned with plant nitrogen demand (Amir et al., 2005). In addition, increased stem diameter and biomass further indicate improved nutrient uptake efficiency and sustained vegetative growth under PHA-CUF application.

The superior performance of CRF in promoting fresh biomass and plant height compared with IRF can be attributed to its ability to supply nutrients more gradually, thereby reducing nutrient loss and maintaining availability throughout the nursery period (Goh et al., 2003). Overall, these results demonstrate that PHA-based controlled-release fertilizers can provide nutrient availability comparable to commercial CRFs and superior to conventional IRF, leading to improved early growth performance of oil palm seedlings.

Both PHA-based CUF formulations produced higher palm seedling biomass and plant height than the commercial CRF. This improvement is likely attributable to the gradual and sustained nutrient-release behavior of PHA-CUF, which provides a more stable nutrient supply that better matches the physiological requirements of nursery-stage oil palm seedlings (Murugan et al., 2020). In addition, formulation B resulted in slightly greater biomass and height than formulation A, suggesting that differences in pellet structure and nutrient diffusion behavior between the two formulations may influence nutrient availability during plant growth. A more sustained nutrient supply can help prevent nutrient loss and maintain adequate nitrogen availability throughout the cultivation period, thereby supporting continuous photosynthesis, vegetative growth, and root development (Laycock et al., 2013).

Throughout the three-month cultivation period, soil pH remained stable at 7.0 ± 0.00 across all treatments, indicating that none of the fertilizer types significantly affected soil acidity. Soil electrical conductivity (EC) was also measured to evaluate nutrient availability in the soil environment. The highest EC value was observed in the IRF treatment (550.0 ± 5.6 μS/cm), whereas EC values in the CRF and PHA-CUF treatments ranged from 65.4 to 72.1 μS/cm. The elevated EC in the IRF treatment reflects its rapid nutrient dissolution and the resulting high concentration of soluble ions in the soil solution. In contrast, the lower EC values observed for CRF

Table 2
Effect of PHA-based fertilizers (PHA-CUF-A and PHA-CUF-B) on the growth of nursery oil palm. Data are compared with commercial intermediate-release fertilizer (IRF, 46:0:0), commercial controlled-release fertilizer (CRF, 13:13:13), and a no-fertilizer control (PHA-B) with rice husk, no urea).

Parameters	Unit	PHA-CUF-A	PHA-CUF-B	IRF	CRF	Control
Plant height	Cm	$24.9 \pm 3.0^{a,b}$	25.2 ± 2.3^{a}	21.0 ± 1.8^{d}	22.5 ± 2.7^{c}	18.6 ± 2.3^{e}
Leave number		$5.3 \pm 0.5^{a,b}$	5.6 ± 0.5^{a}	3.3 ± 0.5^{c}	3.3 ± 0.5^{c}	$3.0 \pm 0.0^{c,d}$
Chlorophyll content	mg/g leaf fresh weight	3.1 ± 0.1^{b}	3.5 ± 0.1^{a}	3.5 ± 0.2^{a}	2.5 ± 0.1^{c}	1.5 ± 0.0^{d}
Stem thickness	Cm	$1.0 \pm 0.1^{a,b}$	1.1 ± 0.1^{a}	$1.0 \pm 0.1^{a,b}$	$1.0 \pm 0.1^{a,b}$	0.7 ± 0.1^{c}
Fresh biomass weight	G	14.0 ± 1.0^{b}	14.7 ± 1.2^{a}	12.0 ± 1.0^{d}	13.4 ± 1.4^{c}	7.5 ± 1.0^{e}
Soil pH		7.0 ± 0.0^{a}	7.0 ± 0.0^{a}	7.0 ± 0.0^{a}	7.0 ± 0.0^{a}	7.0 ± 0.0^{a}
Soil conductivity	μS/cm	$70.8 \pm 2.3^{b,c}$	69.1 ± 1.2^{c}	550.0 ± 5.6^{a}	65.1 ± 3.4^{b}	65.4 ± 2.3^{d}

Values represent mean ± standard deviation (n = 10). Different letters within a row indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

and PHA-CUF treatments indicate slower and more controlled nutrient release, which reduces excessive ion accumulation in soil while maintaining nutrient availability over time through diffusion-controlled release mechanisms (Du et al., 2006).

