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Short-Term Responses of Soil Microbial Functional Diversity to Organic Amendments in Paddy Soils

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ABSTRACT

Soil microbial functional diversity is critical to soil health and agricultural sustainability. This study evaluated the short-term responses of soil microbial functional diversity to organic amendments in paddy fields at IADA Kemasin Semerak. Soil samples were collected at three stages: before amendment application (T1), four weeks post-application (T2), and after paddy harvest (T3). The effects of organic amendments on soil chemical properties were evaluated by measuring soil pH, electrical conductivity, total nitrogen, soil organic carbon, and available phosphorus. Microbial functional diversity was assessed using the Biolog Ecoplate system, focusing on microbial activity, species richness, species evenness, and Shannon diversity index. Substrate utilization was further classified into five functional guilds. The application of organic amendments significantly increased soil pH and the availability of phosphorus. Results indicated a significant increase in microbial activity and species richness following amendment application, while no significant differences were observed in species evenness or diversity index across sampling stages. Organic amendment application enhanced microbial substrate utilization across all functional groups compared with the pre-amendment treatment. The greatest increases were observed for carboxylic and acetic acids (133.8%) and amino acids (127.0%). Microbial substrate utilization remained elevated following paddy harvest, with carbohydrates showing the highest increase (149.9%) relative to the pre-amendment condition. The application of organic amendments and following paddy harvest significantly increased utilization of L-arginine, L-asparagine, L-phenylalanine, pyruvic acid methyl ester, D-mannitol, N-acetyl-D-glucosamine, D-cellobiose, D-galacturonic acid, 4-hydroxybenzoic acid, γ-hydroxybutyric acid, D-malic acid, Tween 40, and Tween 80. In conclusion, organic amendments exert positive short-term effects on soil microbial functional diversity, particularly by enhancing microbial activity, species richness, and substrate utilization capacity.

***Key words**: microbial activity, microbial diversity, species richness, Ecoplate, paddy*

INTRODUCTION

Soil ecological processes are predominantly driven by soil microorganisms, which regulate essential ecosystem functions. Microbial metabolic activity and functional diversity are closely associated with biogeochemical cycles and are considered key indicators for evaluating soil ecological functions and productivity (Song *et al*., 2022). Beyond diversity alone, the strength of microbial functional diversity, referring to the intensity and range of substrate utilization and metabolic capacity, provides deeper insight into the resilience, adaptability, and efficiency of microbial communities in supporting soil ecosystem services (Ramond *et al*., 2025). Soil microbial functional diversity plays a crucial role in maintaining soil health and fertility, as microorganisms are actively involved in nutrient cycling, organic matter decomposition, and the regulation of soil structure (Adomako *et al., 2022*; Chen *et al*., 2024). In agricultural ecosystems, particularly in paddy fields, the diversity and functional strength of microbial populations are essential for sustaining crop productivity and maintaining long-term soil quality. These microbial attributes determine how effectively soils respond to environmental changes and management practices (Chen *at al*., 2026). Organic amendments, such as compost,

manure, and crop residues, are widely applied to enhance soil fertility, improve soil structure, and promote sustainable agricultural practices. By enriching the soil with organic matter and diverse carbon sources, these amendments can stimulate microbial activity, alter community composition, and enhance both the diversity and functional strength of soil microbial communities. The introduction of varied substrates through organic inputs may support a broader spectrum of microbial metabolic functions, thereby improving ecosystem functionality (Shu *et al*., 2022).

To assess microbial functional diversity and its strength, the Biolog system has been extensively used as a culture-based approach that evaluates the community-level physiological profiling (CLPP) of soil microbes. This system measures the utilization patterns of different carbon substrates, providing quantitative indices such as average well color development (AWCD), richness, and diversity indices, which collectively reflect microbial metabolic activity and functional potential (Liu *et al*., 2026). As such, the Biolog system serves as a practical and sensitive tool for detecting short-term changes in microbial functional dynamics following soil amendments. Despite the recognized benefits of organic amendments, their short-term effects on soil microbial functional diversity in paddy fields remain insufficiently understood. This knowledge gap limits the ability to optimize the timing, type, and application rates of organic inputs for maximizing microbial-driven soil functions. In particular, the early-stage responses of microbial communities following amendment application are critical, as they may determine subsequent nutrient availability and crop performance. Therefore, this study was conducted to evaluate the short-term response of soil microbial functional diversity and its functional strength to the application of organic amendments in paddy fields. Assessments were carried out at three key stages: prior to the application of organic amendments, four weeks after amendment application, and after paddy harvest, five months after planting, using the Biolog system.

