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Comparative Analysis of Rhizosphere Microbial Diversity and Growth Response of *Eleutherine bulbosa* under Organic and Inorganic Fertilizer Regimes

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Abstract
The transition towards sustainable agriculture requires bio-based alternatives to synthetic fertilizers that simultaneously promote soil health and plant productivity. This study explores the impact of *Eleutherine bulbosa*-derived composts (leaf and bulb) in comparison with Black Soldier Fly (BSF) frass, inorganic NPK fertilizer, and an untreated control on the rhizosphere microbiome and agronomic performance of *E. bulbosa*. Using 16S rRNA amplicon sequencing and PICRUSt2 functional inference, we assessed bacterial community structure, diversity, and predicted metabolic pathways across treatments. Alpha diversity metrics revealed that NPK-treated soil exhibited the highest species richness, while BSF treatments maintained more even microbial communities. Beta diversity analysis indicated distinct clustering of microbial communities under inorganic versus organic treatments. Notably, bulb compost enriched beneficial taxa such as *Caulifigura coniformis* (0.03% relative abundance), while NPK treatments favored copiotrophic species like *Lactococcus sp.* (0.07%). Predicted functions under NPK emphasized rapid growth pathways (e.g., purine biosynthesis), whereas organic inputs enhanced nutrient cycling (e.g., TCA cycle, sulfate assimilation, nitrate reduction). BSF compost produced the highest total plant biomass and chlorophyll content, while bulb compost yielded the most bulbs, and leaf compost led to tallest plants. These findings demonstrate that *E. bulbosa*-derived composts promote beneficial microbial functions and plant productivity, supporting a circular farming model where agricultural waste is recycled for self-sustaining cultivation.
Keywords: *Eleutherine bulbosa*; compost; organic fertilizer; rhizosphere; microbiome; circular farming

Introduction
The intensive application of synthetic fertilizers has long supported global agricultural productivity but at the cost of environmental degradation, soil microbial disruption, and biodiversity loss. Organic alternatives, especially those derived from agricultural waste, are gaining attention as components of circular farming systems that aim to restore ecological balance. *Eleutherine bulbosa* (commonly known as Bawang Dayak) is a traditional medicinal plant native to Southeast Asia. Although its bulb is highly valued for pharmacological applications (Kamarudin et al., 2021), its leaves and spent bulbs are often discarded during harvesting and processing, contributing to organic waste.

The potential to upcycle this biomass into compost offers a novel dual-function strategy: reduce agricultural waste while creating plant-based fertilizers. While numerous studies have evaluated the medicinal properties of *E. bulbosa*, there is limited research on the use of its biomass as fertilizer or its effects on soil microbial communities. Moreover, while the benefits of Black Soldier Fly (BSF) frass and other organic inputs have been documented (Beesigamukama et al., 2020; Baweja et al., 2020;

Brempong and Addo-Danso, 2022), a systematic comparison involving self-derived fertilizers and their functional impact on the rhizosphere microbiome remains unexplored for *E. bulbosa*.

This study aims to bridge these gaps by evaluating the efficacy of *E. bulbosa* bulb and leaf composts relative to BSF compost, NPK fertilizer, and a control. We assessed bacterial diversity, predicted microbial metabolic functions, and plant morphological traits. The findings contribute to understanding the role of plant-based fertilizers in sustainable and medicinal plant agriculture.

Materials and methods

Fertilizer Treatments and Experimental Setup

Five treatments were applied: (1) control (no fertilizer), (2) NPK 15:15:15 inorganic fertilizer, (3) BSF frass, (4) *E. bulbosa* leaf compost, and (5) *E. bulbosa* bulb compost. Each treatment was adjusted to the same electrical conductivity (EC = 1.2 dS/m) to normalize nutrient levels (Barrett et al., 2020) (Table 1). Plants were grown for six weeks in controlled conditions.