Visual observations of oil palm seedlings after three months of cultivation (Fig. 3) further supported the growth data. Seedlings grown without fertilizer (control, Fig. 3A) exhibited the poorest growth, with small plant size, pale leaves, and limited root development. Plants receiving IRF (Fig. 3B) showed moderate improvements in vegetative growth. In contrast, seedlings treated with CRF (Fig. 3C) and both PHA-CUF formulations (Fig. 3D and E) displayed markedly enhanced growth, darker green leaves, and well-developed root systems. Overall, PHA-CUF treatments demonstrated growth performance comparable to or better than commercial CRF, highlighting their potential as effective controlled-release fertilizers for palm nursery cultivation.

3.3. Determination of PHA-CUF and PHA degradation

SEM was employed to examine the surface morphology and structural characteristics of the PHA-CUF formulations. The SEM

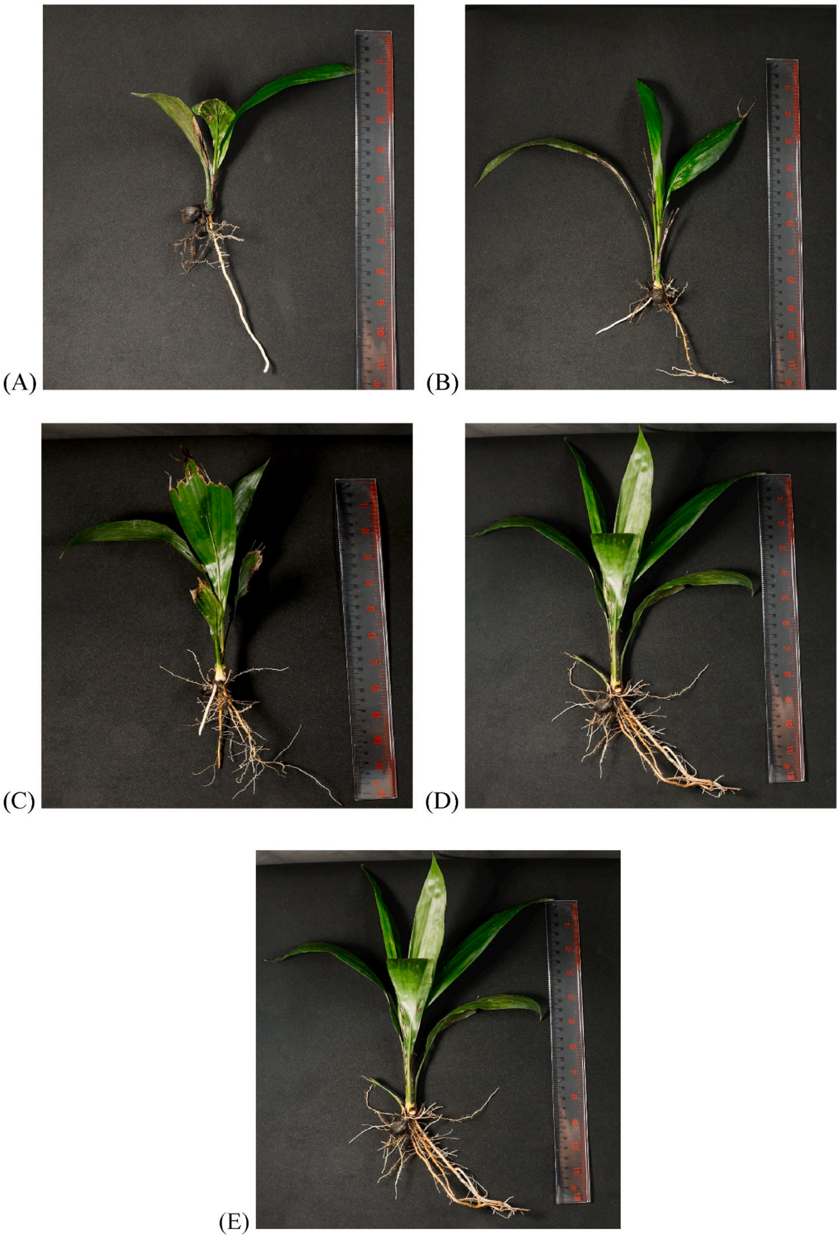

Fig. 3. The appearance of palm nursery plant using control (PHA, rice husk without urea fertilizer), A; intermediate release fertilizer (46:0:0), B; commercial controlled release (13:13:13), C; PHA-CUF-A, D; and PHA-CUF-B, E after three months cultivation.

images revealed a porous and heterogeneous surface structure (Fig. 4A), which is favorable for water penetration and gradual nutrient diffusion. In addition, fertilizer particles embedded within the polymer–husk matrix could be observed (Fig. 4B), indicating that urea was physically entrapped within the composite structure. Such morphology is consistent with controlled-release behavior, in which water ingress and nutrient diffusion occur through interconnected pores and microcracks within the matrix.

After three months of soil incubation, clear surface alterations were observed on the PHA-CUF granules (Fig. 4C), including increased surface roughness, formation of microvoids, and partial fragmentation of the outer layer. These changes indicate progressive physical and biological degradation of the polymer matrix under nursery soil conditions. Although SEM does not allow direct identification of enzymatic activity, the observed structural deterioration is consistent with previously reported biodegradation behavior of PHA-based materials in soil environments, where microbial colonization and extracellular enzymes contribute to polymer surface erosion and mass loss over time (Rakkan et al., 2022; Choonut et al., 2020).

A qualitative comparison between the two PHA-CUF formulations suggested slight differences in surface erosion patterns after soil