MATERIALS AND METHODS

Study site and experimental design

A field experiment was conducted in a paddy rice field in Integrated Agricultural Development Area (IADA) Kemasin Semerak , Bachok district of Kelantan, Malaysia during off-season from July 2024 to November 2024. The study was conducted in a 24 research plots size 5m x 5m each and was arranged in a randomized complete block experimental design with three replicates. Soil amendments consist of biofertilizer-based amendments, biochar-based organic fertilizer and compost.

Soil sampling

Soil sampling was carried out at a depth of 0–20 cm using a soil auger. Composite soil samples were collected at three distinct time points: prior to the application of organic amendments (T1), four weeks after the application of the amendments (T2), and following the paddy harvest, which occurred five months after planting (T3). Following collection, soil samples designated for chemical analyses were air-dried, finely ground, and sieved through a 2-mm mesh to obtain a uniform sample matrix. Separate composite soil samples intended for microbial functional diversity analysis were aseptically transferred into sterile plastic bags and stored at 4 °C until laboratory processing.

Soil chemical properties

The collected soil samples were analyzed for selected soil fertility parameters. Soil pH and electrical conductivity (EC) were determined in a soil-water suspension at a ratio of 1:10 (w/v) following standard laboratory procedures. Soil organic carbon (SOC) was determined using the

Walkley and Black wet oxidation method as described by Nelson and Sommers (1982). Total nitrogen (TN) was analyzed using the Kjeldahl digestion method. Available phosphorus was extracted using the Bray and Kurtz (1945) method and quantified colorimetrically using a UV-visible spectrophotometer.

Microbial Functional Diversity analysis

Microbial functional diversity was assessed based on microbial activity and community-level physiological profiling (CLPP) using the Biolog EcoPlate™ system. The EcoPlate™ contains 31 ecologically relevant carbon substrates that enable the characterization of microbial metabolic potential. A characteristic metabolic fingerprint is generated from the interaction between the soil microbial community and the range of carbon sources (Garland, 1997). Soil suspensions were prepared by mixing 100 g of fresh soil with 1 L of sterile distilled water, followed by agitation for 30 min. The suspension was then filtered through a 0.45 µm membrane filter to obtain a uniform microbial extract. Subsequently, 100 µL of the filtrate was inoculated into each well of the EcoPlate™ and incubated at 27°C. Microbial utilization of carbon substrates resulted in cellular respiration, leading to the reduction of a tetrazolium dye and the development of a purple color. The intensity of color in each well was measured as optical density (OD) at 590 nm using a microplate reader. To ensure that the measured response reflected substrate utilization rather than residual soluble organic carbon from the rhizosphere, OD readings were recorded after 48 h of incubation (Gomez *et al*., 2006).

Average well color development (AWCD) was used as an indicator of overall microbial activity and was calculated from optical density (OD) readings at 590 nm after 48 h of incubation. AWCD was determined using the following equation (Gomez *et al*., 2006):

$$AWCD = \sum ODi / 31$$

where ODi represents the optical density value of each well in the EcoPlate™, and 31 corresponds to the total number of carbon substrates assessed.

Richness (R) and Shannon diversity index (H) were calculated following the method described by Garland (1997). Richness was defined as the number of oxidized carbon substrates utilized by the microbial community, with a positive response identified when the optical density (OD) of a Biolog EcoPlate™ well reached or exceeded a threshold value of 0.25.

The Shannon diversity index (H) was calculated using the following equation:

$$H=-\sum pi\ (\ln pi)$$

where p_i represents the proportional activity of each substrate, calculated as the ratio of the optical density of each well (OD_i) to the sum of optical densities across all wells (ΣOD_i), and ln denotes the natural logarithm.