Table 1: Amount of fertilizer (g) dissolved to achieve a 1.2 dS/m EC reading

Type of fertilizer	Fertilizer amount (g)	EC reading (dS/m)
NPK	4	1.2
BSF	11	1.2
Bulb compost	31	1.2
Leaves compost	35	1.2

Soil Sampling and DNA Extraction

At the end of the experiment, rhizosphere soil samples were collected from each treatment replicate. Total genomic DNA was extracted using a commercial soil DNA kit and subjected to 16S rRNA gene amplicon sequencing targeting the V3-V4 regions using Illumina MiSeq.

Bioinformatics and Statistical Analysis

Raw reads were processed using QIIME2 and DADA2 for quality filtering and ASV clustering. Taxonomy was assigned using the SILVA database. Alpha and beta diversity were analyzed using Chao1, Shannon indices (Haegeman et al., 2013) and Bray-Curtis PCoA (Kers & Saccenti, 2022; Wang et al., 2022), respectively. Predicted microbial functions were inferred using PICRUSt2 (Douglas et al., 2020), and pathway comparisons were performed with STAMP (Parks et al., 2014).

Plant Growth and Yield Measurements

Morphological traits were measured at harvest: plant height, number of leaves, number of tillers, chlorophyll SPAD value, fresh bulb weight, number of bulbs, and bulb diameter (Marlin et al., 2022).

Results

Microbial Diversity and Community Structure

According to Figures 1 and 2, the Chao1 richness index showed the highest species count in NPK-treated soils, while the Shannon diversity indicated more balanced evenness under BSF and bulb compost treatments. PCoA (Figure 3) revealed distinct clustering of NPK samples, separated from organic treatments (BSF, leaf, and bulb compost).