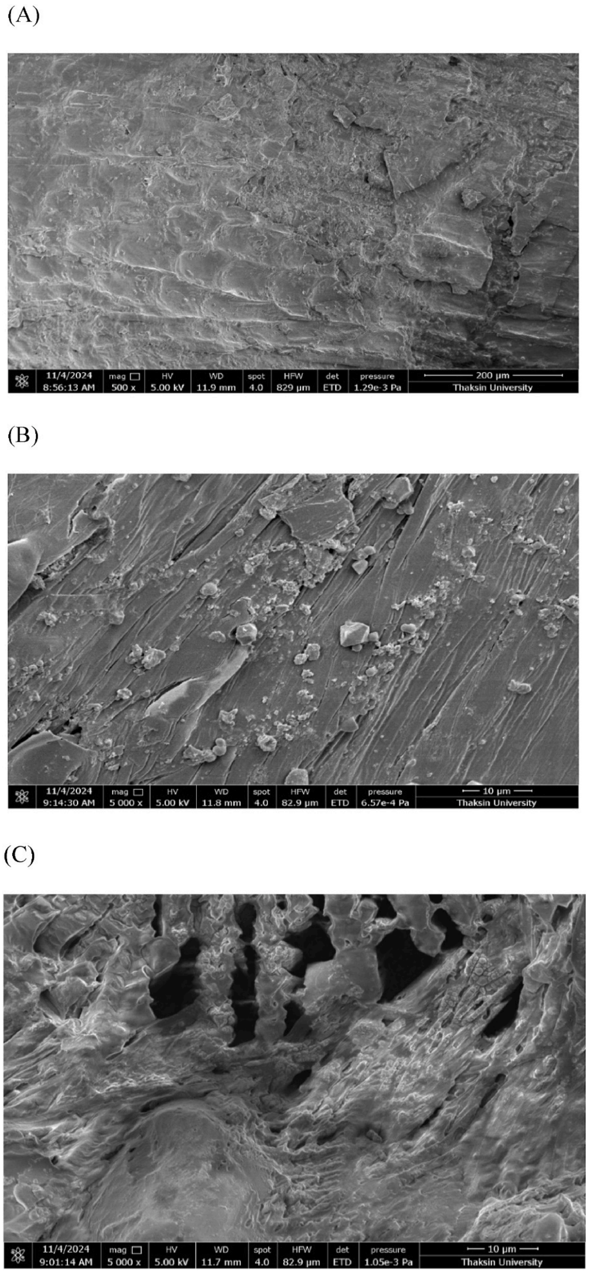

Fig. 4. Scanning electron micrographs (SEM) of PHA-based controlled release urea fertilizer (PHA-CUF) before use (A, 500 ×), the attachment of urea fertilizer (B, 5000 ×) and after three months cultivation (C, 5000 ×).

exposure. However, since SEM provides only surface-level and qualitative information, no definitive conclusions regarding relative degradation rates can be drawn from these observations alone. Therefore, the degradation behavior is discussed in terms of overall matrix breakdown rather than specific polymer compositional effects. Importantly, the observed surface deterioration confirms that the PHA-based coating is biodegradable under nursery conditions, supporting its suitability as an environmentally friendly carrier for controlled-release fertilizer applications.

3.4. Effect of PHA-CUF on soil microbial community

The effects of different fertilizer treatments on soil microbial communities were evaluated using amplicon-based metagenomic sequencing (Fig. 5). Due to budgetary and technical constraints, sequencing was performed only on three representative treatments: control (no fertilizer), IRF, and PHA-CUF. These treatments represent baseline soil conditions, conventional fertilizer practice, and the novel controlled-release formulation, respectively. Although both bacterial 16S rDNA and fungal ITS sequencing were conducted, the fungal ITS read counts did not meet the quality threshold required for reliable taxonomic assignment. Therefore, only bacterial community data are presented. Preliminary ITS results showed no apparent differences among treatments, suggesting that the major detectable shifts occurred within bacterial communities rather than fungal populations.