Shannon evenness (E) was then derived from the Shannon diversity index (H) and substrate richness (R) following Zak *et al*. (1994), and was calculated as:

$$E= H / \ln R$$

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Substrate utilization

Substrate utilization by the microbial community was assessed based on optical density (OD) readings from each EcoPlate™ well after 48 h of incubation. The substrates included in the EcoPlate™ were further classified into five functional guilds according to Weber and Legge (2009), reflecting different categories of ecologically relevant carbon sources (**Table 1**).

Table 1: Substrates in Biolog Ecoplate™ categorized into five functional guilds

Functional guild	Substrates
Amines and Amides	Phenylethylamine
	Putrescine
Amino Acid	L-Arginine
	L-Asparagine
	LPhenylalanine
	L-Serine
	L-Threonine
	Glycyl-L-Glutamic Acid
Carbohydrates	b-Methyl-D-Glucoside
	Pyruvic Acid Methyl Ester
	D-Xylose
	i-Erythritol
	D-Mannitol
	N-Acetyl-D-Glucosamine
	D-Cellobiose
	Glucose-1- Phosphate
	α-D-Lactose
	D,L-α-Glycerol Phosphate
Carboxylic and acetic acid	D-Galactonic Acid γ-Lactone
	D-Galacturonic Acid
	2-Hydroxy Benzoic Acid
	4-Hydroxy Benzoic Acid
	γ- Hydroxybutyric Acid
	D-Glucosaminic Acid
	Itaconic Acid
	α-Ketobutyric Acid
	D-Malic Acid
Polymer	Tween 40
	Tween 80
	α- Cyclodextrin
	Glycogen

Statistical analysis

Analysis of variance and multiple comparisons of means Tukey's HSD test were used for the interpretation of AWCD, R, H, and E. All statistical analyses were performed with Minitab 17.

RESULTS AND DISCUSSION

Soil chemical properties

The application of organic amendments significantly increased soil pH and the availability of phosphorus (**Table 2**). In contrast, electrical conductivity (EC), total nitrogen (TN), and soil organic carbon (SOC) were not significantly influenced by the addition of these amendments.

Table 2: Soil chemical properties

Treatment	pH	EC (µs/cm)	Total Nitrogen (%)	OrganicCarbon (%)	Available Phosphorus (µg/g)
T1	4.080±0.094b	291.363±31.219a	0.259±0.025a	2.534±0.269a	31.391±3.674b
T2	5.622±0.214a	237.250±21.505a	0.298±0.014a	2.466±0.091a	53.188±3.573a
T3	5.215±0.151a	238.475±18.769a	0.261±0.011a	2.369±0.100a	45.188±1.523a

Notes: The mean of pH, EC, total nitrogen, organic carbon, available phosphorus and data are expressed in means ± SE. The mean comparison was performed using Tukey's HSD. The mean with the same letter is not significant ($p \leq 0.05$). T1: Before the organic amendment application; T2: After the organic amendment application; T3: After paddy harvest

The observed increase in soil pH is consistent with previous findings reported by Rendana *et al*. (2022) and Xu *et al*. (2025), who demonstrated that the combined application of chemical fertilizers and organic amendments can enhance soil pH and phosphorus availability. The increase in soil pH may be attributed to the input of base cations from organic materials, which help neutralize soil acidity and improve buffering capacity, as reported by Ye *et al* (2025). In addition, humic substances and biochar contribute to pH regulation through their ability to adsorb H^+ ions and release base cations, thereby mitigating acidic conditions. The integration of organic and mineral amendments, such as humic acid in combination with clay minerals or biochar, has been shown to further enhance soil pH and nutrient availability in paddy systems (Rendana *et al*., 2022). Despite these improvements, the lack of significant changes in EC, TN, and SOC suggests that the short-term application rate or duration of organic amendments may not have been sufficient to alter these parameters markedly. Alternatively, these properties may require longer-term accumulation and stabilization processes before measurable differences become apparent.

Microbial functional diversity

The results of this study indicated that AWCD and R increased following the application of organic amendments (T1) as shown in **Table 3**. However, no significant differences were observed for these two parameters after the paddy harvest. Additionally, no significant differences were found in H or E between the different treatment groups.