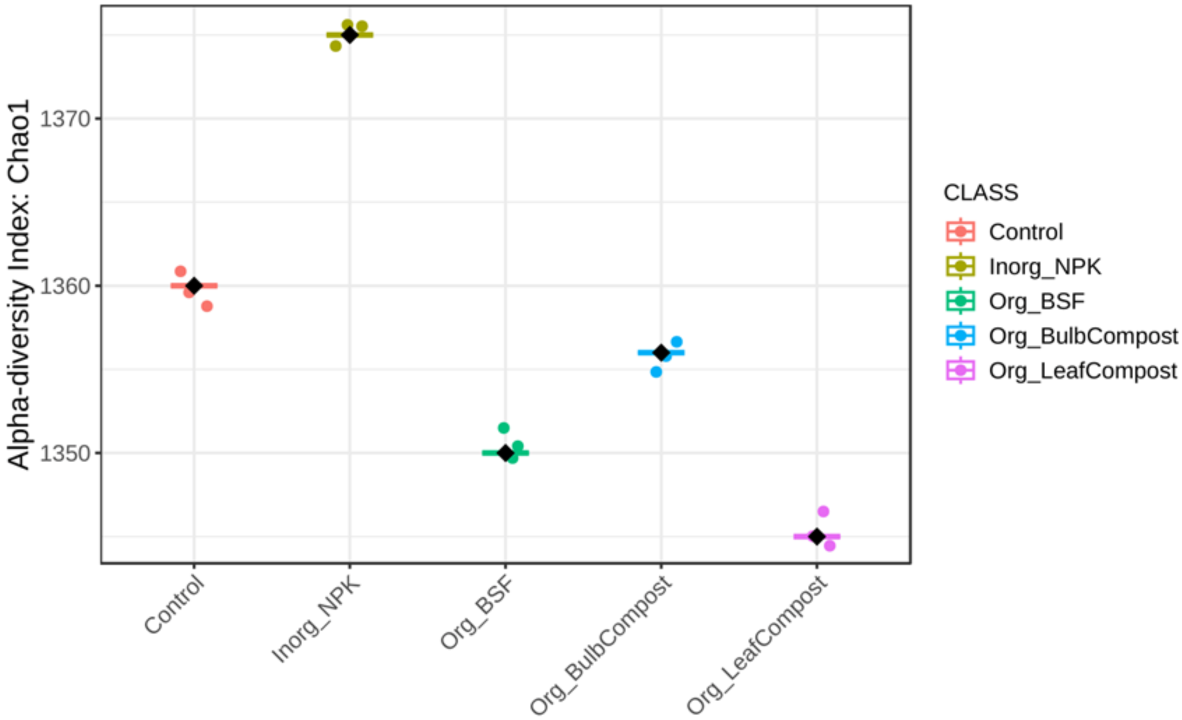

Figure 1 Chao1 alpha diversity metrics plot between treatments

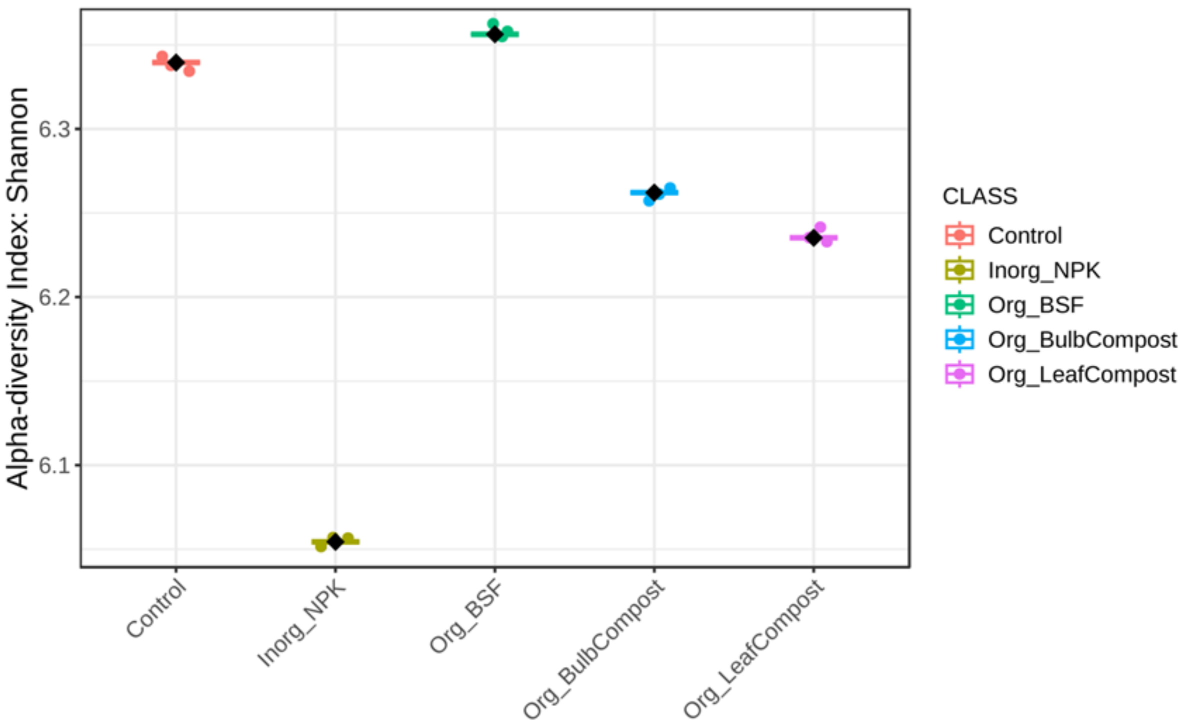

Figure 2 Shannon alpha diversity metrics plot between treatments

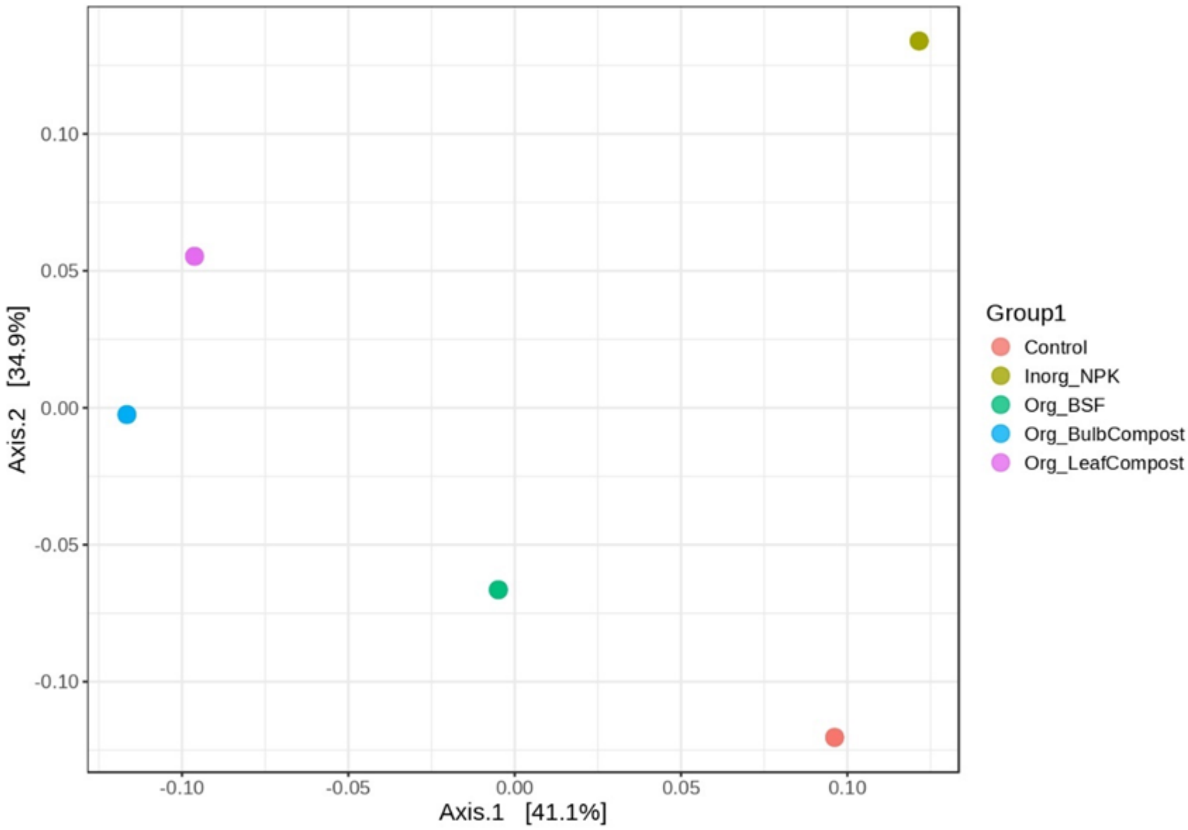

Figure 3 Beta diversity Bray-Curtis PCoA plot between treatments

Taxonomic Composition
Control and compost-treated soils were dominated by *Planctomycetaceae* and *Xanthobacteraceae*, while NPK-treated soils showed a rise in *Streptococcaceae* (notably *Lactococcus sp.*, 0.07% abundance) according to Figure 4. Bulb compost increased *Caulifigura coniformis* abundance to 0.03%, higher than all other treatments (Figure 5).