Only one archaeal phylum, Methanobacteriota, was detected and occurred at very low abundance in all samples. The microbial communities were dominated by bacteria, with detected phyla including Acidobacteriota, Actinomycetota, Bacillota, Bacteroidota, Bdellovibrionota, Chloroflexota, Cyanobacteriota, Fibrobacterota, Nitrospirota, Planctomycetota, Pseudomonadota, Spirochaetota, Thermomicrobiota, Verrucomicrobiota, and several minor groups. Differences in the relative abundance of several dominant phyla were observed among treatments, indicating that fertilizer type is associated with changes in overall community structure during nursery cultivation. However, because sequencing was performed on a limited number of treatments and replicates, these results should be interpreted as indicative trends rather than definitive shifts in population dynamics.

Across all treatments, Bacillota and Pseudomonadota were the dominant phyla, consistent with their broad ecological distribution and metabolic versatility in agricultural soils. A relative decrease in Bacillota was observed following fertilizer application, while Actinomycetota showed higher relative abundance in IRF-treated soil, which is consistent with previous reports linking this group to nitrogen-enriched environments. In the PHA-CUF–treated soil, a broader range of bacterial phyla was detected, including several low-abundance groups that were not observed in the control or IRF treatments (Table 3). This observation suggests that the PHA-CUF treatment was associated with a more taxonomically diverse bacterial community under the tested conditions.

Notably, higher relative abundances of Cyanobacteriota and Acidobacteriota were observed in the PHA-CUF treatment compared with IRF and control soils. Members of these phyla are commonly reported in soils rich in organic substrates and are frequently associated with carbon turnover and nutrient cycling processes (Singh et al., 2016; Kielak et al.). However, as this study relied solely on