Table 3: Microbial functional diversity comprised of AWCD, H, R and E

Treatment	AWCD	H	R	E
T1	0.608±0.154b	2.743±0.349a	15.875±3.446b	0.999±0.159a
T2	1.252±0.200a	3.268±0.032a	29.75±0.650a	0.964±0.005a
T3	1.243±0.146a	3.219±0.037a	27.167±1.403a	0.982±0.008a

Notes: The mean of AWCD, H, R, E, and data are expressed in means ± SE. The mean comparison was performed using Tukey's HSD. The mean with the same letter is not significant ($p \leq 0.05$). T1: Before the organic amendment application; T2: After the organic amendment application; T3: After paddy harvest

Organic amendments positively influenced AWCD and R, while H and E were not significantly affected. Organic inputs, including compost, manure, crop residues, and cover crops, have been shown to significantly enhance soil microbial activity and species richness by supplying essential nutrients, improving soil structure, and stimulating the production of soil enzymes (Tang *et al*., 2020; Cui *et al*., 2023). Organic materials are rich in carbon, nitrogen, phosphorus, and other essential nutrients. When organic inputs decompose, they release nutrients such as carbon, nitrogen, and phosphorus into the soil, which are readily available for microbes. This nutrient boost stimulates microbial growth and activity, as microbes thrive when nutrient availability is high.

The soil chemical properties observed in this study demonstrated an increase in soil pH and available phosphorus following the application of organic amendments. These improvements may explain the observed increase in AWCD and microbial species richness. Soil pH is widely recognized as one of the main factors regulating microbial community structure and diversity. A previous study by Zhou *et al* (2024) reported that bacterial richness exhibits a unimodal relationship with soil pH, with diversity generally increasing from acidic to near-neutral conditions across different biomes. Therefore, the increase in soil pH in the present study may have created a more favorable environment for microbial proliferation and metabolic activity. In addition, the enhanced availability of phosphorus may have contributed to the increase in microbial biomass and diversity. A global meta-analysis conducted by Chen and Xiao (2023) demonstrated that phosphorus addition significantly increases microbial biomass in soil ecosystems. Greater microbial biomass is commonly associated with elevated microbial activity, as reflected by higher AWCD values, which is consistent with findings

reported by Prommer *et al.* (2019) and Wang *et al.* (2024). Furthermore, increased phosphorus availability has also been linked to higher microbial species richness, as reported by Liu *et al.* (2025).

This study also demonstrated that organic amendments did not significantly affect soil microbial diversity or species evenness in paddy fields. The sampling site has been under continuous paddy cultivation for over 20 years, with standard agricultural practices, including the application of chemical fertilizers and pesticides, consistently implemented across all tested areas. Soil microbial communities often occupy specific ecological niches (Malard *et al*., 2022). If these niches are already fully occupied by particular microbial groups, introducing organic amendments may not result in significant changes in diversity. Changes in microbial diversity and species evenness may take time to manifest. In short-term studies or observations, these changes might not yet be evident. This may explain the absence of differences in microbial diversity and species evenness observed in this study. Organic amendments, particularly those that decompose slowly such as compost and manure, might initially have a minimal effect on the microbial diversity. Over time, as organic material breaks down and nutrients become more available, microbial diversity and species evenness could shift.

The sustained application of organic amendments in this study has the potential to enhance microbial diversity and functionality over time, which may subsequently influence paddy yields. A meta-analysis conducted by Shu *et al.* (2022) provides robust evidence that organic amendments significantly enhance microbial diversity compared to mineral-only fertilization. During the sampling period, species richness increased; however, microbial diversity was not affected by the addition of organic matter. This may be attributed to the microbial composition of the tested soil, which was predominantly comprised of oligotrophic bacteria. Oligotrophic bacteria are known to thrive in paddy soils and nutrient-poor environments (Su *et al*., 2023). Although the addition of organic amendments increases soil organic carbon, which typically promotes the growth of copiotrophic bacteria, their abundance may remain lower than that of oligotrophic bacteria due to the overall low levels of soil organic carbon. The increase in soil organic carbon following the application of organic amendments typically becomes evident over the long term, often after 10 years (Bai *et al*., 2023). Consequently, a corresponding increase in the abundance of copiotrophic and other bacterial groups is expected to occur gradually over time.