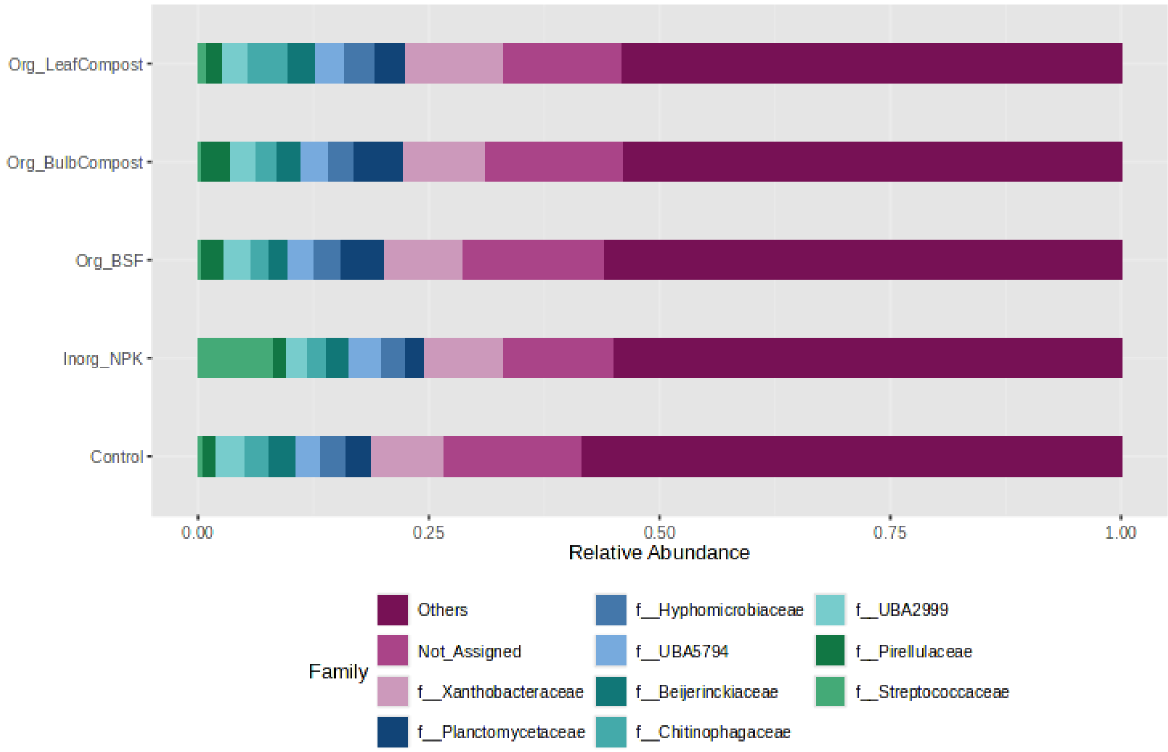

Figure 4 Stacked bar plot showing the average relative abundance of top 10 bacterial families per treatment.