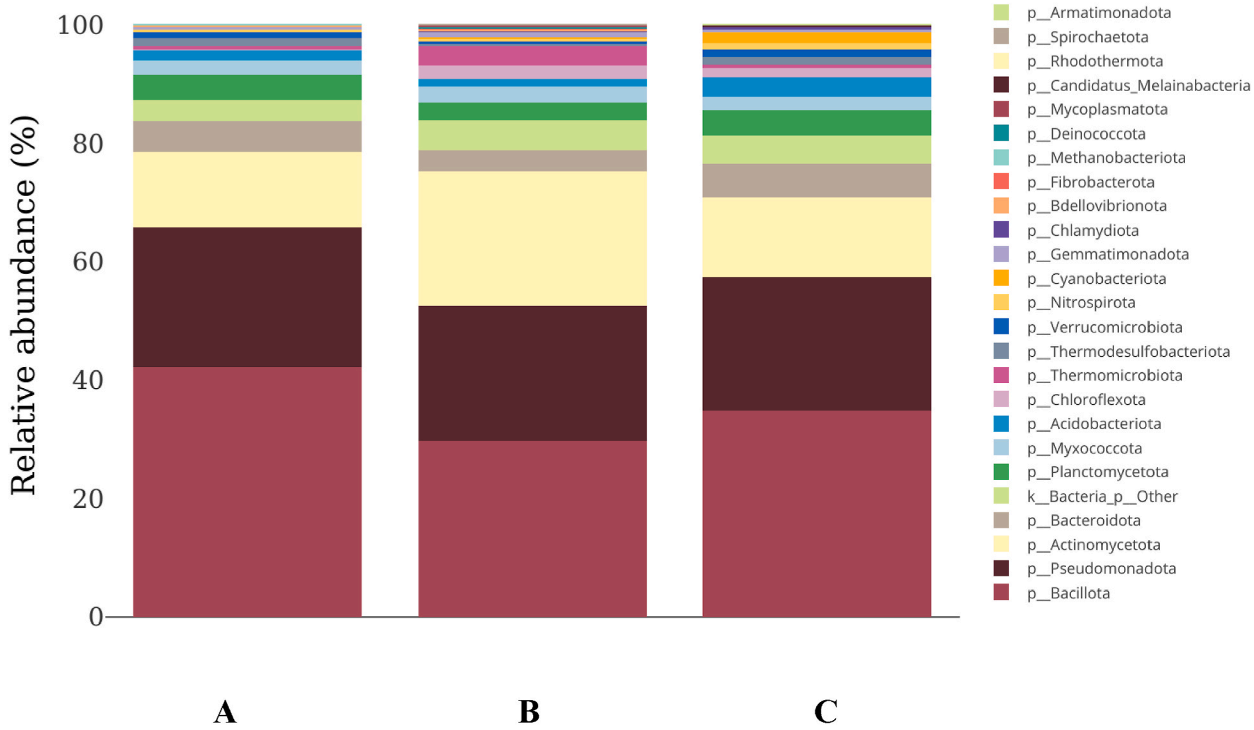

Fig. 5. Phyla of microbial community present in the soil treated with different fertilizer formulations, including cultivation soil (without any fertilizer, A), soil with IRF (B), and soil with PHA-CUF-B (C).

Table 3

Taxonomy abundance count of microbial community present in the soil treated with different fertilizer formulations, including cultivation soil (without any fertilizer), soil with IRF, and soil with PHA-CUF-B.

Kingdom	Phylum	Soil without fertilizer	Soil with IRF	Soil with PHA-CUF-B
Archaea	Methanobacteriota	8	5	5
Bacteria	Other	449	619	590
	Acidobacteriota	194	170	409
	Actinomycetota	1563	2800	1660
	Armatimonadota	0	0	3
	Bacillota	5154	3638	4268
	Bacteroidota	614	429	686
	Bdellovibrionota	5	34	10
	Candidatus_Melainabacteria	0	0	4
	Chlamydiota	18	34	55
	Chloroflexota	67	284	197
	Cyanobacteriota	0	18	238
	Deinococcota	0	17	0
	Fibrobacterota	3	20	10
	Gemmatimonadota	41	106	48
	Mycoplasmotota	0	16	0
	Myxococcota	311	331	287
	Nitrospitota	86	79	117
	Planctomycetota	511	361	516
	Pseudomonadota	2902	2774	2738
	Rhodothermota	0	0	5
	Spirochaetota	0	0	2
	Thermodesulfobacteriota	155	58	169
	Thermomicrobiota	26	388	48
	Verrucomicrobiota	120	36	162

taxonomic profiling, no direct functional conclusions can be drawn regarding nitrogen fixation, organic matter degradation, or other metabolic activities. Therefore, the observed associations should be interpreted as potential ecological correlations rather than evidence of specific functional enhancements induced by the fertilizer formulation. Future studies incorporating functional gene analysis, metatranscriptomics, or enzyme activity measurements would be required to verify these ecological roles.