Substrate utilization

Organic amendment application (T2) increased microbial substrate utilization across all functional groups compared with the pre-amendment treatment (**Figure 1**). Among the substrate groups, carboxylic and acetic acids and amino acids exhibited the greatest increases in utilization, with percentage changes of 133.776% and 127.040%, respectively.

The enhanced utilization of these substrates suggests that organic amendment stimulated microbial metabolic activity and promoted the decomposition of readily available carbon and nitrogen compounds in the soil. Organic amendment application increased soil organic matter content, which promotes microbial decomposition and the production of low-molecular-weight organic acids (Cyle *et al*., 2021). The accumulation of these compounds may shift microbial substrate utilization toward carboxylic and acetic acids, as observed in the present study. Organic acids are considered readily available carbon sources and are rapidly assimilated by soil microorganisms for energy production and metabolic processes. Recent findings demonstrated that soil microbes may preferentially utilize organic acids over sugars in rhizosphere environments due to their rapid uptake and respiration rates (Wiesenbauer *et al*., 2025). Organic amendment application also increased total nitrogen content (Xu *et al*., 2025), which may explain the greater utilization of amino acids following amendment application. Increased nitrogen availability can stimulate microbial metabolism and enhance the utilization

of amino acid substrates, which serve as important sources of both carbon and nitrogen for soil microorganisms (Liu *et al*., 2023).

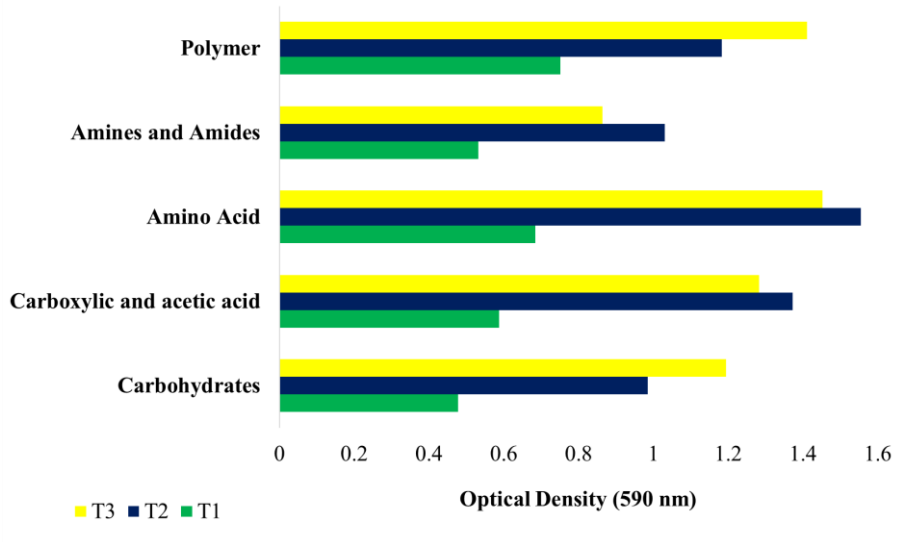


Figure 1. Substrate utilization patterns of soil microbial communities in IADA Kemasin Semerak paddy field

Microbial substrate utilization following paddy harvest (T3) also remained higher than that observed before organic amendment application. In particular, carbohydrates showed the highest utilization, with percentage increases of 149.912%. The increased utilization of this substrate after harvest may be associated with the accumulation of plant residues and root exudates, which provide complex carbon sources for soil microbial communities. The incorporation of crop residue on the soil surface with or without tillage has increased the metabolic capacity of soil microorganisms to exogenous carbon sources such as carbohydrates, as demonstrated by Cheng *et al*. (2022).