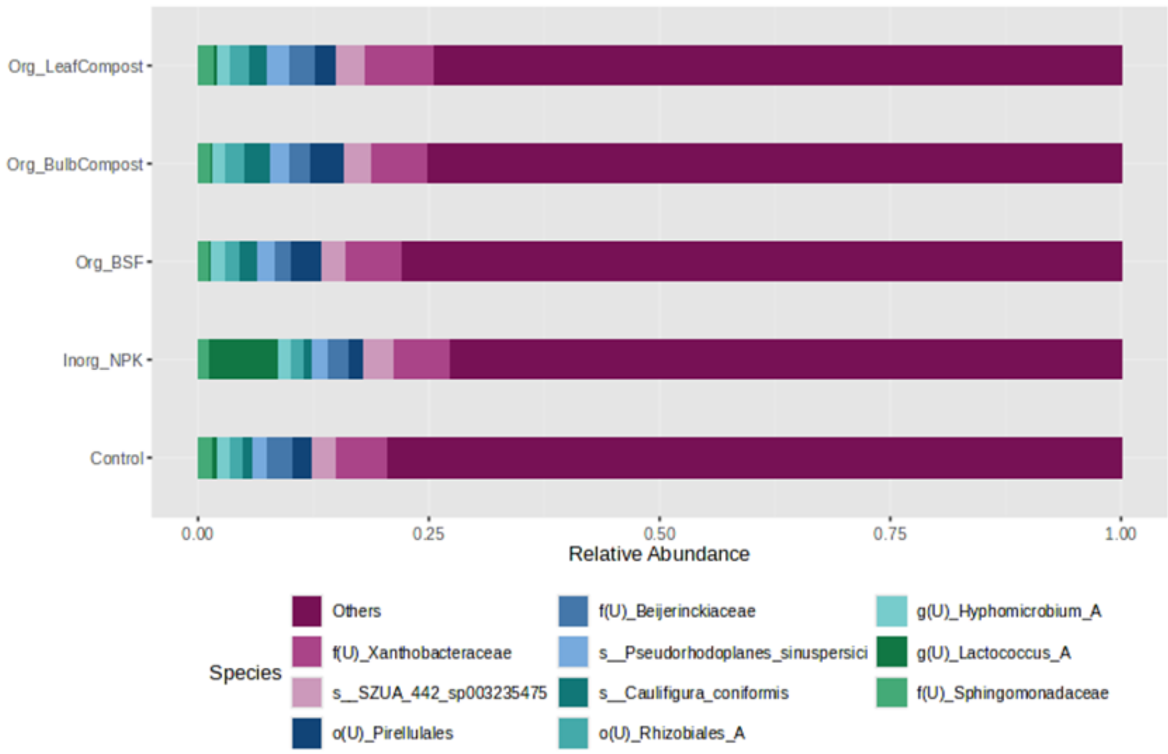

Figure 5 Stacked bar plot showing the average relative abundance of top 10 bacterial species per treatment.

Predicted Functional Pathways

STAMP analysis showed that NPK treatment enriched growth-associated pathways such as purine metabolism and DNA replication (Figure 6). Conversely, BSF and *E. bulbosa* composts enhanced TCA cycle, sulfur assimilation, and nitrate reduction—functions linked to nutrient turnover and plant-microbe symbiosis (Figure 7).

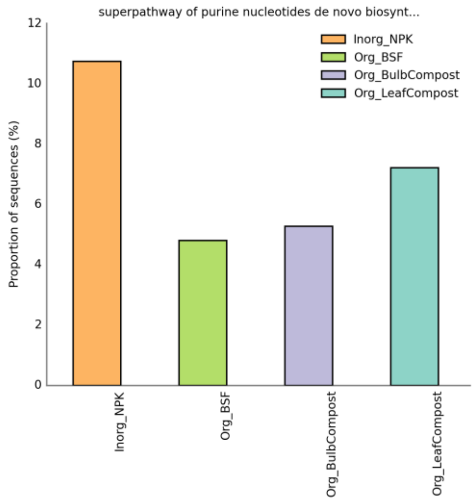

Figure 6 Relative abundance of the purine metabolism pathways across fertilizer treatments

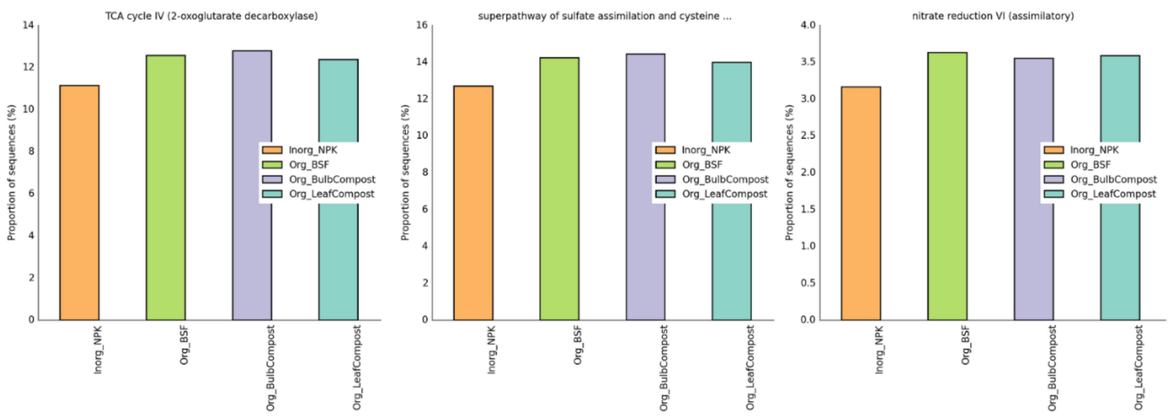

Figure 7 Relative abundance of the TCA cycle (left), sulfate assimilation (middle) and nitrate reduction (right) pathways across fertilizer treatments

Plant Morphological Performance

According to Tables 2 and 3, BSF compost yielded the highest total plant biomass (40.77 ± 3.4 g), chlorophyll content (27.55 ± 2.6 SPAD), and fresh bulb weight (26.79 ± 1.2 g). Bulb compost generated the most bulbs (4 ± 0.4), while leaf compost supported the tallest plants (42.28 ± 3.2 cm). All organic treatments performed better than the control in all measured parameters.