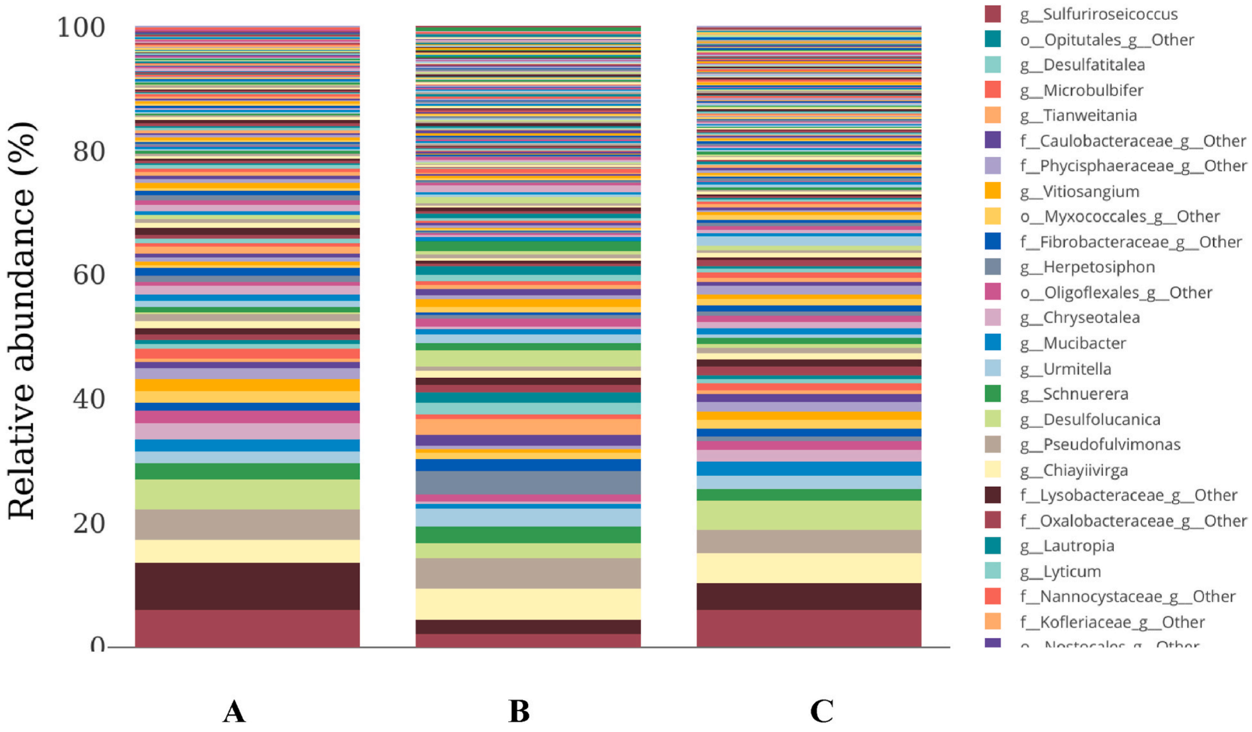

Fig. 6. Top twenty relative abundance at the genus levels present in the soil treated with different fertilizer formulations, including cultivation soil (without any fertilizer, A), soil with IRF (B), and soil with PHA-CUF-B (C).