The application of organic amendments (T2) and following paddy harvest (T3) significantly stimulated a broad spectrum of microbial metabolic functions. Notably, there was an increased utilization of a diverse array of substrates, including L-arginine, L-asparagine, L-phenylalanine, pyruvic acid methyl ester, D-mannitol, N-acetyl-D-glucosamine, D-cellobiose, D-galacturonic acid, 4-hydroxybenzoic acid, γ-hydroxybutyric acid, D-malic acid, Tween 40, and Tween 80 (**Figure 2**). This enhanced substrate utilization suggests a stimulation of both energy and nitrogen metabolism, sustained nitrogen-associated microbial activity, improved carbon cycling, and an increased microbial adaptation to complex organic substrate.

Amino acids are important low-molecular-weight organic compounds that serve as readily available sources of carbon and nitrogen for soil microorganisms. The addition of organic amendments likely increased soil organic matter and nitrogen availability, thereby promoting microbial activity and amino acid metabolism. Previous studies have demonstrated that organic fertilizer application can significantly alter microbial carbon-source utilization patterns, particularly for amino acids. A study by Liu *et al*. (2023) reported that organic fertilizer substitution enhanced the microbial utilization of amino acids, especially L-asparagine, and improved soil nutrient status and microbial functional diversity in agricultural soils. The enhanced utilization of L-arginine may indicate increased microbial enzymatic and hydrolytic activities after organic amendment application. L-arginine addition stimulated soil hydrolytic and protease activities, indicating increased microbial decomposition capacity and nutrient cycling (Khosrozadeh and Nourbakhsh, 2023).

Malaysian Journal of Soil Science 2026 Vol. 30: 336-347
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Functional Guild	Substrates	T1	T2	T3
Amines and Amides	Phenylethylamine			
	Putrescine			
Amino Acid	L-Arginine			
	L-Asparagine			
	LPhenylalanine			
	L-Serine			
	L-Threonine			
	Glycyl-L-Glutamic Acid			
Carbohydrates	b-Methyl-D-Glucoside			
	Pyruvic Acid Methyl Ester			
	D-Xylose			
	i-Erythritol			
	D-Mannitol			
	N-Acetyl-D-Glucosamine			
	D-Cellobiose			
	Glucose-1- Phosphate			
	α-D-Lactose			
	D,L-α-Glycerol Phosphate			
Carboxylic and acetic acid	D-Galactonic Acid γ-Lactone			
	D-Galacturonic Acid			
	2-Hydroxy Benzoic Acid			
	4-Hydroxy Benzoic Acid			
	γ- Hydroxybutyric Acid			
	D-Glucosaminic Acid			
	Itaconic Acid			
	α-Ketobutyric Acid			
	D-Malic Acid			
Polymer	Tween 40			
	Tween 80			
	α- Cyclodextrin			
	Glycogen			

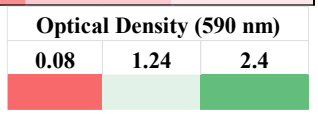


Figure 2. Heatmap illustrating microbial substrate utilization patterns categorized by functional groups

Pyruvic acid methyl ester, D-mannitol, N-acetyl-D-glucosamine, and D-cellobiose represent readily available carbon compounds associated with carbohydrate metabolism, microbial residue turnover, and cellulose degradation. The elevated utilization of D-mannitol may be associated with increased microbial activity and changes in soil enzymatic processes after amendment application. A study by Yu *et al*. (2023) demonstrated that mannitol amendment significantly altered soil microbial communities and enhanced soil enzymatic activities, indicating that sugar alcohols can serve as important microbial carbon substrates and promote nutrient cycling processes. The greater utilization of D-cellobiose suggests enhanced cellulolytic activity and decomposition of plant-derived organic matter. D-cellobiose is an intermediate product of cellulose degradation and is commonly utilized by microorganisms involved in carbon cycling. Increased utilization of this substrate may indicate stimulation of microbial populations associated with organic residue decomposition following organic

amendment application. Similar findings have been reported in studies showing that organic amendments promote copiotrophic microbial groups and accelerate carbon turnover in agricultural soils (Cui *et al*., 2023; Liu *et al*., 2023). Enhanced utilization of N-acetyl-D-glucosamine may reflect increased microbial turnover and decomposition of chitin and peptidoglycan derived compounds in soil. N-acetyl-D-glucosamine is an amino sugar derived from fungal cell walls and microbial residues and serves as an important source of carbon and nitrogen for soil microorganisms. Previous studies have demonstrated that amino sugars released from microbial cell wall decomposition are rapidly utilized by soil microbes and contribute substantially to soil organic nitrogen cycling (Ren *et al*., 2024). Similarly, the increased utilization of pyruvic acid methyl ester indicates elevated microbial respiration and energy metabolism, as pyruvate-related compounds are key intermediates in microbial carbon metabolism.