Table 2: Mean Comparison for growth parameters of *E. bulbosa* cultivated for 6 weeks

Treatment	Plant height (cm)	Number of tillers	Number of leaves	Chlorophyll content (SPAD)
T_0	30.80 ± 1.9^b	1.2 ± 0.3^c	1.5 ± 0.3^c	23.28 ± 1.5^a
T_1	39.70 ± 2.8^a	10.8 ± 1.2^a	21.0 ± 1.1^a	27.55 ± 2.6^a
T_2	34.98 ± 1.9^{ab}	5.3 ± 1.3^b	10.0 ± 0.9^b	25.38 ± 2.1^a
T_3	42.28 ± 3.2^a	5.0 ± 1.2^b	8.5 ± 1.0^b	27.23 ± 2.9^a
T_4	28.73 ± 3.1^b	1.8 ± 0.3^c	2.3 ± 0.5^c	23.03 ± 1.4^a

Data was expressed as mean ± standard error (SE); T_0 = control, T_1 = black soldier fly frass fertilizer, T_2 = *E. bulbosa* bulb compost, T_3 = *E. bulbosa* leaves compost, T_4 = NPK fertilizer; different alphabet indicated significantly different at P≤ 0.05 using Post Hoc Duncan Multiple Range Test (DMRT); n =5

Table 3: Mean Comparison for yield parameters of *E. bulbosa* cultivated for 6 weeks

Treatment	Total plant weight (g)	Fresh bulb weight (g)	Bulb diameter (mm)	Number of bulbs
T_0	19.19 ± 1.3^b	15.71 ± 0.8^c	22.24 ± 3.4^{ab}	1.0 ± 0.0^c
T_1	40.77 ± 3.4^a	26.79 ± 1.2^a	27.03 ± 4.2^a	3.3 ± 0.5^{ab}
T_2	34.80 ± 2.1^a	21.36 ± 1.3^b	23.62 ± 1.7^{ab}	4.0 ± 0.4^a
T_3	25.69 ± 2.5^b	17.91 ± 1.2^{bc}	25.21 ± 4.0^{ab}	2.0 ± 0.7^{bc}
T_4	19.72 ± 1.4^b	11.90 ± 1.4^d	16.17 ± 1.1^b	1.3 ± 0.3^c

Data was expressed as mean ± standard error (SE); T_0 = control, T_1 = black soldier fly frass fertilizer, T_2 = *E. bulbosa* bulb compost, T_3 = *E. bulbosa* leaves compost, T_4 = NPK fertilizer; different alphabet indicated significantly different at P≤ 0.05 using Post Hoc Duncan Multiple Range Test (DMRT); n =5

Discussion

This study highlights the dual agronomic and ecological benefits of *E. bulbosa*-derived composts. The composts enhanced microbial taxa involved in nutrient cycling and suppressed fast-growing copiotrophs favored by NPK fertilizers. The microbial shifts observed suggest a more stable and functionally enriched rhizosphere under organic inputs. BSF compost remains a potent organic option due to its high nutrient content and chitin-associated biostimulant properties (Kemboi et al., 2022), but bulb and leaf composts derived from *E. bulbosa* offer locally accessible alternatives with comparable benefits.

Our findings align with prior reports indicating that organic amendments promote beneficial microbes and enhance plant physiological traits (Baweja et al., 2020; Brempong and Addo-Danso al., 2022). The enrichment of TCA cycle and nitrogen assimilation pathways suggests potential synergies in nutrient mobilization and uptake. These functional predictions support the hypothesis that composted *E. bulbosa* biomass contributes positively to soil microbial ecology and plant health.

Conclusion

E. bulbosa-derived composts significantly enhanced microbial diversity, functional potential, and plant performance, supporting their viability as circular fertilizer inputs. These findings contribute to the development of bio-based, sustainable cultivation systems for medicinal plants and advocate the reuse of agricultural by-products as functional amendments.

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