At the genus level, variations in community composition were also observed among treatments (Fig. 6), further indicating that fertilizer type is associated with shifts in bacterial community structure. Overall, the metagenomic results suggest that PHA-CUF application is associated with a distinct and more taxonomically diverse soil bacterial community compared with IRF and unfertilized control soils, while remaining within the range of naturally occurring soil microbiota during nursery cultivation.

The proportion of the “Others” group (unclassified or low-abundance taxa) increased from 50.48% in the control soil to 58.82% under IRF treatment, while a slightly lower value (56.16%) was observed in soil treated with PHA-CUF. This pattern suggests that IRF application was associated with a broader but less structured redistribution of minor bacterial taxa, whereas PHA-CUF treatment was associated with a community structure more comparable to that of the unfertilized soil. Similar trends were observed for several genera, including Sporosarcina and Corynebacterium (Table 4), indicating that fertilizer type may influence the relative balance between dominant and minor microbial populations.

Soils treated with PHA-CUF retained relatively higher proportions of bacterial families commonly reported in agricultural soils, such as Bacillaceae (e.g., Lysinibacillus) and Peptostreptococcaceae (e.g., Romboutsia), compared with IRF-treated soils. However, because this study relied on taxonomic profiling without functional assays, these observations should be interpreted as compositional associations rather than evidence of improved soil health or microbial function. Nevertheless, the results suggest that PHA-CUF application does not induce abrupt microbial shifts compared with IRF, which may be advantageous in maintaining soil microbial stability during nursery cultivation.

A similar trend was reported by Murugan et al. (2020), who evaluated a PHA-based controlled-release fertilizer containing P (3HB-co-3HHx) and NPK fertilizer (17:8:9) in oil palm nurseries and observed both improved plant growth and altered soil microbial composition compared with conventional fertilizers. The higher plant growth reported in their study compared with the present work may be attributed to the balanced and sustained supply of nitrogen, phosphorus, and potassium, which supports coordinated root and shoot development and overall plant physiological performance. In contrast, the present study focused on urea-based formulations to specifically evaluate nitrogen-release behavior, which represents the most widely used nitrogen fertilizer globally and provides a relevant baseline for assessing controlled-release performance.

It should be noted that microbial sequencing in this study was limited to three representative treatments, which restricts statistical power and generalization of microbial diversity patterns. Therefore, the microbial results should be considered exploratory and hypothesis-generating rather than conclusive. Future studies incorporating additional treatments, biological replicates, and functional microbial analyses will be required to better elucidate the ecological impacts of PHA-based fertilizer systems.

From an economic and scalability perspective, the estimated production cost of PHA-based CUF (USD 4–6 per kg) remains higher than that of conventional polymer coatings such as polyethylene or polyurethane (< USD 2 per kg). However, the biodegradability and renewable origin of PHA provide important environmental advantages by reducing long-term soil plastic accumulation. With ongoing development of low-cost PHA production using waste substrates and optimized fermentation processes, the cost gap is expected to decrease. Moreover, the PHA-CUF fabrication process, which involves melt-compounding and pelletizing, is compatible with existing industrial fertilizer coating technologies, suggesting favorable scalability once PHA production becomes more economically competitive.