D-galacturonic acid, 4-hydroxybenzoic acid, γ-hydroxybutyric acid, D-malic acid, Tween 40, and Tween 80 are compounds associated with plant-derived organic matter decomposition, lipid metabolism, and aromatic compound transformation, suggesting that organic amendment stimulated microbial processes involved in carbon cycling and organic matter turnover. The elevated utilization of D-galacturonic acid may reflect enhanced degradation of pectin-rich plant residues after amendment application. D-galacturonic acid is a major component of pectin derived from plant cell walls and is commonly utilized by microorganisms involved in organic matter decomposition. Previous studies have shown that organic amendments enrich beneficial metabolites and stimulate microbial pathways associated with plant residue degradation and soil quality improvement (Fallah *et al*., 2023). The increased utilization of 4-hydroxybenzoic acid suggests enhanced microbial capacity for degrading aromatic compounds. Aromatic acids are important intermediates produced during lignin and phenolic compound decomposition, and their utilization indicates active microbial transformation of recalcitrant organic matter. Organic amendment application has been reported to shift microbial community composition and improve microbial carbon use efficiency, particularly for complex organic substrates and heterocyclic compounds (Luo *et al*., 2023).

Similarly, the higher utilization of γ-hydroxybutyric acid and D-malic acid may indicate increased microbial respiration and tricarboxylic acid cycle activity, as these compounds are closely related to microbial energy metabolism. Organic amendments can stimulate microbial enzymatic activity and increase the availability of low-molecular-weight organic compounds, thereby enhancing microbial metabolic processes and substrate turnover (Liu *et al*., 2023). The increased utilization of Tween 40 and Tween 80 further suggests enhanced lipid and ester degradation activity in amended soils. Tween substrates are commonly used as indicators of microbial lipolytic activity, and greater utilization may reflect stimulation of microorganisms capable of degrading fatty acid esters and complex organic compounds.

CONCLUSION

This study evaluated the short-term responses of soil microbial functional diversity to organic amendments in paddy fields. Organic amendment application significantly increased soil pH, available phosphorus, microbial activity and microbial richness, whereas microbial diversity and evenness remained statistically unchanged. Organic amendment application enhanced microbial substrate utilization across all functional groups, with the greatest increases observed for carboxylic and acetic acids and amino acids. Following paddy harvest, microbial substrate utilization remained elevated compared with the pre-amendment condition, particularly for carbohydrates. The application of organic amendments and subsequent paddy cultivation promoted the utilization of a broad range of carbon substrates. Notably, increased utilization of L-arginine, L-asparagine, L-phenylalanine, pyruvic acid methyl ester, D-mannitol, N-acetyl-

D-glucosamine, D-cellobiose, D-galacturonic acid, 4-hydroxybenzoic acid, γ-hydroxybutyric acid, D-malic acid, Tween 40, and Tween 80 highlights the enhanced metabolic versatility of the soil microbial community following amendment application. In conclusion, organic amendments exert positive short-term effects on soil microbial functional diversity, particularly by enhancing microbial activity, species richness, and substrate utilization capacity. Nevertheless, sustained application of organic amendments may be required to achieve long-term improvements in microbial diversity and ecosystem functioning. These improvements could contribute to enhanced nutrient cycling, soil health, and the sustainability of rice production systems. Long-term studies involving repeated amendment applications, combined with microbial community characterization using high-throughput sequencing and direct measurements of soil biochemical processes, are recommended to determine the persistence of these responses and their implications for soil fertility and sustainable rice production. Long-term studies involving repeated amendment applications, combined with microbial community characterization using high-throughput sequencing and direct measurements of soil biochemical processes, are recommended to determine the persistence of these responses and their implications for soil fertility and sustainable rice production.

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