4. Conclusion

This study evaluated the performance of PHA-based controlled-release urea fertilizers (PHA-CUF) formulated with rice husk as a hydrophilic filler to improve water uptake and nutrient-release behavior. The PHA-CUF formulations effectively supported oil palm seedling growth during the nursery stage and showed agronomic performance comparable to or better than conventional fertilizer treatments. In addition, metagenomic analysis indicated that PHA-CUF application was associated with a distinct soil bacterial community structure compared with immediate-release fertilizer, while remaining within the range of typical agricultural soil microbiota. The incorporation of rice husk contributed to water absorption, pellet integrity, and initial nutrient diffusion, demonstrating the feasibility of using low-cost lignocellulosic residues to enhance the functional performance of biodegradable fertilizer carriers. The PHA matrix served as a physical barrier that slowed urea dissolution, resulting in more gradual nutrient release compared with uncoated urea, which is beneficial for reducing nutrient losses and improving nutrient-use efficiency during early plant growth. It should be noted that this study was conducted at nursery scale under controlled conditions, which may not fully represent environmental variability encountered under field conditions. Therefore, long-term biodegradation behavior, nutrient-release kinetics under different soil types, and effects on crop yield remain to be investigated. Future studies should include field-scale trials, optimization of PHA–lignocellulosic composite formulations, and evaluation of alternative agricultural residues to improve both performance and economic feasibility. From a practical perspective, large-scale implementation of PHA-based fertilizers is currently constrained by production costs associated with microbial fermentation and polymer recovery. Further research should focus on reducing production costs through the use of low-cost or waste-derived carbon substrates, improved bioprocess efficiency, and simplified downstream processing. In addition, comprehensive techno-economic and life-cycle assessments are necessary to evaluate the environmental benefits and commercial viability of PHA-based controlled-release fertilizer systems. Addressing these factors will be critical for advancing biodegradable polymer technologies toward sustainable agricultural applications.

CRediT authorship contribution statement

Nassareeya Binlateh: Investigation, Formal analysis, Data curation. **Nuchnaphat Noocharoen:** Investigation, Formal analysis, Data curation. **Sorapong Benchasri:** Supervision, Methodology, Conceptualization. **Kanokphorn Sangkharak:** Writing – review &

Table 4
Top twenty relative abundance at the genus levels present in the soil treated with different fertilizer formulations, including cultivation soil (without any fertilizer), soil with IRF, and soil with PHA-CUF-B.

Kingdom	Phylum	Class	Order	Family	Genus	Soil without fertilizer	Soil with IRF	Soil with PHA-CUF-B
Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Lysinibacillus	5.99%	2.23%	6.01%
Bacteria	Bacillota	Clostridia	Peptostreptococcales	Peptostreptococcaceae	Romboutsia	7.62%	2.16%	4.24%
Bacteria	Other	Other	Other	Other	Other	3.67%	5.06%	4.83%
Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Niallia	4.79%	4.90%	3.86%
Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Bacillus	5.00%	2.33%	4.64%
Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Priestia	2.51%	2.72%	1.91%
Bacteria	Pseudomonadota	Alphaproteobacteria	Geminicoccales	Geminicoccaceae	Arboricoccus	1.93%	2.96%	2.17%
Bacteria	Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	Hyphomicrobiaceae	Pedomicrobium	2.07%	0.80%	2.16%
Bacteria	Bacteroidota	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	2.46%	0.33%	1.98%
Bacteria	Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	Hyphomicrobiaceae	Methylothermalis	2.09%	1.19%	1.47%
Bacteria	Bacillota	Bacilli	Bacillales	Caryophanaceae	Sporosarcina	0.00%	3.76%	0.63%
Bacteria	Actinomycetota	Other	Other	Other	Other	1.21%	1.86%	1.28%
Bacteria	Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	Hyphomicrobiaceae	Other	1.80%	1.00%	1.52%
Bacteria	Bacillota	Clostridia	Peptostreptococcales	Peptostreptococcaceae	Other	2.09%	0.70%	1.20%
Bacteria	Pseudomonadota	Gammaproteobacteria	Other	Other	Other	1.77%	0.44%	1.68%
Bacteria	Actinomycetota	Actinomycetes	Micromonosporales	Micromonosporaceae	Other	0.88%	1.79%	1.16%
Bacteria	Actinomycetota	Actinomycetes	Mycobacteriales	Corynebacteriaceae	Corynebacterium	0.69%	2.45%	0.61%
Bacteria	Bacillota	Bacilli	Bacillales	Caryophanaceae	Solibacillus	1.46%	0.71%	1.16%
Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Other	0.70%	1.97%	0.63%
Bacteria	Actinomycetota	Acidimicrobiia	Acidimicrobiales	Ilumatobacteraceae	Desertimonas	0.79%	1.81%	0.69%
Others	Others	Others	Others	Others	Others	50.48%	58.82%	56.16%

editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Grammaly in order to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Data availability

No data was used for the research described in the article.

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